Articles

Mazindol Analogues as Potential Inhibitors of the Cocaine Binding Site at the Dopamine Transporter

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A series of mazindol (**2**) and homomazindol (**3**) analogues with a variety of electron-donating and electron-withdrawing groups in the pendant aryl group and the benzo ring C, as well as H, methoxy, and alkyl groups replacing the hydroxyl group were synthesized, and their binding affinities at the dopamine transporter (DAT) on rat or guinea pig striatal membranes were determined. Several active analogues were also evaluated for their ability to block uptake of DA, 5-HT, and NE and inhibit binding of [^{125}I] RTI-55 at HEK-hDAT, HEK-hSERT, and HEK-hNET cells. Mazindane (**26**) was found to be a pro-drug, oxidizing (5-H → 5-OH) to mazindol on rat striatal membranes and HEK-hDAT cells. The 4′,7,8-trichloro analogue (**38**) of mazindol was the most potent and selective ligand for HEK-hDAT cells (DAT K_i = 1.1 nM; SERT/DAT = 1283 and NET/DAT = 38). Experimental results strongly favor the cyclic or ol tautomers of **2** and **3** to bind more tightly at the DAT than the corresponding keto tautomers.

Introduction

R-Cocaine (**1a**), a potent stimulant, is a major drug of abuse and has contributed directly or indirectly to many severe medical and social problems in the United States.[1] The majority of evidence in animal and human studies provide support that the reinforcing and other behavioral effects caused by R-cocaine are related to its binding to a site at the dopamine transporter (DAT),[2] with possible contribution due to interaction at the serotonin transporter (SERT).[3] The action of cocaine at the DAT inhibits the uptake of dopamine (DA) into presynaptic axon terminals and gives rise to an increase of extracellular DA that is thought to be the primary mediator involved in the reinforcing effect of cocaine.[2]

The search for substances that block the binding site of cocaine at the DAT, to prevent its reinforcing and behavioral properties, has resulted in the identification of several series of diverse compounds.[4] A number of potent DAT uptake inhibitors have one or two phenyl or substituted phenyl groups attached directly or distal to a heterocycle containing one or two N-atoms or an O-atom. Examples of this group of compounds are cocaine-like analogues,[5] β-aryltropanes,[6] piperdine analogues of cocaine,[7] benztropines,[8] GBR12909 analogues,[9] methylphenidate,[10] and mazindol (**2**). Recently, phenylbicyclo [2.2.2]-octane[11] and phenylindane[12] compounds with an N-atom attached to the carbon ring and non-nitrogen analogues[13] (bicyclo-[3.2.1]-octanes) of WIN 35,428 (**1b**) have also been reported to be potent DAT uptake inhibitors.

Our laboratory has focused on mazindol (**2**), a substance developed as an appetite suppressant,[14] as a starting point to develop a selective inhibitor of cocaine binding because of its ability to inhibit binding of [^{3}H] cocaine (**1a**), [^{3}H] WIN 35,428 (**1b**), and [^{3}H] RTI-55 to the DAT in nanomolar range.[15] In clinical studies, mazindol abuse in humans[16] is rare, and it has been found to be of possible use in preventing relapse in methadone-maintained cocaine abusers.[17] We have reported on structure−activity relationship (SAR) of analogues containing one or more halogen atoms (F, Cl, Br, I) in ring C or the pendant aryl group (D), six (**3**; homomazindol), and seven-membered ring A derivatives,[18] and structural modification of the carbonyl group of the benzophenone keto tautomer of mazindol that exists in strong acidic media[19] (Figure 1).

This paper describes SAR studies where (a) the pendant aryl group (ring D) in mazindol (**2**) and homomazindol (**3**) is substituted in the 3′- and 4′-positions by groups other than halogen atoms and also replaced by 2-, 3-, or 4-pyridyl, (b) ring C of mazindol contains substituents in positions 6−9, and (c) the OH of mazindol or homomazindol is replaced by an H, alkyl, or alkoxy group. In addition, evidence is presented that the carbinolamine or cyclic ol tautomer of mazindol (**M** in Figure 1) is the active tautomer that interacts at the

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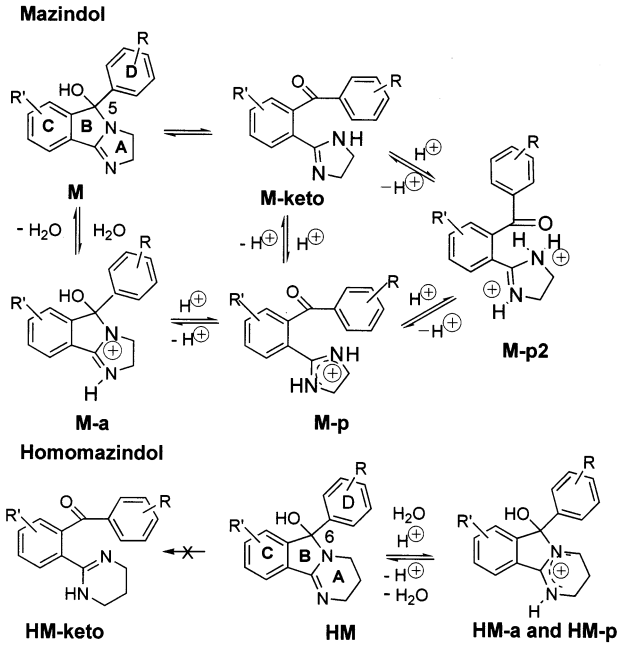


Figure 1. Tautomeric forms of substituted mazindol (**M**) and homomazindol (**HM**) analogues in neutral and strong acid media.

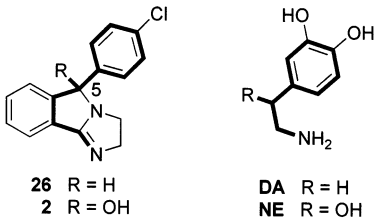
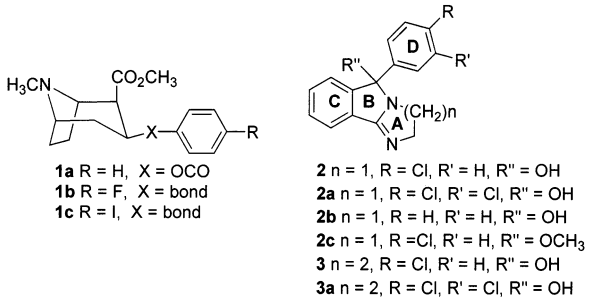


Figure 2. Structural similarities (bold lines) between mazindol (**2**), mazindane (**26**), dopamine (**DA**), and norepinephrine (**NE**).

cocaine binding site on the DAT (Figure 2), and an interaction model is proposed (Figure 3).



