Synthesis and Pharmacology of Potential Cocaine Antagonists. 2. Structure−Activity Relationship Studies of Aromatic Ring-Substituted Methylphenidate Analogs

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As part of a program to develop medications which can block the binding of cocaine to the dopamine transporter, yet spare dopamine uptake, a series of aromatic ring-substituted methylphenidate derivatives was synthesized and tested for inhibitory potency in [^{3}H]WIN 35,428 binding and [^{3}H]dopamine uptake assays using rat striatal tissue. Synthesis was accomplished by alkylation of 2-bromopyridine with anions derived from various substituted phenylacetonitriles. In most cases, *erythro* compounds were markedly less potent than the corresponding (±)-*threo*-methylphenidate (TMP; Ritalin) derivatives. The *ortho*-substituted compounds were much less potent than the corresponding *meta*- and/or *para*-substituted derivatives. The most potent compound against [^{3}H]WIN 35,428 binding, *m*-bromo-TMP, was 20-fold more potent than the parent compound, whereas the most potent compound against [^{3}H]dopamine uptake, *m*,*p*-dichloro-TMP, was 32-fold more potent. *Threo* derivatives with *m*- or *p*-halo substituents were more potent than TMP, while electron-donating substituents caused little change or a small loss of potency. All of the derivatives had Hill coefficients approaching unity, except *m*,*p*-dichloro-TMP, which had an n_H of 2.0. Although the potency of the (±)-methylphenidate derivatives in the two assays was highly correlated (R^2 = 0.986), the compounds *m*-chloro-, *m*-methyl-, and *p*-iodo-TMP were 4−5-fold more potent at inhibiting [^{3}H]-WIN 35,428 binding than [^{3}H]dopamine uptake (cocaine has a ratio of 2.3). These and other compounds may be promising candidates for further testing as potential partial agonists or antagonists of cocaine.

Introduction

Abuse of stimulant drugs (primarily of cocaine) is a major continuing problem in the United States.[1] This work aims to contribute to the solution of this problem by developing treatment agents (partial agonists or antagonists) directed toward the stimulant recognition site on the dopamine transport complex, where the reinforcing effect of these agents is thought to be mediated.[2−4] These drugs act primarily by blocking the re-uptake of dopamine into the presynaptic neuron, thus increasing the concentration of dopamine in the synapse. Our goal is to synthesize drugs, which at least in part can block the binding of cocaine, yet allow dopamine uptake. Although many studies suggest that dopamine and stimulant drugs compete for the same site on the dopamine transporter,[5] both binding[6,7] and thermodynamic[8] studies suggest that there may be separate or overlapping, but not identical, stimulant and substrate binding sites. Cloning of the dopamine transporter has allowed site-directed mutagenesis experiments which suggested separate residues are important for cocaine binding and for dopamine transport.[9] In addition, the results of experiments involving protection of the transporter with different ligands from alkylation by *N*-ethylmaleimide[10−12] have been interpreted in terms of substrate and inhibitors having different binding sites. Very recently, a detailed molecular model of the dopamine transporter has been constructed.[13] This model predicts 12 transmembrane-spanning α-helices (TMH) and separate binding sites for dopamine (TMH 1,7 and 10−12) and cocaine (1,7 and 9−11). Simoni *et al.*[14] have shown that two methoxy-substituted cocaine analogs are 4 times and 2.5 times weaker as inhibitors of dopamine uptake than would be predicted from their ability to inhibit [^{3}H]mazindol binding. He *et al.*[15] showed that the ratio of the inhibition of [^{3}H]-cocaine binding to [^{3}H]dopamine uptake for a series of BTCP analogs varied from 0.5 to 9. Newman *et al.*[16] have synthesized a tropane analog which has a ratio of uptake to binding inhibition of 6 and has little effect on locomotor stimulation in mice. Furthermore, this compound did not substitute for cocaine in rats trained to discriminate 10 mg/kg of cocaine from saline. Behavioral data[17] for 4′-iodococaine suggests that this compound has little effect on locomotor stimulation, but is able to block the effects of cocaine. Our recent work[18] has shown that the relationship between binding and uptake can be changed by simple structural modifications. Taken together, these studies support the possibility that a drug could be developed which can block the cocaine binding site, but not interfere with dopamine uptake.

(±)-*threo*-Methylphenidate (**1a**, Ritalin, methyl ritalinate) was selected as a promising candidate for drug development based on several factors. It binds potently and somewhat selectively to the dopamine transporter[2,7,19] and has been used to study the stimulant binding site.[20] It is a well-known stimulant that, paradoxically, is used to treat hyperactive children. In spite of its extensive use, little is known about structure−activity relationships for methylphenidate analogs.

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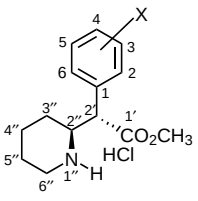
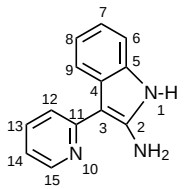
First synthesized in 1944[21] and recognized as a stimulant in 1954,[22] early chemical work is described primarily in the patent literature[23] and was centered on the separation and interconversion of the so called (a) isomer and the biologically more active (b) isomer. The absolute (2*R*,2′*R*; *threo*) stereochemistry of the most active enantiomer of methylphenidate (**1a**) was proven by chemical techniques.[24,25] A series[26] of alkyl esters of (±)-*threo*-ritalinic acid was made in 1961 and shown to be less potent stimulants than **1a**. In 1974 Faraj *et al.*[27] reported on the synthesis of *p*-hydroxymethylphenidate (**1b**, mixture of *erythro* and *threo* isomers) and its relationship to the metabolism of **1a**. Compound **1b** (mixture) was tested for locomotor activity in mice; it was inactive by ip administration and weakly active by the *intraventricular* (icv) route compared to **1a**. Pure compound **1b** was synthesized by Patrick *et al.*[28] They showed, as judged by locomotor stimulation in rats, that when given icv the *threo* isomers **1a** and **1b** were more potent than the corresponding *erythro* isomers and that **1b** was more potent than **1a**. In addition, **1b** had a longer duration of action than **1a**. Wolters *et al.*[29] synthesized **1c** but did not consider which isomer they produced; the probable *erythro* **1c** showed little biological activity. Very recently Pan *et al.*[30] have reported the synthesis of *o*-, *m*-, and *p*-bromomethylphenidate (**1d**, **1e**, and **1f**). Compared to **1a**, these compounds are reported to be 6, 20, and 9 times more potent, respectively, as inhibitors of the binding of the cocaine analog [^{3}H]WIN 35,428; although they all cause locomotor stimulation in rats given 20 mg/kg (ip), their maximum effects are less than **1a**. [These three compounds have been resynthesized by Pan *et al.* and retested with somewhat different results. Retesting in the laboratory of Dr. Gatley (private communication) gave binding results for **1e** and **1f** in substantial agreement with his earlier results; however, **1d** was about 20 times *less* potent than **1a** rather then 6 times *more* potent. The reasons for the discrepancy with **1d** are under investigation.] These authors have also presented some preliminary results[31] of their further work in this area, including the synthesis of **1b**, **1g**, and **1h**. This work was part of their program[32] to develop specific PET probes for the dopamine transporter. They also suggest that **1g** may be a potential cocaine antagonist.



