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Pharmacophore-Based Discovery, Synthesis, and Biological Evaluation of 4-Phenyl-1-arylalkyl Piperidines as Dopamine Transporter Inhibitors

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Abstract—Pharmacophore-based discovery, synthesis, and structure–activity relationship (SAR) of a series of 4-phenyl-1-arylalkyl piperidines are disclosed. These compounds have been evaluated for their ability to inhibit reuptake of dopamine (DA) into striatal nerve endings (synaptosomes). The lead compound **5** and the most potent analogue **43** were found to have significant functional antagonism. © 2001 Elsevier Science Ltd. All rights reserved.

Because of its powerful reinforcing properties, (*R*)-cocaine (**1**) has great abuse potential. The level of cocaine abuse has reached epidemic proportions worldwide in recent years, and immediate therapies are needed for its treatment.[1] Cocaine reinforcing and stimulant properties have been associated with its ability to bind to monoamine transporter systems, particularly the dopamine transporter (DAT). Several studies have shown that compounds with DAT inhibitory activity may serve as potential therapeutic agents for cocaine abuse treatment.[1c] While no specific pharmacotherapy is currently available in clinic, two potent DAT inhibitors are now in clinical trials for the treatment of cocaine abuse.[2] Over the last 20 years, extensive chemical and pharmacological studies have been performed on several classes of DAT inhibitors, including tropanes (mainly compounds of the WIN series, **2**), benzotropanes, piperazines (also called GBR series, **4**) and most recently piperidines (**3**).[3]

We are interested in the discovery of novel DAT inhibitors that can be used as either cocaine antagonists or 'partial agonists',[4] and have used for this purpose a novel 3D-database pharmacophore searching approach. Novel DAT inhibitors are then evaluated as potential cocaine antagonists in an in vitro functional antagonism assay. In this functional antagonism assay, the IC_{50} value of cocaine in the presence of approximate IC_{10} to IC_{50} concentrations of candidate antagonist compounds was then compared to the IC_{50} value of cocaine alone. Significant differences in IC_{50} values were compared to theoretical IC_{50} values expected from models of 'same site' antagonism.[5] IC_{50} values greater than those expected for 'same site' antagonism were taken as evidence of functional antagonism. This test was performed under pre-incubation conditions to allow slowly equilibrating compounds to reach equilibrium. Further, any artifactual differences in IC_{50} (K_i) due to differences in temperature, tissue preparation, 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 insures 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.

We have recently reported the discovery of hydroxypiperidines as a novel class of DAT inhibitors with

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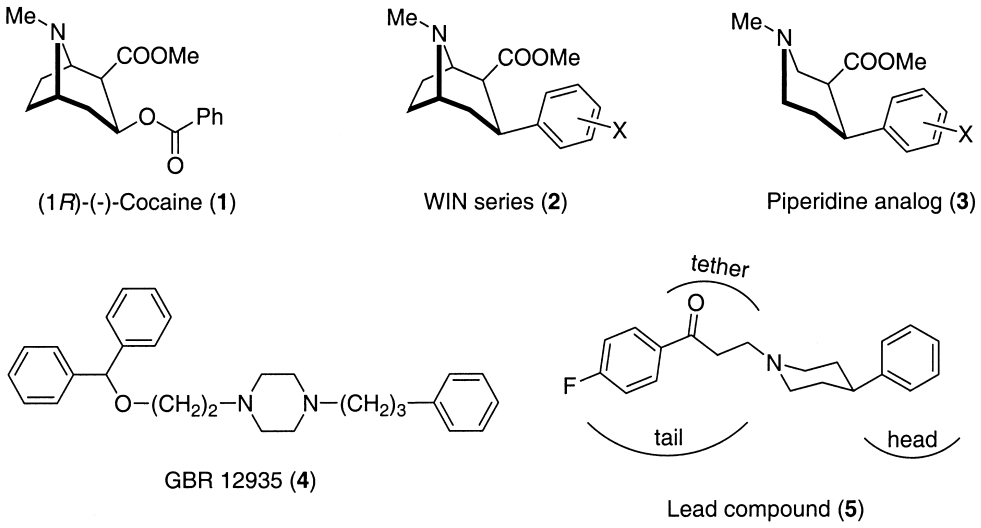