Chemistry

The synthesis of the novel racemic mazindol and homomazindol analogues in Tables 1 and 2 is given in Schemes 1−4. No attempt was made to resolve mazindol or any of its analogues because of their potential to form the keto tautomer (Figure 1), thereby destroying the chiral center.

Preparation of the *N*,*o*-dilithio derivative of 2-phenyl-4,5-dihydro-1H-imidazoline by a previously reported[20] procedure and treatment with the appropriate methyl benzoate or methylpyridylcarboxylate gave **4**, **7**, **8**, **11**, **16**, **18**, **20**, and **21** and **23** and **24**, respectively (Scheme 1). Compounds **4**, **7**, **8**, **23**, and **24** were established to exist in the ol tautomer form in d_6-DMSO by the presence of a ^{13}C NMR signal between 87.6 and 88.9 ppm which is characteristic of the C-5 atom and lack of a signal in the 194−196 ppm region, which is the C-atom of the carbonyl group in the keto tautomer. The compounds containing one (**11**), two (**16**, **18**, or **20**), or three (**21**) groups with oxygen atoms connected directly to the benzene ring gave ^{13}C NMR signals characteristic of the C-5 and carbonyl group, indicating the presence of both tautomeric forms. The amount of each tautomer was estimated to be 50:50 for the 3′,4′-$(OCH_3)_2$ (**16**) and 3′,4′,5′-$(OCH_3)_3$ (**21**) derivatives and 60:40 ol to keto for the 3′,4′-methylene- (**18**) and ethylenedioxy (**20**) derivatives by comparing the ratio of ^{1}H NMR signals of the four H-atoms in their respective imidazoline rings and 55:45 ol to keto for the 4′-OCH_3 (**11**) by comparing the ratio of CH_3 signals.

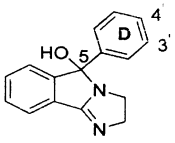
The pyrimidino [2,1-a]-isoindols **15** and **17** were obtained by treating the *N*,*o*-dilithio derivative of 2-phenyl-1,4,5,6-tetrahydropyrimidine with a methyl benzoate, and compounds **12**, **13**, **14**, and **19** were prepared by reacting the Grignard reagent of 4-R-bromobenzene with 3,4-dihydropyrimido-[2,1-a]-isoindole-6(2H)-one in refluxing THF. The 4′-OH derivative **10** was obtained by removal of the benzyl group in **15** by catalytic hydrogenation (Scheme 2). The ^{13}C NMR spectra of these compounds gave a C-6 atom signal at 91.34−91.99 ppm for those isolated as a free base (**10**, **12**, **14**, **15**, and **17**) and 95.57 and 93.1 ppm for those existing as HCl salts (**13** and **19**) and failed to display any signals in the carbonyl region. These findings indicate that the pyrimido-[2,1-a]-isoindol-6-ols exist in the ol form in both the free base and HCl salt forms (Figure 1, **HM-p**).

Preparation of 5H-mazindol (**26**; mazindane) was accomplished by condensing ketoaldehyde **25** with 1,2-diaminoethane in refluxing xylenes by the procedure[21] used to prepare the 5-phenyl analogue. Compound **26** was isolated as the sulfate salt because, like the 5-phenyl analogue, the free base was found to oxidize at C-5 to mazindol on prolonged contact with air.

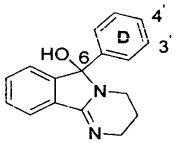
Alkylation of the sodium salt of the ethoxyisoindoline **27** with bromomethyl methyl ether gave **28b**. Heating the known **28a**[22] and **28b** with 3-aminopropanol gave 3-hydroxypropylamines **29a** and **29b**. When these were treated with the $I_2/(C_6H_5)_3$ P complex in the presence of imidazole, the resultant 3-iodopropylamino intermediates cyclized to 6-alkyl-homomazindols **30** and **31** (Scheme 3).

The synthesis of the new ring C mazindol analogues **39**−**45** is given in Scheme 4. Novel 2-arylimidazolines were obtained by treatment of hydroxyamides **32a**−**32c**, prepared from the corresponding acid chlorides and 2-amino-2-methyl-1-propanol, with thionyl chloride in toluene to form oxazolines **33a**−**33c**. Following the procedure of Chadwick,[23] they were treated with iodomethane and the resultant *N*-methyl quaternary salts **34a**−**34c** reacted with 1,2-diaminoethane in refluxing acetonitrile to give **35e**−**35g**. Preparation of the *N*,*o*-lithium salts of the 2-arylimidazolines from *n*-butyllithium in THF was carried out at room temperature except for **35e**, which was treated at 15 °C in order to prevent dilithiation of its electron rich phenyl ring. Treatment of the dilithium salts with methyl 4-chlo-

Table 1. Inhibition of [^{3}H] WIN 35,428 Binding at the Dopamine Transporter[a]



type A



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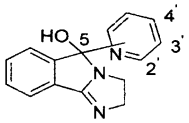


type C

compd[b]	type	substituent	ol: keto ratio[c]	IC_{50}, nM[d] (% inhib, 10^{-6} M)	mazindol/analogue ratio[e]
1a	R-cocaine			89.1 ± 4.8	
2	A	4′-Cl	100:0	8.1 ± 1.2	1.0
				42.6 ± 2.3*	1.0
3	B	4′-Cl	100:0	1.0 ± 0.2	8.1
				4.2 ± 0.6*	10.1
4	A	4′-CH_3	100:0	93.3 ± 8.7	0.1
5	A	3′-CF_3	100:0	230 ± 17	0.04
6	A	4′-CF_3	100:0	290 ± 31	0.03
7	A	4′-$CH_2N(CH_3)_2$	100:0	2770 ± 200	0.003
8	A	4′-CO_2CH_3	100:0	3310 ± 45	0.002
9	A	4′−C_6H_5	100:0	(14.1)[f]	<0.002
10	B	4′-OH	100:0	3.4 ± 0.4	2.4
11	A	4′-OCH_3	55:45	110 ± 7*	0.4
12	B	4′-OCH_3	100:0	2.5 ± 0.1	3.2
13[f]	B	3′-$OCH_2CH{=}CH_2$	100:0	219 ± 12.7	0.2
14	B	4′-$OCH_2CH{=}CH_2$	100:0	71.5 ± 8.2	0.1
15	B	4′-$OCH_2C_6H_5$	100:0	99.8 ± 5.8	0.1
16	A	3′,4′-$(OCH_3)_2$	50:50	6890 ± 698	0.001
17	B	3′,4′-$(OCH_3)_2$	100:0	87.4 ± 13.2	0.1
18	A	3′,4′-OCH_2O	60:40	113 ± 10*	0.4
19[f]	B	3′,4′-OCH_2O	100:0	1.94 ± 0.3	4.2
				1.09 ± 0.1*	39.1
20	A	3′,4′-OCH_2CH_2O	60:40	1260 ± 89*	0.03
21	A	3′,4′,5′-$(OCH_3)_3$	50:50	(5.7)*	<0.002
22	C	2′-isomer	100:0	3700 ± 413	0.002
23	C	3′-isomer	100:0	638 ± 52	0.013
24	C	4′-isomer	100:0	62.5 ± 5.6	0.1