(±)-*threo*-**1**



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1a, X = H; **1b**, X = 4-OH; **1c**, X = 3,4,5-tri-MeO;
1d, X = 2-Br; **1e**, X = 3-Br; **1f**, X = 4-Br; **1g**, X = 4-OMe;
1h, X = 3-1,4-OH; **1i**, X = 4-*t*-Bu; **1j**, X = 2-Cl;
1k, X = 3-Cl; **1l**, X = 4-Cl; **1m**, X = 3,4-di-Cl; **1n**, X = 2-F;
1o, X = 3-F; **1p**, X = 4-F; **1q**, X = 4-I; **1r**, X = 3-Me;
1s, X = 4-Me; **1t**, X = 3-NH_2•HCl; **1u**, X = 4-NH_2•HCl;
1v, X = 4-NO_2; **1w**, X = 2-OH; **1x**, X = 3-OH;
1y, X = 2-OMe; **1z**, X = 3-OMe; **1aa**, X = 3,4-di-OMe

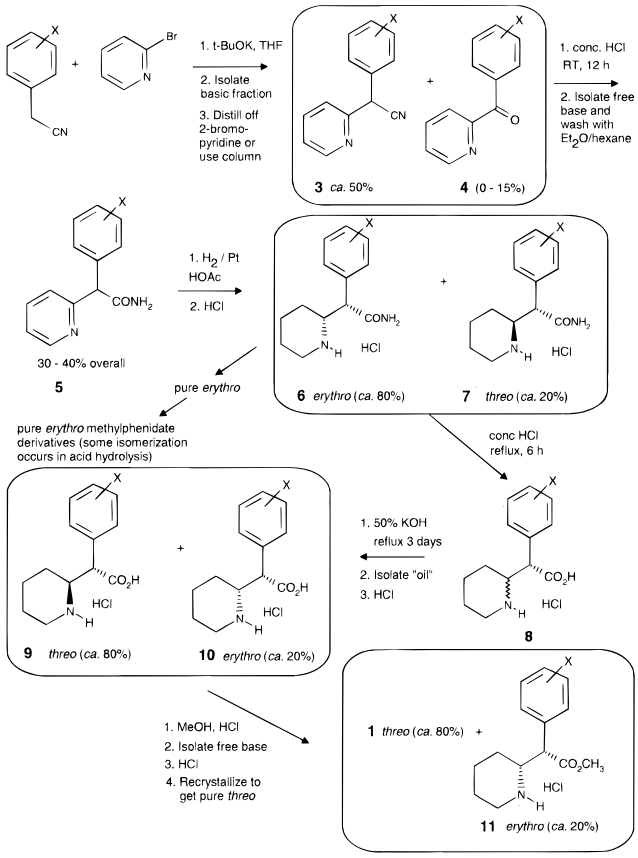
The mechanism for the stimulant properties of methylphenidate has generally been assigned to be the same as cocaine.[33] Availability of the tritiated form of the drug made it possible to show that binding sites for methylphenidate are localized primarily on dopaminergic nerve terminals[19] and are associated with dopamine uptake.[34] The biological relevance of the site recognized by methylphenidate was clarified by Schweri *et al.*[19] who showed that the stimulant properties of **1a** and a series of alkyl esters were directly correlated to their ability to bind to the site. Methylphenidate is self-administered by animals. However, it does not seem to have as great an abuse potential in humans as cocaine despite its widespread availability and may actually reduce craving for cocaine.[35] Although methylphenidate acts in a manner similar to cocaine, there may also be significant differences in the exact manner in which it binds or the exact site where it binds. For example, the entropy of binding[8] ($\Delta S°$, measured using [^{3}H]GBR 12783) for methylphenidate is −25.5 kcal/mol whereas it is −5.6 kcal/mol for cocaine, each at 25°.

On the basis of the above information, we initiated a program to synthesize and evaluate congeners of methylphenidate. The aim was to produce compounds which, because of their unique interactions with the dopamine uptake complex, would be antagonists or partial agonists for the reinforcing properties of cocaine. This paper describes the synthesis of aromatic ring-substituted analogs and compares their potency in the inhibition of [^{3}H]WIN 35,428 binding to their ability to inhibit [^{3}H]dopamine uptake.

Results

Chemistry. Our strategy for synthesis, corresponding generally to the methods previously developed in the literature, was based on the alkylation of 2-bromopyridine with anions derived from various phenylacetonitriles. A summary of this method is shown in Scheme 1. Several significant modifications in the literature procedures were made in order to make the reaction scheme more efficient. The original method of Panizzon[21] called for the use of sodium amide in toluene and 2-chloropyridine for the first step; most subsequent workers have used this method. In our experience, this procedure often gave mixtures of products which sometimes required difficult chromatographic separations. In addition, certain substituent groups would be expected to be incompatible with these conditions. The use of potassium *tert*-butoxide in THF and 2-bromopyridine worked better in our hands. The ketone byproducts **4** that were sometimes produced are easily removed in the next step. The use of concentrated hydrochloric acid was preferable to the standard condition of concentrated sulfuric acid for the hydrolysis of the nitriles **3** to the amides **5**. The yields were generally higher, and the problem of aromatic ring sulfonation, when X = OCH_3, was avoided. Most workers have used the piperidine amides **6** and **7** in the 50% KOH epimerization procedure; we found this to be very unreliable. Variable results from run to run and low yields were often obtained. When the amides were first hydrolyzed and the acids **8** used for epimerization, the reaction proceeded much more reliably. Interestingly, the acids formed an insoluble "oil" in the 50% KOH solution. For the 2-chloro compound, base-catalyzed epimerization did not work well. However, treatment with 6N HCl under reflux for three days produced a *threo*/*erythro* ratio of 60:40. In all cases except the 2-hydroxyl compound, the desired *threo* isomer was obtained by crystallization of the mixture of hydrochloride salts of the methylpheni-

Scheme 1



X=3 and 4-NH_2; 4-*t*-Bu; 2, 3 and 4-Cl; 3, 4-di-Cl; 3, 4-di-OMe; 2, 3 and 4-F; 2, 3 and 4-MeO; 3 and 4-Me.

date derivatives **1** and **11** from various solvents. In several cases the pure *erythro* amides **6** were isolated by crystallization or simple solvent washing. They were hydrolyzed to the *erythro* acids **10** with a small amount of epimerization (*ca.* 10%) and then converted to methyl esters from which the pure *erythro* methylphenidate analogs could be isolated by crystallization.