significant functional antagonism against cocaine.[4] Furthermore, using the same pharmacophore model, several additional classes of novel DAT inhibitors were discovered.[6] Compound **5** represents one such novel lead compound. Compound **5** was found to have a K_i value of 1.56 μM in the inhibition of DA reuptake (Table 1), a relatively weak DAT inhibitor. However, when **5** was tested in our functional antagonism assay as described previously,[4] it exhibits a significant functional antagonism. For example, in the presence of **5** (500 or 1000 nM), the experimental IC_{50} values of cocaine in the inhibition of DA reuptake were significantly increased (Table 2). These values are significantly greater than the theoretical values calculated using the same binding-site model.[5] These data thus suggested that **5** has significant functional antagonism against cocaine. Therefore, despite its weak potency, **5** may represent an interesting lead for further chemical modifications.

Since the pharmacophore model (Fig. 1) used to identify the lead compound was developed based upon cocaine and WIN compounds, we carried out molecular modeling studies to investigate the overlap between the lead compound **5** and WIN 35065. Conformational analysis (generation and energy minimization) was performed using the QUANTA/CHARMm program.[7] Upon the completion of conformation generation and energy minimization, the most stable conformation was identified (the global minimum in vacuum). It is noted, however, that the lowest energy conformation may not be the bioactive conformation. For this reason, other low energy conformations, typically within 5 kcal/mol of the global minimum were identified. It was found that **5** and WIN 35065 have a very good overlap with their low energy conformations with respect to the two binding elements defined in the pharmacophore model (i.e. the nitrogen atom and the phenyl ring at the 4-position of the piperidine **5** overlap very well with the corresponding structural elements of WIN 35065). The *N*-substituent of piperidine **5** has two different orientations, and the energy difference is less than 1 kcal/mol between these two conformations. One conformation has reasonable overlap with the third binding element, the methyl ester in the WIN compound (Fig. 2a). Thus, our molecular modeling suggested that despite the structural differences between **5** and WIN 35065, they may interact with DAT in a similar binding mode, although it is important to keep in mind that these calculations were done in vacuo.

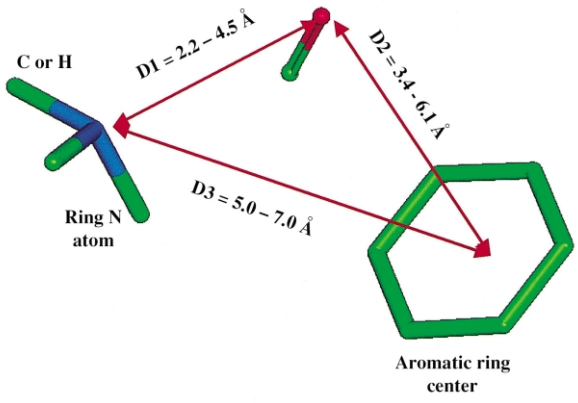


Figure 1. A 3D pharmacophore model derived from cocaine and WIN 35065 compounds used in 3D database pharmacophore search.

Compound **5** has a weak potency and its potency has to be improved significantly in order to have any therapeutic value. For this purpose, we have carried out SAR studies on this class of compounds. Compound **5** may be divided into three basic structural elements, the piperidine ring, the 4-phenyl substituent (head), and the phenyl ring (tail) tethered by a three-carbon linker. Accordingly, we made chemical modifications to the head, the tail, and the linker. The resulting compounds were evaluated for their ability to inhibit DA reuptake. The most potent compound was tested for its functional antagonism. It is of note that some *N*-arylalkyl-substituted tropanes with DAT inhibitory activity,[8] and piperidinylbutyrophenones with potent neuroleptic activity have been reported.[9a] The SAR studies and the evaluation of the in vitro functional antagonism against cocaine of the most potent compound are the central focus of this report.