[a] See Experimental Section for details. [b] The synthesis of compounds **5**, **6**, **9**, and **22** are given in ref 14. [c] Determined by ^{1}H NMR in d_6-DMSO. [d] Values are the mean (±) standard error of four assays in triplicate with rat striatal membranes. Those marked with an * are the mean (± SEM) of three assays with guinea pig striatal membranes. [e] Rat and guinea pig IC_{50} values are compared to the respective rat or guinea pig value for mazindol (**2**). [f] HCl salt.

robenzoate gave **39**−**45**. The ^{1}H and ^{13}C NMR spectra of **39**, **41**, **42**, **44**, and **45** gave signals indicating the presence of the ol tautomer, whereas the 6-methoxy analogue **40** and **43** exist as a 75:25 and 85:15 mixture of ol:keto tautomers.

The tendency of ring C analogues **40**−**43**, which have an oxygen connected directly to the benzene ring, to exist in the keto tautomer is less than that for their ring D analogues **11**, **16**, and **18**. A possible explanation for this trend is that much of the electron density supplied by these groups is being directed to the electron withdrawing imine portion of ring A, thereby favoring the ol tautomer. With the ring D oxygen analogues, the electron density can be more directed to the 5-position, making that carbon atom more electron-rich and less likely to interact with an electron-rich nitrogen atom to form ring B of the ol tautomer.

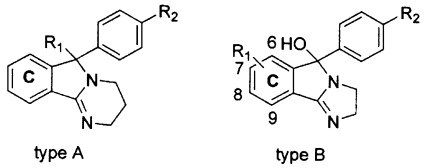
Previously reported[18] ultraviolet spectra in neutral (95% EtOH) and acidic (pH 0.9: 95% EtOH−2NHCl; 9:1) media indicated that halogenated mazindol analogues exist as the carbinolamine in neutral media and as the benzophenone tautomer in strong acidic media. One exception is the 2′-bromo analogue that exists as the carbinolamine form in both neutral and acidic media. The halogenated homomazindols also exist as the carbinolamine in both neutral and strong acidic media. The present findings that mazindol analogues can also exist in the benzophenone form in neutral media (DMSO) and several homomazindol hydrochloride salts exist as carbinolamines leads to a more complex equilibrium (Figure 1). X-ray crystal analysis of the 5-(3-iodophenyl) analog[24a] and the HBr salt of the 5-phenyl analog[24b] (**2**b) confirm the existence of the ol (**M**) and keto (**M-p**) forms in the solid state. Other possible forms of mazindol and homomazindol include the previously proposed[25a] diprotonated keto form (**M-p2**) and the protonated amidine forms **M-a** and **HM-a**, which are present in H_2O due to their pK_a values (8.55 ± 0.05 for mazindol[25a]) and can also be present under the proper conditions, given in Figure 1. The zwitterion[25b] (-0^- and $C{=}NH^+$) forms of mazindol and homomazindol, which are suggested from solid-state IR studies, have not been included in Figure 1.

Pharmacology. The compounds listed in Tables 1 and 2 were tested for their ability to displace [^{3}H] WIN 35,428 (**1b**) from the DAT of rat or guinea pig striatal membranes.[26] Those found to have similar or greater activity than mazindol were evaluated for selectivity to inhibit binding of [^{125}I] RTI-55 (**1c**) and monamine uptake in HEK cells expressing human recombinant DA, NE, and 5-HT transporters (Tables 4 and 5).

Results and Discussion

Mazindol Series: Ring D. Interpretation of the [^{3}H] WIN 35,428 binding data (Table 1) for the mazindol series is complicated because several of the compounds (**11**, **16**, **19**, **20**, and **21**) most likely exist in the ol and keto tautomers under the conditions (pH 7.40) that the assay was carried out. For the purpose of SAR analysis, these compounds are discussed separately.

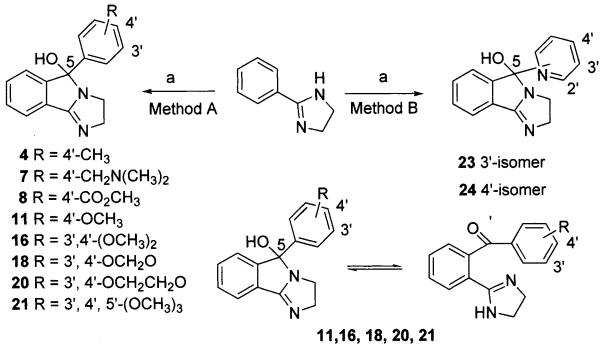
Table 2. Inhibition of [^{3}H] WIN 35,428 Binding at the Dopamine Transporter by Ring C and C-5 or C-6 Substituted Compounds[a]



compd[b]	type	R_1	R_2	ol:keto ratio[c]	IC_{50}, nM[d] (% inhib, 10^{-6} M)	ratio[e] mazindol/ analogue
1a	R-cocaine				89.1 ± 4.8	
2	B	H	Cl	100:0	8.1 ± 1.2	1.0
					42.6 ± 2.6*	1.0
2b	B	H	H	100:0	66.0 ± 8.9	0.1
2c[f]	B	H	Cl		1009 ± 84.7	0.008
3	A	OH	Cl	100:0	1.0± 0.2	8.1
					4.2 ± 0.6*	10.1
26[g]	B	H	Cl		29.7 ± 3.1*	1.4
30	A	CH_3	Cl		125 ± 11*	0.3
31	A	CH_2OCH_3	Cl		(5%)*	<0.002
36	B	6-Cl	H	100:0	57.1 ± 8.3	0.1
37	B	7-Cl	H	100:0	86.4 ± 14	0.1
38	B	7, 8-Cl_2	Cl	100:0	13.6 ± 1.5	0.6
39	B	9-F	Cl	100:0	12.3 ± 2.5*	3.5
40	B	6-OCH_3	Cl	75:25	67.1 ± 3.4*	0.6
41	B	7-OCH_3	Cl	100:0	13.3 ± 0.8*	3.2
42	B	9-OCH_3	Cl	100:0	4.75 ± 0.8*	9.0
43	B	6,7-OCH_2O	Cl	85:15	359 ± 29*	0.1
44	B	6,8-$(CH_3)_2$	Cl	100:0	67.1 ± 3.7*	0.6
45	B	9-C_6H_5	Cl	100:0	390 ± 17*	0.1