The alkylation procedure above failed for 4-nitro- and 4-(trifluoromethyl)phenylacetonitrile; no condensation product could be isolated. With 2-aminophenylacetonitrile only the 2-aminoindole **2** could be isolated (this compound does not appear to be described in the literature and its structure is based on the IR, MS, and ^{1}H-NMR spectral data). (±)-*threo*-4-Nitromethylphenidate (**1v**) was synthesized by the direct nitration of (±)-*threo*-methylphenidate with fuming nitric acid. A summary of some selected properties of the compounds synthesized in this study is shown as Table 1.

Pharmacology. All of the compounds that were synthesized in this study were tested for their ability to inhibit the binding of [^{3}H]WIN 35,428 to rat striatal tissue membrane preparations, as well as the uptake of [^{3}H]dopamine into rat striatal synaptosomes. These results are summarized in Table 2.

Discussion

Chemistry. Significant refinements of the literature conditions gave a synthetic scheme which was more reproducible with higher overall yields. Both *erythro* and *threo* isomers of methylphenidate analogs can be produced by this method, although a direct stereoselective synthesis of the *threo* isomer would be highly desirable. A summary of selected properties of the compounds synthesized in this study is shown as Table 1.

Table 1. Selected Properties of Compounds Synthesized in This Study

compd	X	pos	overall yield, %[a]	mp, °C	recryst sol[b]	anal.
1a	H		18	199−200	B	
1i	$C(CH_3)_3$	4	18	221.5−222.5	G	C, H, N, Cl
1s	CH_3	4	13	204.5−205	A	C, H, N, Cl
1r	CH_3	3	19	200−201	A	C, H, N, Cl
1j	Cl	2	18	192.5−193.5	B	C, H, N, Cl
1k	Cl	3	22	205.5−206.5	D	C, H, N, Cl
1l	Cl	4	7	201−203	A	C, H, N, Cl
1m	di-Cl	34	13	214−215	B	C, H, N, Cl
1n	F	2	20	205.5−206.5	A	C, H, N, Cl
1o	F	3	26	213−214	A	C, H, N, Cl
1p	F	4	12	208.5−210.5	A	C, H, N, Cl
1t	NH_2·HCl	3	12	225.5−227.5	C	C, H, N, Cl
1u	NH_2·HCl	4	24	211 (dec)	C	C, H, N, Cl
1v	NO_2	4		189−191	F	C, H, N, Cl
1w	OH[c]	2				
1x	OH	3	16	201.5−202	F	C, H, N, Cl
1b	OH	4	8	211−212[d]	F	C, H, N, Cl
1y	OMe	2	28	189.5−192	C	C, H, N, Cl
1z	OMe	3	22	203−204.5	C	C, H, N, Cl
1g	OMe	4	10	193.5−195[d]	C	
1aa	di-OMe	34	13	214.5−216	E	C, H, N, Cl
11a	$C(CH_3)_3$	4	6	199−202	A	C, H, N, Cl
11c	Cl	2	23	186.5−188	B	C, H, N, Cl
11d	Cl	3	15	200−201	C	C, H, N, Cl
11e	OMe	2	28	190−191	C	C, H, N, Cl

[a] Free base mixture. [b] A, EtOAc/MeOH (2:1); B, EtOAc/MeOH (1:1); C, EtOAc/MeOH (1:2); D, MeOH; E, acetone/MeOH (1:1); F, acetone; G, acetone/MeOH (2:1). [c] Mixture *erythro* and *threo*. [d] Literature[28] mp 222−224 °C.

This method failed completely with 4-(trifluoromethyl)- and 4-nitrophenylacetonitriles. Apparently, the intermediate enolates from these compounds are not reactive enough toward the relatively poor electrophile 2-bromopyridine. (±)-*threo*-4-Nitromethylphenidate was made by direct nitration of (±)-*threo*-methylphenidate. The product of this reaction was difficult to purify because of the formation of the 3-nitro isomer (presumed impurity based on [^{1}H]-NMR analysis).

In our experience the 50% KOH epimerization step worked best with the acids **8** rather than the amides **6** and **7**. The potassium salts of **8** are insoluble in 50% KOH and float on top as an "oil". This oil is relatively easy to separate, and after esterification the contaminating inorganics can be separated from the free base. After epimerization, the *threo*/*erythro* ratio (**9:10**) varied from about 1:1 for 4-*tert*-butyl and 3,4-dimethoxy to about 20:1 for 2-fluoro, but was generally about 4:1. The less soluble *threo* hydrochloride salt was easily separated by crystallization. In cases where less of the *threo* isomer was produced, purification was more difficult.

The assignment of *threo* and *erythro* to the isomers of methylphenidate congeners was based on several factors. First, by analogy with **1a**, the hydrogenation step (**5** to **6** and **7**) would be expected to produce a preponderance of the *erythro* isomers in all cases. In fact, the hydrogenation reaction always produced an approximate 80/20 mixture of isomers. Further analysis of these mixtures was always consistent with the major isomer being *erythro*. Also, based on ^{1}H-NMR analysis of the intermediates and final products in the synthesis of **1a**, a clear pattern for the two isomers was evident. This pattern was confirmed in all of the congeners synthesized in this study (see supplemental material). An example of the pattern is shown in Figure 1.

Table 2. Inhibition of [^{3}H]WIN 35,428 Binding and [^{3}H]Dopamine Uptake of Methylphenidate Derivatives and Related Compounds [Value ± SEM (*n*)]