The synthesis of compounds, outlined in Scheme 1, was accomplished by standard procedures.[9] 4-Piperidone hydrochloride was converted to its *N*-benzyl derivative **7** using benzyl bromide and potassium carbonate in DMF at 70 °C in 70% yield. 1-Benzyl-4-piperidone (**7**) was reacted with suitably substituted aryl Grignard reagents in THF at 0 °C to give compounds **8**, **9**, or **10** in good yields. One-step removal of the 4-hydroxyl and *N*-benzyl groups under hydrogenolysis conditions always gave mixtures. The 4-hydroxyl group was therefore first removed in a two-step sequence to give compounds **14** and **15** in quantitative yield. Compound **16** was prepared by debenzylation of compound **8** in 92% yield. To avoid the problem of reduction of the chlorine substituent under palladium catalyzed hydrogenation conditions in compound **13**, we have chosen Wilkinson's catalyst for the double bond reduction and 1-chloroethyl chloroformate for *N*-debenzylation.[10] Accordingly, compound **18** was prepared in good yield by reduction of the double bond in compound **13** using Wilkinson's catalyst, followed by the *N*-debenzylation using 1-chloroethyl chloroformate. Compounds **14**, **15**, **16**, and **18** were alkylated with various arylalkyl halides in DMF using potassium carbonate at 60 °C to give compounds **32**–**43**. The alkylation yields vary from 53 to 87% depending on the arylalkyl halide used (Scheme 3 and Table 1). The preparation of arylalkyl halides **21**, **22**, **25**, **28**, and **31** is shown in Scheme 2; the remaining arylalkyl halides were purchased from commercial suppliers.