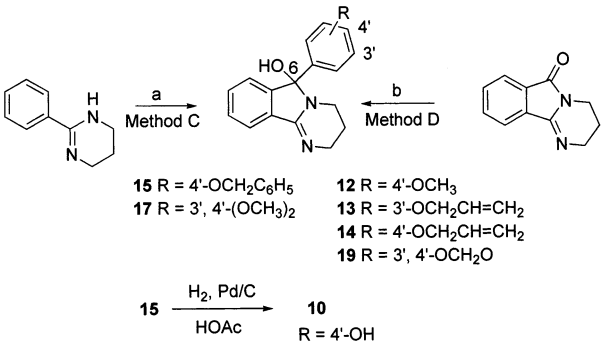
[a] See Experimental Section for details. [b] The synthesis of compounds **36**, **37**, and **38** are given in ref 14 and **2c** in ref 24. [c] Determined by ^{1}H NMR in d_6-DMSO. [d] Values are the mean (±) standard error of four experiments in triplicate with rat striatal membranes. Those marked with an * are the mean (± SEM) of three assays at five concentrations with guinea pig striatal membranes. [e] Rat and guinea pig IC_{50} values are compared to the respective rat or guinea pig value for mazindol (**2**). [f] OH in B replaced by OCH_3. [g] H_2SO_4 salt; OH in B replaced by H.

Scheme 1[a]



[a] Reagents/conditions. (a) (i) n-BuLi, THF, 50 °C, 3 h; (ii) methyl-R-benzoate or methylpyridylcarboxylate, 50 °C, 3−6 h.

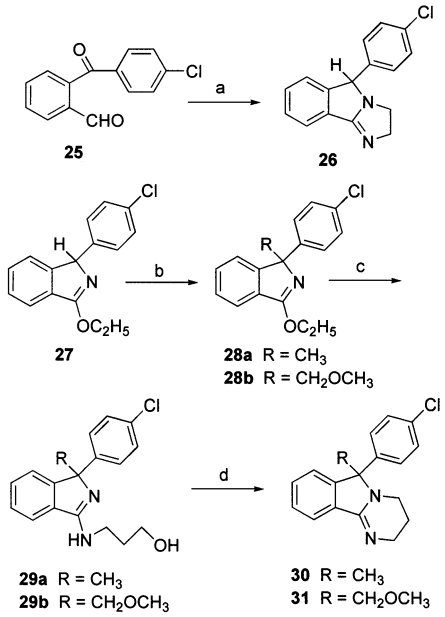
Scheme 2[a]



[a] Reagents/conditions. (a) (i) n-BuLi, THF, 35 °C, 4 h; (ii) methyl R-benzoate, THF, 50 °C. (b) (i) R-bromobenzene, Mg, THF, reflux, 2 h; (ii) THF, reflux, 5 h.

Replacement of the 4′-Cl in mazindol by a CH_3 (**4**), 3′-CF_3 (**5**), or 4′-CF_3 (**6**) gave an 11-, 28-, and 36-fold loss in activity. The dimethylaminomethyl (**7**), carbomethoxy (**8**), and phenyl (**9**) analogues all showed a considerable loss (340-, 410-, and >450-fold). Exchange of the 4′-chlorophenyl group for a 2- (**22**), 3- (**23**), or 4-pyridyl (**24**) group resulted in a 460-, 80-, and 8-fold loss of activity, respectively.

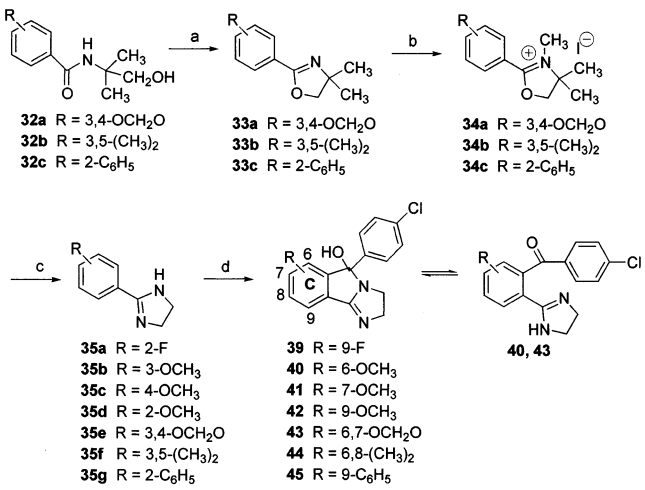
Scheme 3[a]



[a] Reagents/conditions. (a) 1,2-diaminoethane, toluene, N_2, reflux, 8 h. (b) (i) NaH, DMF, N_2, rt, 2 h; (ii) CH_3OCH_2Br, DMF, rt, overnight. (c) 3-Aminopropanol, 180 °C, 3 h. (d) I_2, imidazole, $(C_6H_5)_3P$, DMF, 0 °C, rt, 1 h.

Comparison of the [^{3}H] WIN 35,428 binding inhibition of the 4′-CH_3 and 4′-OCH_3 analogues of threomethylphenidate,[10b] WIN 35,428,[27] and 3 α-(diphenylmethoxy) tropane[28] with the mazindol analogues (**4**, **11**) shows a

Scheme 4[a]



[a] Reagents/conditions. (a) $SOCl_2$, toluene, rt, 48 h. (b) MeI, rt, 72 h. (c) 1,2-diaminoethane, MeCN, reflux, 10 h. (d) (i) n-BuLi, THF, N_2, rt or 15 °C, 2−3 h; (ii) methyl 4-chlorobenzoate, THF, rt or 15 °C, 3−4 h.

Table 3. Comparison of the Inhibition of [^{3}H] WIN 35,428 Binding Activity of Mazindol and Homomazindol Analogues Containing the Same Substituent(s)

			ratio of IC_{50} activity[a]	
compd	ring A size	substituent(s)	relative to mazindol	6-ring/ 5-ring
2	5	4′-Cl	1.0	8.1
3	6	4′-Cl	8.1	
2a	5	3′,4′-Cl_2	3.2	1.5
3a	6	3′,4′-Cl_2	4.8	
11	5	4′-OCH_3	0.4	8
12	6	4′-OCH_3	3.2	
16	5	3′,4′-$(OCH_3)_2$	0.001	100
17	6	3′,4′-$(OCH_3)_2$	0.1	
18	5	3′,4′-OCH_2O	0.4	98
19	6	3′,4′-OCH_2O	39.1	

[a] See Table 1 and ref 18 for IC_{50} values.

similar trend in loss of activity (1.5−11-fold) compared to the 4′-Cl derivative.