			IC_{50}, nM			
compd	X	pos	[^{3}H]WIN 35,428 binding	[^{3}H]dopamine uptake	Hill coefficient for WIN binding	discrimination ratio[a]
1a	H		83.0 ± 7.9 (4)	224 ± 19 (4)	0.90 ± 0.09	2.7
1d[b]	Br	2	1870 ± 135 (2)	3410 ± 290 (2)	0.93 ± 0.00	1.8
1e[b]	Br	3	4.2 ± 0.2 (2)	12.8 ± 0.20 (2)	1.14 ± 0.07	3.1
1f[b]	Br	4	6.9 ± 0.1 (2)	26.3 ± 5.8 (3)	1.15 ± 0.07	3.8
1i	*t*-Bu	4	13500 ± 450 (2)	9350 ± 950 (2)	1.12 ± 0.08	0.7
1j	Cl	2	1950 ± 230 (3)	2660 ± 140 (2)	0.98 ± 0.02	1.4
1k	Cl	3	5.1 ± 1.6 (3)	23.0 ± 3.0 (2)	0.95 ± 0.12	4.5
1l	Cl	4	20.6 ± 3.4 (5)	73.8 ± 8.1 (5)	1.17 ± 0.09	3.6
1m	di-Cl	3,4	5.3 ± 0.7 (2)	7.0 ± 0.6 (2)	2.07 ± 0.05	1.3
1n	F	2	1420 ± 120 (2)	2900 ± 300 (2)	0.90 ± 0.02	2.1
1o	F	3	40.5 ± 4.5 (2)	160 ± 0.00 (2)	0.85 ± 0.10	4.0
1p	F	4	35.0 ± 3.0 (2)	142 ± 2.0 (2)	0.97 ± 0.02	4.1
1q[b]	I	4	14.0 ± 0.1 (2)	64.5 ± 3.5 (3)	1.10 ± 0.04	4.6
1r	Me	3	21.4 ± 1.1 (2)	100 ± 18 (2)	1.01 ± 0.11	4.7
1s	Me	4	33.0 ± 1.2 (2)	126 ± 1 (2)	1.05 ± 0.02	3.8
1t	NH_2·HCl	3	265 ± 5 (2)	578 ± 160 (2)	1.06 ± 0.13	2.2
1u	NH_2·HCl	4	34.6 ± 4.0 (3)	115 ± 10 (2)	0.96 ± 0.09	3.3
1v	NO_2	4	494 ± 33 (4)	1610 ± 210 (2)	1.17 ± 0.10	3.3
1w[c]	OH	2	23100 ± 50 (2)	35800 ± 800 (2)	1.04 ± 0.04	1.6
1x	OH	3	321 ± 1.0 (2)	790 ± 30 (2)	1.09 ± 0.02	2.5
1b	OH	4	98.0 ± 10 (2)	340 ± 70 (2)	1.07 ± 0.12	3.5
1y	OMe	2	101000 ± 10000 (3)	81000 ± 2000 (2)	0.94 ± 0.09	0.8
1z	OMe	3	288 ± 53 (2)	635 ± 35 (2)	1.11 ± 0.16	2.2
1g	OMe	4	83 ± 11 (2)	293 ± 48 (2)	0.83 ± 0.10	3.5
1aa	di-OMe	3,4	810 ± 10 (2)	1760 ± 160 (2)	1.12 ± 0.00	2.2
11a	*t*-Bu	4	41300 ± 250 (2)	52500 ± 3500 (2)	1.10 ± 0.18	1.3
11b[b]	Br	2	38100 ± 1900 (2)	59000 ± 5000 (2)	1.06 ± 0.01	1.5
11c	Cl	2	52500 ± 1500 (2)	61000 ± 3000 (2)	0.92 ± 0.01	1.2
11d	Cl	3	378 ± 73 (2)	1540 ± 40 (3)	1.03 ± 0.00	4.1
11e	OMe	2	139000 ± 6500 (2)	290000 ± 5000 (2)	0.87 ± 0.10	2.1
COC[d]			173 ± 13 (2)	404 ± 26 (2)	1.03 ± 0.01	2.3

[a] Ratio of uptake to binding. [b] Samples obtained from Dr. S. John Gatley, Brookhaven National Laboratories. [c] Mixture of *erythro* and *threo* (*ca.* 1/1). [d] (*R*)-Cocaine.

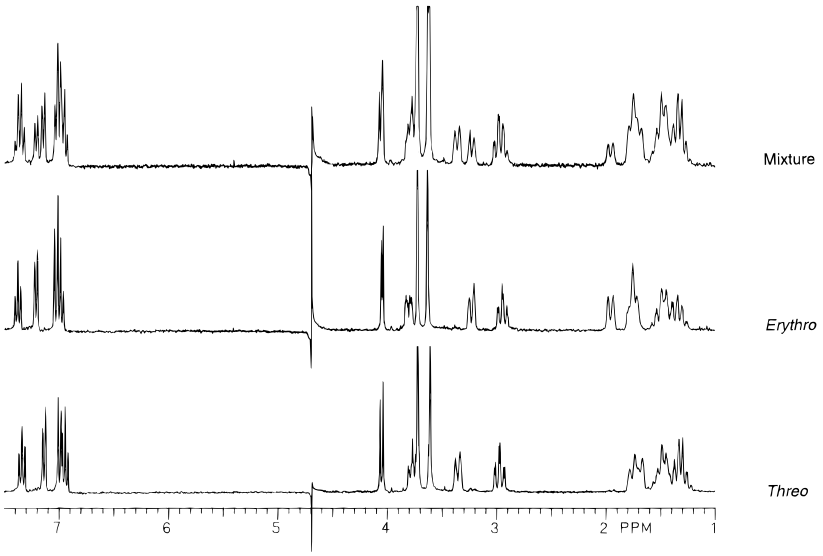


Figure 1. ^{1}H-NMR spectra of *erythro*- and *threo*-2-methoxymethylphenidate and mixtures of the isomers.

Although the relative stereochemistry of the "active" and "inactive" isomers of (±)-methylphenidate (**1a**) has been firmly established by chemical means, a confirmation of the assignments made in this study was desirable. To this end, high-quality crystals of (±)-*threo*-4-chloro- (**1l**) and (±)-*threo*-2-methoxymethylphenidate (**1y**) hydrochlorides were obtained (assignment made by the above-mentioned arguments). The structures of these compounds were solved by X-ray crystallography, the details of which will be published elsewhere. Shown as Figure 2 are the solid state structures of these compounds, which confirm the relative *threo* stereochemistry in each case. In addition, in cases where both isomers of a given compound were isolated, the biological screening clearly showed that the one assigned the *threo* stereochemistry was the more active. No attempt was made to resolve any of the compounds in this study into the individual enantiomers. If promising compounds are identified on the basis of further biological testing, optical resolution will be attempted.

Pharmacology: A. [^{3}H]WIN 35,428 Binding; 1. *Erythro* Isomers. The *erythro* isomers were less active

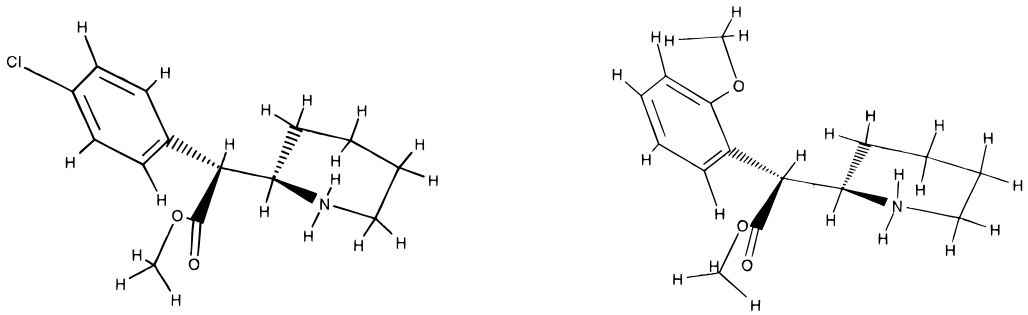


Figure 2. X-ray structures of (±)-*threo*-4-chloro- (left) and 2-methoxymethylphenidate (right) hydrochlorides (with chloride removed).