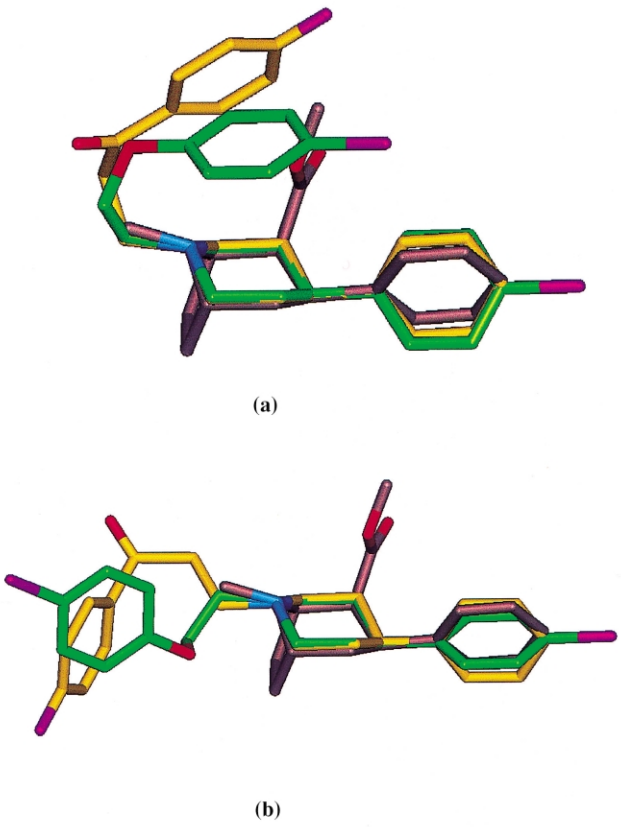
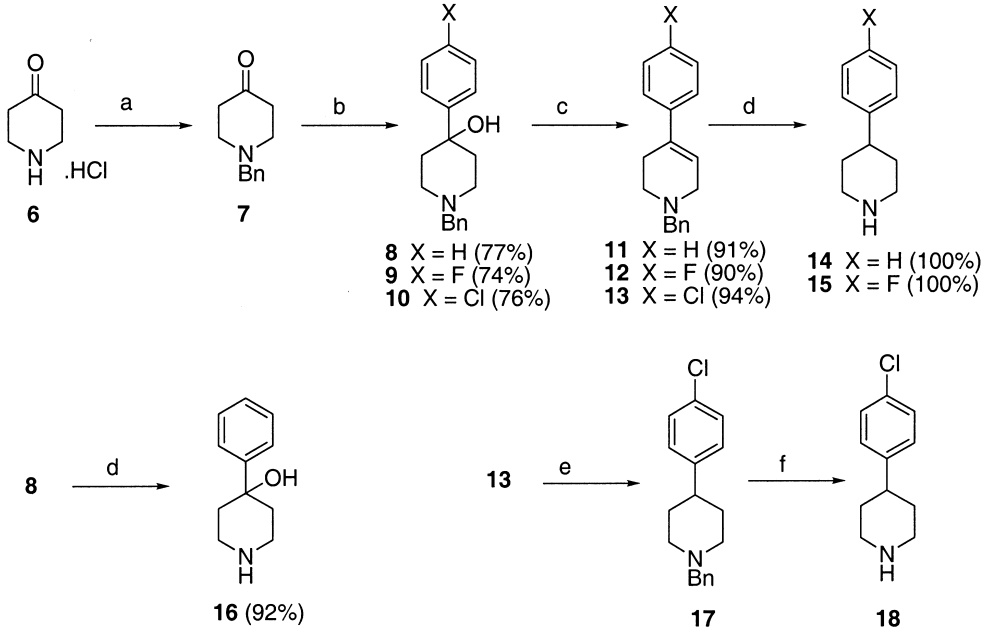
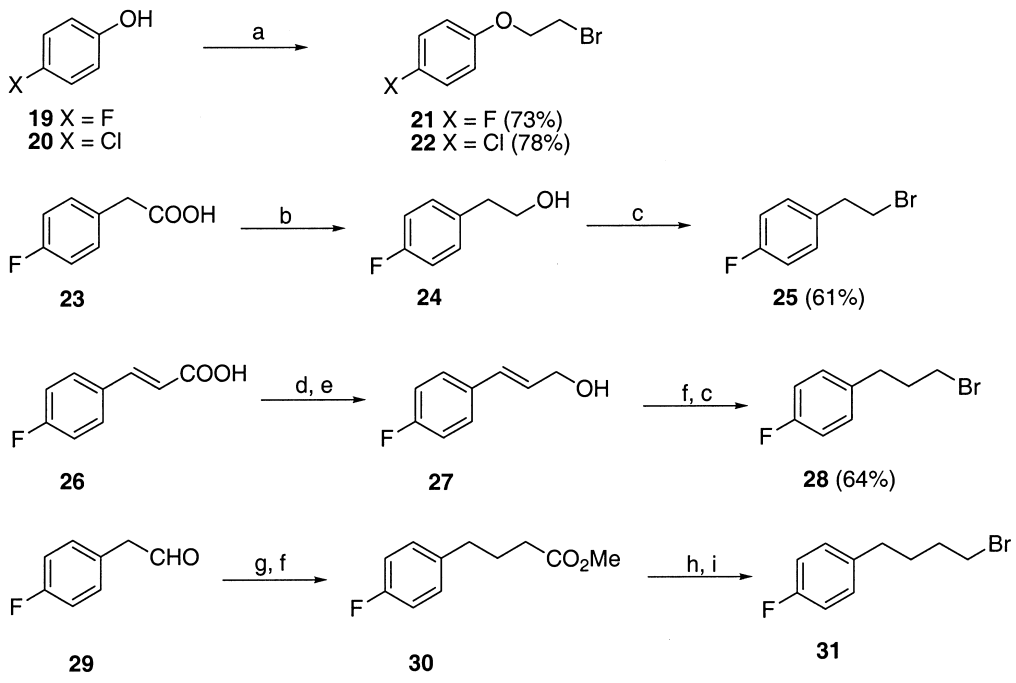


Figure 2. Superposition of two low energy conformations of lead compound **5** (yellow), and compound **43** (green) on WIN 35065 compound (gray).