The large drop in activity (57-fold) with the 2′-pyridyl isomer **22** relative to the 4′-pyridyl (**24**) mimics the "ortho effect" seen when a halogen (F, Cl, Br) is placed in the 2′ or ortho position of the pendant aryl group in mazindol.[18] This suggests that electronic factors, as well as substituent size, play a role in the "ortho effect" seen in the mazindol and other classes of DA uptake inhibitors that have free rotating aryl groups.[18] A second possible explanation for the large loss in activity with the 2-pyridyl analogue is the formation of an H-bond between the OH and the 2-pyridyl N-atom, making the OH less available for interaction at the WIN binding site (Figure 3).

Homomazindol Series: Ring D. In the homomazindol series, replacement of the 4′-Cl by an OH (**10**) group led to ca. 3.5-fold loss of activity. The methoxy analogue **12** showed a slight increase but was still 2.5-fold weaker. Increasing the size and electron density to an alloxy (**14**) or benzyloxy (**15**) or transferring the allyloxy group to the 3′-position (**13**) led to further loss of activity, with maximum loss (100-fold) occurring with the benzyloxy analogue. Addition of a second OCH_3 group at the 3′-position (**17**) of **12** resulted in an 87-fold loss of activity, while the methlyenedioxy **19** was ca. 2−4-fold more active than homomazindol (**3**).

The activity trend found with the 4′-OH (**10**) and 4′-OCH_3 (**12**) analogues are similar to that found in the methylphenidate series where a 5-fold and 40-fold loss of activity relative to the 4′-Cl analogue was reported.[10b]

Tautomeric Mazindol Series: Ring D. A comparison of the inhibition of binding of [^{3}H] WIN 35,428 by mazindol and homomazindol compounds containing the same substituent(s) in position 4′ or 3′,4′ of the pendant aryl group is given in Table 3. In all examples the homomazindol analogues were more active (1.5−103-fold) than the corresponding mazindols. Those mazindol compounds (4′-Cl, 3′,4′-Cl_2) that exist in the ol form in neutral media showed less difference (8.1- and 1.5-fold) than the 4′-OCH_3 (8-fold), 3′,4′-$(OCH_3)_2$ (100-fold), and 3′,4′-OCH_2O (98-fold) derivatives that exist as a mixture of ol and keto isomers. The greater activity of the homomazindol analogues compared to the mazindol series can be explained by assuming that the ol tautomer interacts more strongly than the keto (Figure 1) at the WIN 35,428 binding site. The very weak binding displayed by the 3′,4′-ethylenedioxy (**20**) and 3′,4′,5′-trimethoxy (**21**) analogues can also be explained by the presence of a large amount (40% and 50%) of keto tautomer.

Mazindol Series: Ring C. When the 4′-Cl in ring D of mazindol is removed and placed in either the 6 (**36**) or 7 (**37**) position of ring C, a 7- and 11-fold loss of activity occurred. If these are compared with the unsubstituted ring D analogue **2b**, the presence of a 6- or 7-Cl had no effect. Addition of two Cl atoms (**38**) at positions 7, 8 of mazindol resulted in a slight loss (0.3-fold) loss in activity. A flourine added to position 9 (**39**) of mazindol gave a 3.5-fold increase in activity (Table 2).

Placement of a methoxy group in position 6 (**40**), 7 (**41**), or 9 (**42**) of mazindol resulted in a small loss of activity (0.3-fold) for the 6-isomer and a 3- and 9-fold increase in activity for the 7- and 9-isomers, respectively. This is an interesting activity change when compared to the 6- and 7-chlorine isomers. For the Cl compounds, the 6-isomer was more active (1.5×) than the 7-isomer, whereas the 7-methoxy isomer was more active (5.0×) than the 6-isomer. This difference between the Cl and methoxy isomers is most likely caused by the 6-methoxy isomer existing partly (25%) in the keto form, while the 6-Cl is in the ol form. The large loss (8-fold) in activity that occurs with the 6,7-methylenedioxy derivative (**43**) of mazindol is most likely due to its partial existence (15%) in the keto form and possible H-bonding with the C-5 hydroxyl group.

The addition of methyl groups at positions 6 and 8 (**44**) resulted in a small loss (0.35-fold) in activity, while the presence of a phenyl at position 9 (**45**) gave a 9-fold loss of activity relative to mazindol.

Replacement of OH Group. The methoxy analogue of mazindol **2c** was less potent (125×) than mazindol and mazindane (**26**), the 5H-analogue, was 1.4× more potent in blocking the binding of WIN (Table 2). The 6-methyl (**30**) homomazindol was 0.3- and 30-fold less active than mazindol and homomazindol, respectively, while the 6-methoxymethyl derivative **31** showed extremely weak inhibition (<500×) of WIN binding compared to homomazindol.

Table 4. Inhibition of [^{125}I] RTI-55 Binding at the DA, 5-HT, and NE Transporter Sites on HEK-hDAT, HEK-hSERT, and HEK-hNET Cells[a]

	binding,[b] K_i, nM			selectivity ratio	
compd	hDAT	hSERT	hNET	SERT/DAT	NET/DAT
2	45 ± 1	50 ± 15	18 ± 2	1.1	0.4
2b	1070 ± 270	1760 ± 180	100 ± 29	1.6	0.1
3	1.7 ± 0.8	39 ± 11	18 ± 2	23	10.6
10	87.1 ± 5.6	77 ± 21	380 ± 140	0.9	4.4
12	36.2 ± 1.9	55.9 ± 6.8	84 ± 23	1.5	2.3
19	2.55 ± 0.7	25.6 ± 2.5	8.8 ± 3.6	10.0	3.5
26	23 ± 6	26 ± 5	170 ± 75	0.9	7.4
38	1.05 ± 0.26	1347 ± 600	40 ± 21	1283	38
39	23.1 ± 3.7	38.1 ± 5.0	0.90 ± 0.43	1.65	0.04
40	10.3 ± 1.2	1990 ± 420	23.1 ± 6.3	193	2.2
41	6.0 ± 1.5	1030 ± 110	23.0 ± 6.6	172	3.9
42	2.9 ± 1.3	870 ± 180	6.8 ± 2.7	300	2.3
cocaine[c]	490 ± 48	328 ± 31	1940 ± 280	0.7	4

[a] See experimental Section for details. [b] Values are the mean ± standard error of two or three independent experiments, each conducted with triplicate determinations. [c] Values are the average of 10 independent experiments, each conducted with triplicate determinations.