than the corresponding *threo* isomers. The ratio of IC_{50} values (*erythro*/*threo*) varied: **11d**/**1k** (X = 3-Cl) 74; **11c**/**1j** (X = 2-Cl) 27; **11b**/**1d** (X = 2-Br) 20; **11a**/**1i** (X = 4-*t*-Bu) 3.0; **11e**/**1y** (X = 2-MeO) 1.4. For the highly active **1k** the value is consistent with the ratio of 76 reported by us for the enantiomers of methylphenidate (**1a**) in displacing [^{3}H]methylphenidate binding.[19] For the less active compounds the ratio was generally much lower. These values supported the assignments of *erythro* and *threo* for these and, by inference, the other compounds in this study.

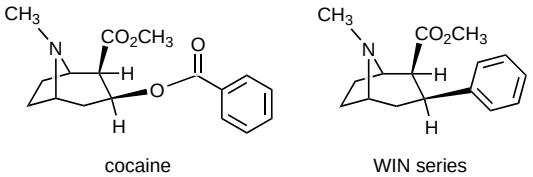
2. *Threo* Isomers; a. 2-Position Isomers. All of the derivatives with substituents in the 2-position were much less active than those with the same substituent in the 3- or 4-positions. This loss in activity (for example, ratio of IC_{50} for position 2/4) was largest with methoxy (1200) followed by bromo (270), chloro (95), and fluoro (41). This effect is roughly correlated with the size of the substituent and thus appears to be steric and not electronic in nature. As can be seen in Figure 2, the solid state conformations of (±)-*threo*-4-chloro- and -2-methoxymethylphenidate hydrochlorides are quite similar, suggesting that a substitution at the 2-position does not induce a large conformational change around the C1 to C2′ bond. For example, the torsion angles C2−C1−C2′−HC2′ for the above compounds are −1° and 2°, respectively. In addition, the proton coupling constants between HC2′ and HC2″ support the notion that there are not large differences between the solution conformations of these and other 2- and 4-substituted compounds. The average *J* (HC2′ to HC2″) is 9.3 Hz for 4-substituted and 8.8 Hz for 2-substituted compounds. These *J* values are quite consistent with the solid state conformations, which show that the torsion angles HC2′−C2′−C2″−HC2″ for the above compounds are 164° and 162°, respectively. Our initial interpretation of this data is that the "2-position" effect is from a direct steric interaction with the receptor and not caused by a significant conformational change in the ligand itself. Few 2-substituted analogs of stimulant drugs have been synthesized. Davies *et al.*[36] made one 2-methyl-substituted compound which was an analog of the WIN series of compounds. Compared to the 4-methyl, the 2-methyl compound was 160 times less active, a ratio similar to the compounds synthesized in this study.

b. 3- and 4-Position Isomers. The 3- and 4-substituted compounds with electron-withdrawing substituents tended to have increased binding potency, whereas those with electron-releasing groups were little affected or were less potent than the parent compound. However, substantial differences exist between the effects at the 3- and 4-positions. For F, Cl, Br, and CH_3 groups, all of the compounds were more potent than methylphenidate; 3-substitution produced compounds that were the same or more potent than 4-substitution. Thus, the most potent compounds tested in this study are 3-chloro- and 3-bromomethylphenidate (IC_{50} 5.1 and 4.2 nM, respectively). For NH_2, OCH_3, and OH groups, substitution in the 4-position did not change potency greatly, whereas in the 3-position the corresponding compounds were all less active than methylphenidate (average decrease by a factor of 3.4). For the larger nitro and *tert*-butyl groups, substitution in the 4-position gave less active compounds. This effect was particularly large with the *tert*-butyl compound (**1i**) which was about 160 times less active than the unsubstituted compound. Only two 3,4-disubstituted derivatives were synthesized; the dichloro compound had the same activity as the 3-chloro, while the dimethoxy was 3-fold less active than the 3-methoxy compound.

3. Hill Coefficients. The n_H values (see Table 2) for most of the compounds were close to unity, ranging from 0.83 for **1g** to 1.17 for **1l**. The only exception was **1m** (X = 3,4-di-Cl), which had an n_H of 2.07. The increased n_H does not appear to be a function of disubstitution alone, as an elevated n_H was not observed for **1aa** (X = 3,4-di-MeO). Interestingly, preliminary results in our laboratories show a similar effect for another 3,4-dichloro-substituted compound from an unrelated class of stimulant compounds. Additional experiments are planned to address the possibility that these results are artifacts of the high lipid solubility of dichloro-substituted compounds.

4. Comparison to Literature Results. The results reported here for the 2-, 3-, and 4-substituted bromo compounds and the 4-substituted hydroxy and methoxy compounds are comparable, for the most part, to those reported by others.[28,30,31] The greatest discrepancy is with **1g** which in our hands is 3-fold less potent than the available literature value.[31]

Comparison to Cocaine and WIN Compounds. The effect of aromatic ring substitution has been little studied for cocaine analogs,[37] but much better studied for WIN analogs.[38,40] For cocaine a 4-amino substituent



lowered activity by a factor of about 9, whereas for the

WIN compound a 4-amino substituent increased activity by a factor of 2.3[42] (for comparison, with methylphenidate a 4-amino substituent increased activity by a factor of 2.5). A 4-iodo substituent in cocaine[39] lowered activity by a factor of about 3, whereas for the WIN compound a 4-iodo substituent increased activity by a factor of 18 (for comparison, with methylphenidate a 4-iodo substituent increased activity by a factor of 6). For the WIN series, other substituents[42] in the 4-position have an effect similar to that in the methylphenidate series. Less data is available for 3-position substituents for the WIN series, but the effects are roughly similar to those observed in methylphenidate. For example, the 3-F- and 3-Cl-substituted WIN compounds are about 1.5 and 4 times more active than the unsubstituted compound, whereas the 3-F- and 3-Cl-substituted methylphenidate compounds are about 2 and 15 times more active than methylphenidate. It would thus appear that the aromatic ring structure−activity requirements for methylphenidate are similar to those for WIN analogs and very different than those for cocaine. It is interesting to note that the N″ to C4 distance in **1l** (X = 4-Cl) is 6.5 Å (from X-ray structure) and is very similar to the corresponding nitrogen to carbon distance, 7.0 Å, in the WIN compound and much shorter than the corresponding distance in cocaine, 9.0 Å (both calculated using PCMODEL, Serena Software).