The synthesized compounds were tested for their ability to inhibit the reuptake of DA, and the data are summarized in Table 1.[4] Compound **32** differs from the lead compound **5** only by a 4-fluoro substituent in the 'head' phenyl group and is slightly less potent than **5**. To investigate the effect of the length of the linker, compound **33** (one carbon shorter than **32**) and **34** (one carbon longer than **32**) were synthesized and tested. Both compounds **33** and **34** are less potent than **32** but the effect is moderate. To investigate the effect of the carbonyl group in the linker, the carbonyl group in **32**



Scheme 1. Reagents and conditions: (a) K_2CO_3, $PhCH_2Br$, DMF, 70 °C, 70%; (b) (*p*-X)PhMgBr, THF, 0 °C; (c) P_2O_5, toulene, reflux; (d) H_2, Pd/C, EtOH; (e) $(Ph_3P)_3RhCl$, H_2, toulene, 70 °C, 91%; (f) (1) 1-chloroethyl chloroformate, 1,2-dichloroethane, reflux, 2 h; (2) MeOH, reflux, 82% over two steps.

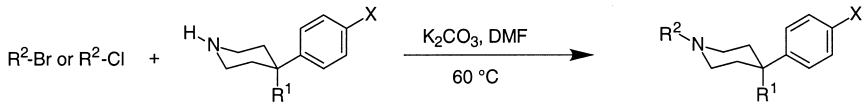


Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, 1,2-dibromoethane, 70 °C; (b) 1 M BH_3•THF, THF, 0 °C to reflux, 90%; (c) PBr_3, ether, 0 °C; (d) $SOCl_2$, MeOH, 0 °C; (e) 1 M DIBAL-H, CH_2Cl_2, −78 °C, 85%; (f) Pd/C, H_2, EtOH, 100%; (g) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, room temperature, 64%; (h) DIBAL-H, CH_2Cl_2, 0 °C, 81%; (i) Ph_3P, CBr_4, CH_2Cl_2, room temperature, 76%.

was replaced by a methylene group, and the resulting compound **35** was found to be slightly more potent than **32**. To further investigate the effect of the length of the linker, compounds **36**–**38** were synthesized and tested. Compound **36** with a one-carbon linker is at least 5-fold less potent than **35**, suggesting the importance of the length of the linker. While compound **37** with a two-carbon linker has a potency comparable to that of **35**, compound **38** with a four-carbon linker is slightly less potent than **35**. These data suggest that the optimal length of the linker is either two- or three-carbon atoms, although the effect is only marginal except for compound **36**. To investigate the electronic effect of the linker, the carbonyl group in the linker was replaced by an oxygen atom. The resulting compound **39** was 3-fold more potent than **32**. To investigate the effect of the 4-fluoro substituent in the 'head' phenyl ring, compound **40** without the 4-fluoro substituent was synthesized and found to be 3-fold less potent than **39**. An additional hydroxyl group at the 4-position of the piperidine ring has a detrimental effect since compound **41** is 7-fold less potent than compound **40** without the hydroxyl group. It is known from the literature that a *para* chloro substituent in the phenyl ring of the WIN series is optimal for activity.[11] To investigate the effect of the substituents on both the 'head' and the 'tail' phenyl rings, compounds **42** and **43** were synthesized and tested.[12] Compound **42** with a 4-chloro substituent in the 'tail' phenyl ring is slightly more potent than compound **39** with a 4-fluoro substituent. However, compound **43** with a 4-chloro substituent in both the 'head' and the 'tail' phenyl rings is 9-fold more potent than compound **39** and 7-fold more potent than compound **42**. These data suggest that a substituent on the 'head' phenyl ring significantly improves the potency of the compounds relative to the substituent on the 'tail' phenyl ring. 4-Chloro substituted compound **43** is a reasonably potent DAT inhibitor with a K_i value of 90 nM, and is 17-fold more potent than the lead compound **5**.