Table 5. Inhibition of DA, 5-HT and NE Uptake to HEK-hDAT, HEK-hSERT, and HEK-hNET Cells[a]

	uptake[b], IC_{50}, nM			discrimination ratio IC_{50}/K_i uptake/binding[c]		
compd	hDAT	hSERT	hNET	DAT	SERT	NET
2	43 ± 2.0	94 ± 32	4.9 ± 0.5	1.0	1.9	0.3
2b	730 ± 180	2140 ± 450	2.8 ± 0.92	0.7	1.2	0.03
3	3.7 ± 0.4	53 ± 7	4.9 ± 0.5	2.2	1.4	2.2
10	59.0 ± 3.6	60 ± 19	1.9 ± 0.15	0.7	0.8	0.005
12	30.4 ± 2.4	94 ± 34	4.1 ± 1.4	0.8	1.7	0.05
19	2.21 ± 0.3	83 ± 29	0.62 ± 0.25	0.8	3.2	0.07
26	6.0 ± 0.7	15 ± 5	6.9 ± 1.5	0.3	0.6	0.04
38	6.0 ± 0.6	444 ± 159	10 ± 4	5.7	0.3	0.3
39	91 ± 38	129 ± 51	5.0 ± 1.9	4.0	3.4	5.6
40	60 ± 15	3600 ± 1300	1.73 ± 0.32	5.8	1.8	0.07
41	16.7 ± 4.3	1960 ± 720	1.46 ± 0.48	2.8	1.9	0.06
42	42.0 ± 5.9	280 ± 52	4.7 ± 1.6	14.5	0.3	0.7
cocaine[d]	296 ± 37	338 ± 67	233 ± 47	0.6	1	0.1

[a] See Experimental Section for details. [b] Values are the mean ± standard error of two or three independent experiments, each conducted with triplicate determinations. [c] See Table 4 for binding values. [d] Values are the average of 10 independent experiments, each conducted with triplicate determinations.

To determine if mazindane was oxidized by air under the condition of the binding assay (pH 7.4), the sulfate salt was dissolved in DMSO, DMSO/H_2O (1:1), or TRIS−NaCl buffer (pH 7.4) and then exposed to air at ambient temperature for 48 h. Analysis of the samples by LC−UV-MS showed only the presence of mazindane. Mazindane sulfate was then tested at a high dose (10^{-6} M) under regular assay conditions ([^{3}H] WIN 35,428 excluded). The rat striatal membranes from the assay were immediately washed with DMF and the filtrate freeze-dried. Only mazindol was detected in the residue by the LC−UV-MS procedure, indicating that mazindane is rapidly oxidized at C-5 by an oxidase or monooxygenase present in the rat striatal membranes.

In summary, replacement of the 4′-Cl in ring D of mazindol (**2**) by a variety of functional groups (Table 1) failed to increase its ability to inhibit WIN binding at the DAT, while in the homomazindol (**3**) series, only the 3′,4′-methylenedioxy group (**19**) gave a 2−4-fold increase of activity. The reason the oxygen containing analogues **11**, **16**, **18**, **20**, and **21** caused such large decreases in activity is most likely due to the presence of significant amounts of the keto tautomer (Figure 1: **M-keto**).

In ring C, placement of small electronegative group (F, OCH_3) at position 9 resulted in compounds (**39** and **42**) that were more effective than mazindol in inhibiting WIN binding at the DAT.

Selectivity and Discrimination. Selectivity and discrimination on nine of the most active inhibitors of WIN binding was determined by measuring their ability to displace [^{125}I] RTI-55 binding and block the uptake of DA, NE, and 5-HT at the DAT, SERT, and NET of HEK cells expressing cDNA for the human transporter (Tables 4 and 5).

All of these compounds, except **10**, were more potent (1.2−41-fold) than mazindol in blocking RTI binding at the DAT, but only the 4′,7,8-trichloromazindol analogue **38** (K_i = 1.1 nM) was equal to homomazindol. No correlation was found between the order of binding activity on rat striatal membranes (Tables 1 and 2) and HEK-hDAT cells (Table 4). Weaker (1−46-fold) affinity for binding at hSERT was observed for all compounds except **19** and **26**, which were 1.5× and 1.9× more potent than homomazindol and mazindol respectively, while at the hNET, **19** and **39** were more effective, 2.0 and 20-fold, in binding than mazindol or homomazindol, respectively.

Several of the compounds displayed a greater selectivity for binding at the hDAT over the hSERT (**38**, **40**, **41**, **42**) or hNET (**38**, **42**). The most selective, at both the hSERT and hNET, was **38** with 1160× and 56× more selectivity over the hSERT and 95× and 3.6× more selectivity over the hNET than mazindol and homomazindol, respectively.

As a group, the compounds were more active as inhibitors of NE uptake, followed by inhibition of DA uptake, and weakest at inhibiting 5-HT uptake (Table 5). The most active uptake inhibitor of both DA and NE

was **19** with IC_{50} values of 2.21 and 0.62 nM, respectively. Compound **26** showed the best activity at inhibiting 5-HT uptake with IC_{50} of 15 nM.

Most of the compounds (**38**-**42**) gave better uptake/binding (K_i/IC_{50}) discrimination ratios at the hDAT than mazindol or homomazindol, with **42** showing the best at 14.5 K_i/IC_{50}. Discrimination ratios better than mazindol or homomazindol at the hSERT were found for **19**, **39**, and **40** while only **39** gave a better K_i/IC_{50} ratio at the hNET.

To determine if mazindane (**26**) had been oxidized to mazindol by the HEK-hDAT cells, it was assayed as in the rat striatal membrane study. Only mazindol was detected by the LC−UV−MS analysis, indicating that oxidation had occurred. The variation in radioligand and uptake inhibition seen with **2** and **26** (Tables 2, 4 and 5) could be due to the oxidizing potential of the different membranes and the duration and temperature of each assay. A more likely explanation for the greater activity of **26** in the WIN and RTI binding and DA uptake assays is that some mazindane, as well as mazindol, may be present during the assays and it is more active at the DAT. It is possible that any remaining mazindane was not extracted by the DMF wash because of its high affinity for the DAT. The greater efficacy of mazindane is possibly due to the presence of a H at C-5 which more closely matches the structure of DA, which has only H-atoms on the α-C atom. The greater activity of mazindol in inhibiting binding and NE uptake at the NET could be due to the presence of an OH at C-5 that more closely matches the structure of NE, which has an OH on the α-C atom (Figure 2).