B. [^{3}H]Dopamine Uptake and the Ratio of Uptake to Binding. The inhibition of [^{3}H]dopamine uptake and [^{3}H]WIN 35,428 binding are highly correlated, as the data in Table 2 indicates ($R^2 = 0.986$ for a log−log plot). Despite the high correlation, the rank order of potency for the compounds is not identical in the two assays; for instance, **1m** is the most potent of the various derivatives in the [^{3}H]dopamine uptake assay, but not in the [^{3}H]WIN 35,428 binding assay. Examination of the relative differences in potency of the individual compounds in the two assays reveals some interesting patterns which may prove useful in furthering our understanding of the dopamine transport complex. As an aid for discussion, the term *discrimination ratio* (DR) is defined as the ratio of the IC_{50} for a given compound for [^{3}H]dopamine uptake to its corresponding IC_{50} for [^{3}H]WIN 35,428 binding. The DR is thus a convenient measure of a compound's ability to differentially affect uptake and binding under a defined set of experimental conditions. Compounds with a high DR would be relatively poor inhibitors of dopamine uptake at a given binding potency and therefore might have an increased potential to be cocaine antagonists are partial agonists. Cocaine has a DR value of about 2.3 and methylphenidate about 2.7, and therefore ratios higher than these would be of interest. As shown in Table 2, approximately half of the compounds had DR values greater than 3, with **1r** (X = 3-Me) exhibiting the highest DR (4.7). In general, the more potent compounds exhibited higher DR values, while the less potent *erythro* and 2-substituted derivatives had DR values approaching unity. All of the derivatives bearing halide substitutions at the 3- or 4-position had DR values greater than those of cocaine or methylphenidate; interestingly, although **1m** (X = 3,4-diCl) was one of the most potent compounds in both assays, its DR dropped to 1.3. Compound **1aa** (X = 3,4-di-MeO) had a DR of 2.2, similar to that of the 3-substituted (2.2) and lower than that of the 4-substituted (3.5) methoxy derivatives.

Where comparisons are available, the DR values reported here are similar to those obtained by others[30,31] even though the assay conditions were somewhat different. The largest discrepancy is noted for **1** (X = 4-MeO) (3.5 in the present report versus 12[31]). This large difference in the DR appears to be due primarily to the differences in binding potency, as the results for inhibition of [^{3}H]dopamine transport appear to be quite similar.

The biological significance of the DR remains to be determined. While it is remarkable that small structural modifications can greatly influence the ratio of uptake to binding, the predictive value of this measure as a guide to selecting compounds which will block cocaine's stimulant and addictive effects *in vivo* is, as yet, unknown. Indeed, many authors have questioned the usefulness of the DR as a tool for detecting compounds which can differentially affect stimulant binding and dopamine transport[16,18,40,41] as it requires comparison of binding data collected under equilibrium conditions and uptake data collected under nonequilibrium conditions.[45]

Directions for Future Research

As the first systematic study of the effect of ring substitution on the activity of methylphenidate derivatives, this effort has produced compounds which promise to greatly advance our understanding of the dopamine transport complex. Selected compounds from this series will be examined in a variety of behavioral tests to determine their potential as cocaine antagonists. These results should also help to clarify the predictive value of the DR for the identification of promising test compounds. Future synthetic work will focus on the synthesis of methylphenidate analogues modified in the piperidine ring and at the ester side chain.

Experimental Section

Chemistry. General. Reagents and solvents were mostly reagent grade and were used without further purification. Solvents or reagents that required drying or purification were prepared according to the procedures found in Vogel.[42] Column chromatography was carried out on Fisher Scientific Co. silica gel (Grade 62) or Fisher Scientific neutral alumina (60−325 mesh). Melting points were obtained using a Laboratory Devices Mel-Temp II instrument without corrections. Nuclear magnetic resonance spectra were recorded on a Varian Gemini 300 (300 MHz) NMR spectrometer. Mass spectra were measured on a VG 70-SE, 2 sector, forward geometry instrument. IR spectra were recorded on a Nicolet 520 FT spectrophotometer. Microanalytical data were obtained by Atlantic Microlabs, Atlanta, GA.

Synthesis of Methyl (±)-*threo*- and -*erythro*-(3-Chlorophenyl)(2-piperidyl)acetates (1k and 11d). Typical Reaction Conditions for the Synthesis of Methylphenidate Analogs: (3-Chlorophenyl)(2-pyridyl)acetonitrile (3, X = 3-Cl). To a stirred solution of 12.3 g (0.110 mol) of *t*-BuOK in 60 mL of dry THF under dry N_2 gas was slowly added 11.8 mL (15.2 g, 0.100 mol) of 3-chlorobenzyl cyanide in 25 mL of dry THF. The mixture was stirred at room temperature for 0.5 h, and 15.8 g (9.50 mL, 0.100 moles) of 2-bromopyridine in 20 mL of dry THF was added dropwise during 1 h. The mixture was stirred at room temperature for another 1 h and then heated under reflux overnight. The THF was evaporated and 100 mL of water added while cooling with an ice bath. The aqueous layer was extracted with 3 × 100 mL of EtOAc, and the organic layer was washed with water and then

extracted with 4 × 70 mL of 6 N HCl solution. The aqueous layer was then made basic with 15% NaOH solution to a pH of >11 and extracted with 3 × 200 mL of EtOAc; the organic layer was washed with water and dried to give a mixture that was crystallized from hexane/EtOAc (1:1) to yield 7.73 g (33.9%) of **3** as colorless crystals: mp 83.3−84.3 °C; ^{1}H NMR ($CDCl_3$) δ 8.63 (dd, J = 2.6, 1.5 Hz, 1H), 7.8 (td, J = 7.8, 1.8 Hz, 1H), 7.45−7.26 (m, 6H), 5.29 (s, 1H); MS-CI m/z 229 (M + 1,100). Alternatively, 2-bromopyridine can be distilled out of the mixture to give impure **3** (*ca*. 50%) containing 7% (by ^{1}H NMR analysis) of the ketone **4** (X = 3-Cl).

(3-Chlorophenyl)(2-pyridyl)acetamide (5, X = 3-Cl). With stirring, 1.00 g (4.40 mmol) of **3** (X = 3-Cl) was dissolved in 10 mL of 12 N HCl, heated to 40 °C, and then stirred at room temperature for 15 h. The solution was poured into 50 mL of ice−water and then adjusted to a pH of 10−11 with 15% NaOH solution. The mixture was extracted with 3 × 40 mL of CH_2Cl_2, washed with 50 mL of water, and dried to give 0.97 g (89%) of **5** as a colorless solid: mp 97.2−98.4 °C; ^{1}H NMR (D_2O) δ 8.61 (d, J = 5.0 Hz, 1H), 7.87 (s, br, 1H), 7.68 (td, J = 7.8, 1.7 Hz, 1H), 7.44 (s, 1H), 7.34−7.23 (m, 5H), 6.21 (s, br, 1H), 4.98 (s, 1H); MS-EI m/z 246 (M^+, 3.4), 203 (100), 167 (71).