The most potent compound **43** was then tested for its functional antagonism against cocaine. It was found that in the presence of 20 and 50 nM of **43**, the IC_{50} values of cocaine in inhibition of DA reuptake were increased and these values are significantly greater than the calculated theoretical values, if one assumes that cocaine and **43** bind to the same binding site at the DAT (Table 2). Therefore, compound **43** is not only a reasonably potent DAT inhibitor but also possesses significant functional antagonism. It is of interest that modification of the structure had no effect on antagonism per se. That is, although there was about a 17-fold difference between the potencies of **5** and **43**, there is a similar degree of right-shift in the cocaine inhibition curve when these compounds are tested at similar concentrations relative to their own K_i. The precise mechanism of the functional antagonism is not clear.

In conclusion, a lead compound (**5**) with moderate activity but significant functional antagonism was discovered using a pharmacophore-based 3D-database searching approach. Through chemical modifications, we identified a new analogue (compound **43**), which is 17-fold more potent than the lead compound in the inhibition of DA reuptake with a K_i value of 90 nM. Similar to the lead compound, this more potent analogue **43** was found to have significant functional antagonism. Further pharmacological and behavioral studies are under way to investigate the mechanism of functional antagonism of **43**, and its therapeutic potential for the treatment of cocaine abuse.



Scheme 3.

Table 1. Chemical structures of DAT inhibitors and their potency as inhibitors of DA reuptake

R^2-Br or R^2-Cl	R^1	X	Yield (%)	Compd	[^{3}H]DA uptake K_i (μM)
	H	H	—	Lead (**5**)	1.56 ± 0.06
	H	F	60	**32**	2.67 ± 0.39
	H	F	68	**33**	4.33 ± 0.28
	H	F	73	**34**	3.17 ± 0.19
	H	F	53	**35**	1.86 ± 0.01
	H	F	82	**36**	>10
	H	F	59	**37**	1.36 ± 0.02
	H	F	66	**38**	2.54 ± 0.02
	H	F	68	**39**	**0.84 ± 0.08**
	H	H	63	**40**	2.72 ± 0.03
	OH	H	54	**41**	17.55 ± 1.53
	H	F	87	**42**	**0.63 ± 0.06**
	H	Cl	71	**43**	**0.09 ± 0.01**

Table 2. Experimental and theoretical IC_{50} values of cocaine in the presence of lead compound **5** and compound **43** for [^{3}H]-dopamine uptake by striatal membrane preparations

	[^{3}H]-DA uptake	
Experimental conditions	Experimental IC_{50} (mean ± SEM)	Theoretical IC_{50} (mean ± SEM)
Cocaine	297 ± 22 nM	
Cocaine + lead (**5**) (500 nM)	538 ± 25 nM	383 ± 13 nM
Cocaine + lead (**5**) (1000 nM)	686 ± 4 nM	471 ± 13 nM
Cocaine + compound **43** (20 nM)	490 ± 74 nM	354 ± 17 nM
Cocaine + compound **43** (50 nM)	696 ± 32 nM	441 ± 22 nM

Acknowledgements

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12. Compound **43**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69–1.82 (4H, m), 2.17–2.26 (2H, m), 2.43–2.53 (1H, m), 2.83 (2H, t, $J = 5.9$ Hz), 3.10 (2H, d, $J = 11.7$ Hz), 4.10 (2H, t, $J = 6.1$ Hz), 6.84 (2H, dd, $J = 2.2$ Hz, 6.9 Hz), 7.14 (2H, dd, $J = 1.7$ Hz, 8.3 Hz), 7.21–7.28 (4H, m); ^{13}C NMR ($CDCl_3$) δ 33.2, 41.8, 54.6, 57.4, 66.3, 115.8, 125.6, 128.1, 128.5, 129.3, 131.6, 144.6, 157.3; MS *m*/*z* (%) 348 (M−1, 4), 210 (64), 208 (100).