Interaction Model. The earlier report[18] from our laboratory on halogenated mazindol analogues as inhibitors of [^{3}H] WIN 35,428 binding at the DAT did not allow a distinction as to whether the ol or keto form (**M** and **M-keto** in Figure 1) is the active structure at the binding site because all the compounds evaluated existed in the ol forms under the assay conditions of pH 7.4. The findings in this report that the mazindol analogues **11**, **16**, **18**, **20**, **21**, **40**, and **43** that exist as a mixture of ol and keto tautomers in neutral media show a considerable loss in activity relative to those that exist in the ol form, and all homomazindols exist in the ol form in neutral or acidic media, makes it likely that the keto form has weaker interaction at the DAT binding site.

If the ol tautomer **M-a** of mazindol, present in aqueous media, is further protonated by an acidic group, such as the carboxyl on an aspartic acid unit of the DAT, it could possibly cause a shift to the protonated keto forms **M-p** or **M-p2** (Figure 1). To determine if this might occur, a UV study (220−330 nm) was carried out on mazindol in aqueous buffered media (Table 6). The pH range 1.5−8.0 was selected because it covers the pH of the assay media and the various acidic binding sites found at the DAT. Only the ol form **M-a** (272 nm maximum) was found between pH 1.5 and 8.0.

Two possible interaction models for the mazindol ol tautomer, which differ only in how the OH interacts at the binding site, are given in Figure 3. In the H-bond A model, the H interacts with an electronegative atom X, where X could be O, N, or S, and in the H-bond B model, the O interacts with the H atom on X−H. No substantial evidence is currently available to decide if the H-bond A Model is more probable than the H-bond B model.

Table 6. UV-pH Study of Mazindol in Buffered Aqueous Media[a]

maximum absorbance at 272 nm			
pH[b]	ϵ	pH[b]	ϵ
1.5	4248	5.0	3918
2.0	332	6.0	4026
3.0	3710	7.0	4148
4.0	4194	8.0	3707

[a] See Experimental Section for details. [b] Buffers: formate, 1.5−3.0; acetate, 4.0 and 5.0; phosphate, 6.0−8.0. [c] Maxima (ϵ) for mazindol in 95% EtOH, 268 (4930), and 276 (4930); in 95% EtOH-2N HCl (9:1), 253 nm (12 210), ref 14.

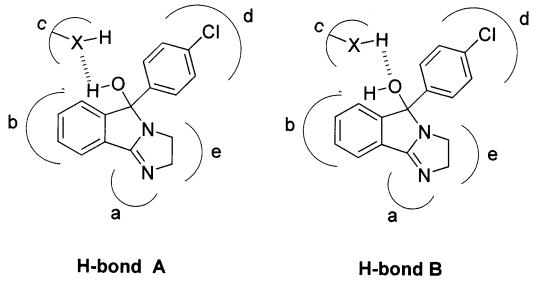


Figure 3. Putative interaction models of the carbinolamine form of mazindol at the dopamine transporter. Interaction site (a) ionic bond, (b and d) aromatic lipophilic, (c) H-bond or ionic, and (e) aliphatic lipophilic.

A previous study[29] of mazindol and structural variants demonstrated the importance of the OH group at C-5 in binding at human and *Drosophila melanogaster* serotonin transporters (SERTs), and Young[30] has completed a preliminary search for a common pharmacophore between cocaine (**1a**) and mazindol using the Chem DBS_3D modules of Chem-X. No correlation was found with the keto form (Figure 1) of mazindol, while both the *R*- and *S*-isomers showed two possible 3-point pharmacophore fits involving the imine N, OH, and 3′−H or 4′-Cl of mazindol and the N, CO_2CH_3, $C_6H_5CO_2$, and phenyl of cocaine. These results complement our findings that the ol form of mazindol most likely interacts at the DAT site where cocaine binds.

Conclusions

Mazindane (**26**) has been found to be a pro-drug, oxidizing (5-H → 5-OH) to mazindol (**2**) on both rat striatal membranes and HEK-hDAT cells.

The 4′,7,8-trichloro analogue (**38**) of mazindol was the most potent and selective for binding at the DAT over the SERT or NET of any mazindol or homomazindol prepared to date.

Experimental results strongly favor the cyclic or ol tautomer **M** and **HM** rather than the keto form (**M-keto** and **HM-keto** in Figure 1), to bind more tightly at the DAT (Figure 3).

Additional in vivo studies to define their potential for treatment of cocaine addiction have been completed for mazindane[31] and are on going for **38**. SAR studies to define the effect of enhanced aliphatic and aromatic lipophilicity have been completed[32] and a three-dimensional quantitative structure−activity relationship (3D-QSAR) at the DAT was carried out[33] on the compounds in this and related papers.

Experimental Section

General. Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are not corrected. Analyses were performed by the Robertson Microlit Laboratories, Inc., Madison, NJ, and are within ±0.4% of theory unless otherwise noted. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at 300 or 500 and 75.5 MHz respectively on a Bruker DPX-300 or DRX-500 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. Mass spectra (MS) were determined on a Micromass Quattro II mass spectrometer using APCI or ES ionization modes (positive or negative) and 0.2% ammonium formate in 50% aqueous acetonitrile as solvent. Thin-layer chromatography (TLC) was carried out on all compounds using glass plates coated with silica gel HF-254 (E. Merck AG). If not otherwise specified, chemicals were obtained from the Aldrich Chemical Co.

Mazindol UV-pH Study. Methanol was added to 2.194 mg of mazindol to a total volume of 10.0 mL. For each spectrum, 200 μL of the stock solution was treated with the appropriate buffer to a total volume of 5000 μL. Buffers were prepared in distilled H_2O as follows: phosphate (pH 6.0−8.0) from 28 mM KH_2PO_4 adjusted with 1N NaOH, acetate (pH 4.0 and 5.0) from 50 mM CH_3CO_2Na adjusted with 0.1N HCl and formate (pH 1.5−3.0) from 50 mM HCO_2NH_4 and adjusted with 1N HCl. Spectra were measured at ambient temperature between 220 and 300 nm on a Shimadzu UV-2101 PC spectrophotometer. Prior to measurement of each sample solution, a spectrum of the appropriate buffer was determined and its absorption was subtracted from the sample absorption. Maximum absorbance (272 nm) values are given in Table 6.

LC−UV-MS Analysis. Mazindane sulfate (**26**) and mazindol (**2**) in the solid form, the residues of freeze-dried solutions of **26** dissolved in DSMO, DMSO/H_2O (1:1), or TRIS NaCl buffer (pH 7.4) that were exposed to air at ambient temperatures for 48 h, and the residues from the freeze-dried DMF or CH_2Cl_2 extracts of rat striatal membranes and HEK-hDAT cells binding assays were each dissolved in ca. 1.0 mL of 40% CH_3CN. A 10 μL sample was injected into a Hewlett-Packard 1050 LC equipped with an Inertsil (ODS-2) column (2.1 mm × 10 cm × 5 μm) maintained at ambient temperature and set at 254 nm UV wavelength, UV/MS split ratio of ca. 5:1 and operated at a column flow rate of 0.4 mL/min. Fractions eluted by 0.1% ammonium formate in H_2O with 1% MeOH and then CH_3CN were analyzed on a micromass Platform II instrument equipped with a positive electrospray ionization detector and set at a scanning range of *m*/*z* 100−1,200 at 1.8 s/scan. Mazindol (*m*/*z* 285; MH^+) eluted at 8.0 min and was found in the rat and HEK samples. Mazindane (*m*/*z* 269; MH^+) eluted at 9.25 min and was found in the DMSO, DMSO/H_2O, and TRIS NaCl samples.