***erythro*- and *threo*-(3-Chlorophenyl)(2-piperidyl)-acetamides (6 and 7, X = 3-Cl).** To a solution of 0.43 g (1.7 mmol) of **5** (X = 3-Cl) in 15 mL of HOAc was added 0.14 g of 5% Pt/C. This mixture was treated with H_2 gas at 30−40 psi for 10 h. The catalyst was removed by filtration and the filtrate evaporated to dryness. Excess concentrated HCl was then added and the mixture again evaporated to dryness to give 0.48 g (98%) of compounds **6** and **7** (83:17 by ^{1}H NMR analysis); washing with EtOH gave 0.29 g (60%) of pure **6** as a white solid: mp 238.7−239.0 °C; ^{1}H NMR (D_2O) δ 7.34−7.19 (m, 4H), 3.66−3.57 (m, 2H), 3.17−3.13 (m, 1H), 2.83−2.79 (m, 1H), 1.96−1.92 (m, 1H), 1.74−1.70 (m, 2H), 1.47−1.40 (m, 3H); MS-CI m/z 253.1 (91, M + 1 − HCl), 170.0 (100).

***erythro*- and *threo*-(3-Chlorophenyl)(2-piperidyl)acetic Acids (8, X = 3-Cl).** A mixture of 5.20 g (0.018 mol) of **6** and **7** (X = 3-Cl) and 100 mL of 6 N HCl solution was heated under reflux for 6 h. The solution was evaporated to dryness to give compounds **8** (71:29 *erythro:threo* by ^{1}H NMR analysis, containing some NH_4Cl): ^{1}H NMR (D_2O) δ 7.33−7.08 (m, 4H), 3.73 (d, J = 8.9 Hz, 1H), 3.62−3.56 (m, 1H), 3.31−3.13 (m, 1H), 2.91−2.75 (m, 1H), 1.99−1.22 (m, 6H); MS-CI m/z 254.1 (57, M + 1 − HCl), 171.0 (100).

***threo*- and *erythro*-(3-Chlorophenyl)(2-piperidyl)acetic Acids (9 and 10, X = 3-Cl).** Under a N_2 atmosphere, the above mixture of compounds **8** (X = 3-Cl, *ca*. 0.018 mol) were mixed with 80 mL of 50% KOH solution and heated under reflux for 4 days, in a Teflon cup. The top oily layer was separated, dissolved in CH_3OH, acidified with concentrated HCl, and evaporated to dryness to give compounds **9** and **10** (83:17 by ^{1}H NMR analysis): ^{1}H NMR (D_2O) δ 7.31−7.08 (m, 4H), 3.84 (d, J = 9.2 Hz), 3.74 (d, J = 9.0 Hz), 3.63−3.56 (m, 1H), 3.32−3.17 (m, 1H), 2.96−2.85 (m, 1H), 1.73−1.18 (m, 6H).

Methyl *threo*-(3-Chlorophenyl)(2-piperidyl)acetate (1k). To a solution of the above mixture of **9** and **10** (X = 3-Cl, *ca*. 0.018 mol) in 193 mL of absolute CH_3OH was slowly added 8 mL of $SOCl_2$, while cooling with an ice bath. The mixture was stirred at room temperature for 1 day and evaporated, water added, and the pH adjusted to *ca*. 11 with 15% NaOH solution. The mixture was extracted with 3 × 120 mL of EtOAc and the organic layer washed with H_2O and dried. Removal of solvent gave 3.53 g (74% from compounds **6** and **7**) of the free base of compounds **1** and **11** (91:9 by ^{1}H NMR analysis) which was dissolved in MeOH, and excess concentrated HCl was added, and the mixture was evaporated to dryness to give a white solid, which was washed with Et_2O and EtOAc to give 3.16 g (90%) of pure **1** (by ^{1}H NMR analysis). The analytical samples was recrystallized from MeOH: mp 197.0−197.9 °C; ^{1}H NMR (D_2O) δ 7.31−7.23 (m, 3H), 7.11−7.08 (m, 1H), 3.84 (d, J = 9.4 Hz, 1H), 3.71−3.64 (m, 1H), 3.58 (s, 3H), 3.33−3.27 (m, 1H), 2.93−2.89 (m, 1H), 1.69−1.22 (m, 6H); MS-CI m/z 268.2 (100, M^+ + 1 − HCl). Anal. Calcd for $C_{14}H_{19}Cl_2NO_2$: C, H, N, Cl.

Methyl *erythro*-(3-Chlorophenyl)(2-piperidyl)acetate (11d). A mixture of 0.25 g (0.87 mmol) of compound **6** (X = 3-Cl) and 10 mL of 6 N HCl solution was heated under reflux for 6 h. The solution was evaporated to dryness to give **9** and **10** (14:86 by ^{1}H NMR analysis), which were mixed with 11 mL of CH_3OH and 0.5 mL of $SOCl_2$. Using a procedure similar to that used for **1** and **11** above, this gave 0.20 g (86%) of the free base of **1** and **11** as a colorless oil which was dissolved in MeOH, and excess concentrated HCl was then added; evaporation to dryness gave a white solid, which was then recrystallized with MeOH/EtOAc (1:2) to give 0.125 g (overall yield 47%) of pure **11** (by ^{1}H NMR analysis) as colorless crystals: mp 199.8−200.2 °C; ^{1}H NMR (D_2O) δ 7.34−77.25 (m, 3H), 7.14 (m, 1H), 3.82 (d, J = 8.9 Hz, 1H), 3.68−3.62 (m, 1H), 3.56 (s, 1H), 3.16−3.11 (m, 1H), 2.83−2.75 (m, 1H), 1.92−1.36 (m, 6H); MS-CI, m/z 268.1 (100, M + 1 − HCl). Anal. Calcd for $C_{14}H_{19}Cl_2NO_2$: C, H, N, Cl.

Isolation of Representative Ketone 4 (X = 3-OMe). A 45% yield of impure **3** (X = 3-OMe) containing some **4** was obtained from 3-methoxyphenylacetonitrile and 2-bromopyridine according to the above general procedure (after removal of unreacted 2-bromopyridine by distillation). Impure **3** was mixed with concentrated HCl to give impure **5** (X = 3-OMe) containing **4**. This mixture was placed on an alumina column and eluted with EtOAc/hexane (2:1) which gave, in the early fractions, a 9% yield of **4** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.73 (d, J = 6.5 Hz, 1H), 8.03 (d, J = 7.7 Hz, 1H), 7.91 (td, J = 7.7, 1.5 Hz, 1H), 7.65−7.62 (m, 2H), 7.52−7.48 (m, 1H), 7.40 (t, J = 8.2 Hz, 1H), 7.16 (dd, J = 8.6, 2.6 Hz, 1H), 3.87 (s, 3H); MS-EI m/z 213 (M^+, 70), 135 (100).

Nitration of 1a. Compound 1v (X = 4-NO_2). To 30 mL of fuming nitric acid at −10 °C, was added 3.9 g (0.015 mol) of (±)-*threo*-ritalinic acid. After stirring for 15 min, ice was added and then ammonium hydroxide until pH = 7. The solid was collected, washed with water, and dried to give 3.1 g (71%) of crude product. A portion (0.50 g) was converted to the methyl ester in the standard manner to yield 0.46 g (87%) of crude hydrochloride salt. Careful crystallization from acetone gave 0.066 g of pure **1** (R = 4-NO_2). Anal. Calcd for $C_{14}H_{19}ClN_2O_4$: C, H, N, Cl.

2-Amino-3-(2-pyridyl)indole (2). Using the same method as for the synthesis of compound **3** above, 7.5 g (0.057 mol) of 2-aminophenylacetonitrile (prepared from Pd-catalyzed hydrogenation of 2-nitrophenylacetonitrile in EtOAc, mp 67−69 °C) yielded 1.9 g of **2** (16%, isolated by alumina chromatography) as a green solid: mp 154−155 °C; ^{1}H NMR ($CDCl_3$) δ 8.51 (H_{14}, dd, J = 5.0, 1.2 Hz, 1H), 7.79 (H_6 and H_9, d, J = 8.3 Hz, 2H), 7.69 (H_{12}, td, J = 7.3, 1.5 Hz, 1H), 7.64 (H-N_1, bs, 1H), 7.16 (H_{11}, d, J = 7.5 Hz), 7.16 (H_7 or H_8, t, J = 7.8 Hz), 7.04 (H_7 or H_8, t, J = 7.7 Hz, 1H), 6.93 (H_{13}, t, J = 7.5 Hz, 1H), *ca*. 2.5 (C_2-NH_2, vbs); MS-EI m/z 209 (M^+, 100); IR (KBr) 3420 (m), 3276 (m), 1598 (s), 1512 (s), 782 (s), 736 cm^{-1} (s).

Demethylation of Methoxy Compounds. Each pure (±)-*threo*-methoxy compound was mixed with excess 48% HBr and refluxed for 4 h under N_2. The solution was evaporated to dryness and converted to methyl ester hydrochloride salts and purified in the standard manner. The compound from **1** (X = 2-OCH_3) gave a mixture of *erythro* and *threo* isomers (*ca*. 1:1) which could not be separated.

Pharmacology: [^{3}H]WIN 35,428 Binding. The synthesized compounds were screened for activity in a striatal tissue preparation using a modification of the [^{3}H]WIN 35,428 binding assay described by Reith and Selmeci.[43] Male Sprague−Dawley rats (Harlan Sprague−Dawley, Indianapolis, IN) weighing 150−300 g were anesthetized using CO_2 gas and sacrificed by decapitation. (Preliminary experiments demonstrated no difference in the K_D or B_{max} of [^{3}H]WIN 35,428 binding in unanesthetized rats versus anesthetized rats; data not shown.) Their brains were quickly removed and placed in ice-cold 0.32 M sucrose. The striatal tissue was removed and homogenized in 20 volumes of 0.32 M sucrose, using 10 up/down strokes of a motorized Potter−Elvejhm homogenizer. The supernatant obtained after centrifugation for 10 min at 0 °C (S_1 fraction) was removed and centrifuged for 20 min at 20000*g* and 0 °C to obtain the P_2 fraction, which was then resuspended in 50 volumes (original wet weight) of ice-cold

25 mM sodium phosphate buffer (pH 7.7) using a Tekmar tissuemizer. Samples containing 750 μL of phosphate buffer, 150 μL of the P_2 suspension, 50 μL of the test compound, 25 μL of water or amfonelic acid (to define nonspecific binding; final concentration, 10 μM), and 25 μL of [^{3}H]WIN 35,428 (final concentration, 2 nM) were incubated for 2 h at 0 °C. The incubation was terminated by vacuum filtration through Whatman GF/B filters presoaked with 0.05% (w/v) polyethylenimine, mounted in Millipore filtration manifolds. A 5 mL aliquot of assay buffer was added to the sample immediately before filtering it, and a second 5 mL aliquot of assay buffer was used to wash the filter. The filters were transferred to scintillation vials, shaken vigorously in the presence of 8 mL of Beckman Ready-Safe scintillation fluid for 30 min, and counted in a liquid scintillation counter.

IC_{50} values (that concentration of test compound required to inhibit 50% of the control specific binding of [^{3}H]WIN 35,-428) were determined from dose−response curves usually containing a range of seven concentrations of the test drug, with triplicate determinations made at each concentration. The IC_{50} for WIN 35,428 was 22.2 ± 4.7 nM (x ± SEM) under these conditions.

[^{3}H]Dopamine Uptake. Accumulation of [^{3}H]dopamine was determined as previously described.[44] Briefly, 250 μL of an S_1 fraction prepared as described above from striatal tissue of unanesthetized rats was diluted 4-fold with a modified Krebs-phosphate buffer (120 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 11 mM glucose, 0.16 mM Na_2EDTA, 1.1 mM ascorbic acid, 0.01 mM pargyline, and 15.5 mM Na_2PO_4, equilibrated with 95% O_2−5% CO_2 and adjusted to pH 7.4 with HCl) and preincubated with 1100 μL of Krebs-phosphate buffer and 100 μL of vehicle or drug for 10 min at 37 °C prior to the addition of 50 μL of [^{3}H]dopamine hydrochloride (Dupont/NEN, Boston, MA, or Amersham Corp., Arlington Heights, IL) which had been previously diluted with sufficient cold dopamine hydrochloride to bring the specific activity to approximately 5 Ci/mmol. The final concentration of dopamine in the samples was approximately 30 nM. The samples were exposed to the [^{3}H]dopamine for exactly 2.0 min. Nonspecific dopamine transport was determined by following the same protocol at 0 °C. Accumulation of [^{3}H]dopamine was terminated by the rapid addition of 5 mL of the chilled Krebs-phosphate buffer to each sample, followed by filtration through a Whatman GF/C filter under vacuum, after which the filter was washed with an additional 5 mL aliquot of buffer. The filters were extracted, and the trapped radioactivity was quantified as described for the [^{3}H]WIN 35,428 binding assay.

The IC_{50} values were determined from dose−response curves usually containing a range of five concentrations of test drug, with [^{3}H]dopamine uptake measured in duplicate samples at each concentration.

Protein Determination. Protein content of the P_2 suspension was determined according to the method of Miller.[45]

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