Method A. Preparation of 5-Aryl-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ols. General Procedure. A stirred solution of 2-phenylimidazoline (0.012 mol) in dry THF (20 mL) under a N_2 atmosphere was treated dropwise with 1.6 M *n*-BuLi in hexanes (22.5 mL, 0.036 mol) over a 0.5 h period. The suspension was heated at 50 °C for 3 h and then treated dropwise with a solution of a substituted methyl benzoate (0.024 mol) in THF (15 mL) over ca. 15 min. The mixture was stirred at 50 °C for an additional 6 h, cooled to 10 °C in an icebath, and then treated dropwise with saturated NH_4Cl solution (15 mL). After the mixture was left to stand overnight at room temperature, the resulting solid was filtered, washed with H_2O (ca. 25 mL), and recrystallized from the appropriate solvent.

5-(4-Methylphenyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (4). 46%, mp 208−209 °C dec (DMF); ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 2.81 (q, 1H), 3.20 (q, 1H), 4.12 (m, 2H), 6.68 (s, 1H), 7.13 (d, 1H), 7.21 (m, 3H), 7.40 (m, 2H), 7.50 (d, 1H), 7.64 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 20.50, 41.25, 60.65, 87.58 (C-5), 119.75, 120.30, 127.65, 128.77, 128.98, 129.35, 130.20, 133.97, 146.96, 161.75 (C═N); MS *m*/*z* 265 (MH^+). Anal. ($C_{17}H_{16}N_2O$) C, H, N.

5-(4-Dimethylaminomethylphenyl)-2,3-dihydro-5H-imidazol-[2, 1-a]-isoindol-5-ol (7). 24%, mp 177−178 °C (CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.05 and 2.06 (s, 6H), 2.88 (q, 1H), 3.29 (q, 1H), 3.35 (s, 2H), 4.12 (m, 2H), 6.73 (s, 1H), 7.27 (d, 2H), 7.32 (d, 2H), 7.46 (m, 3H), 7.68 (dd, 1H); ^{13}C NMR (DMSO-d_6) δ 41.34, 44.93, 45.01, 59.84, 62.99, 63.03, 87.98 (C-5), 121.93, 123.79, 125.76, 127.02, 127.26, 128.02, 128.09, 128.37, 128.56, 128.62, 129.75, 129.94, 130.30, 131.23, 136.68, 138.53, 139.42, 139.66, 143.35, 154.52, 162.90 and 166.95 (C═N); MS *m*/*z* 308 (MH^+). Anal. ($C_{19}H_{21}N_3O$) C, H, N.

Methyl-4-(2,3-dihydro-5-hydroxy-5H-imidazo-[2, 1-a]-isoindol-5-yl) benzoate (8). 23%, mp 169 °C (CH_2Cl_2/pet ether); ^{1}H NMR (DMSO-d_6) δ 2.88 (m, 1H), 3.30 (m, 1H), 3.82 (s, 3H), 4.18 (m, 2H), 6.95 (bs, 1H), 7.23 (d, 2H), 7.61 (d, 1H), 7.72 (d, 2H), 7.91 (d, 1H), 8.05 (s, 1H), 8.22 (dd, 1H); ^{13}C NMR (DMSO-d_6) δ 41.13, 52.48, 59.92, 87.63, (C-5), 122.12, 123.77, 126.59, 127.01, 128.93, 129.73, 130.89, 131.46, 141.83, 154.05, 166.06 and 166.91 (C═N and C═O); MS *m*/*z* 309 (MH^+). Anal. ($C_{18}H_{16}N_2O_3$) C, H, N.

5-(4-Methoxyphenyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol and 4-Methoxyphenyl-[2-(4,5-dihydro-1H-imidazol-2yl)-phenyl]-methanone (11). 37%, mp 196−198 °C (lit,[18] mp 188−190 °C) (DMF); ^{1}H NMR (DMSO-d_6) δ 2.89 (q, 0.60H), 3.30 (q, 0.60H), 3.38 (bs, 1.80H), 4.15 (m, 1.00H), 6.69 (s, 0.55H), 6.83 (s, 0.45H), 6.92 (d, 0.90H), 6.96 (d, 0.90H), 7.28 (m, 0.75H), 7.31 (d, 0.55H), 7.36 (dd, 0.45H), 7.46 (m, 1.10H), 7.55 (d, 1.10H), 7.58 (m, 1.10H), 7.68 (d, 0.55H), 7.73 (d, 0.55H); ^{13}C NMR (DMSO-d_6) δ 41.30, 44.24, 55.03, 55.42, 59.81, 87.86 (C-5), 113.46, 113.56, 121.87, 123.72, 126.96, 127.20, 127.40, 127.71, 128.53, 129.37, 129.77, 129.97, 130.62, 130.65, 131.18, 132.80, 139.94, 154.73, 158.72, 162.39 or 162.92 and 166.94 (C═N), 194.60 (C═O); MS *m*/*z* 297 (MH^+).

5-(3,4-Dimethoxyphenyl-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol and 3,4-Dimethoxyphenyl-[2-(4,5-dihydro-1H-imidazol-2-yl)-phenyl]-methanone (16). 50%, mp 190 192 °C (CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.93 (q, 0.5H), 3.18 (t, 1H), 3.30 (m, 2.5H), 3.75 (s), 3.78 (s), 3.82 (s), 3.84 (s, 6H), 4.18 (m, 1H), 6.73 (s, 0.4H; OH), 6.78−7.08 (d, d, s, d, d, d, d; 2.7H), 7.26−7.31 (m, 1.8 H), 7.48 (m, 0.8H), 7.58 (m, 1H), 7.70 (m, 0.7H); ^{13}C NMR (DMSO-d_6) δ 40.34, 41.29, 44.29, 55.41, 55.47, 55.65, 59.83, 87.91 (C-5), 109.79, 110.37, 110.42, 111.52, 118.20, 121.88, 123.61, 123.74, 126.91, 127.38, 127.82, 128.53, 129.39, 129.72, 130.10, 130.60, 131.14, 133.35, 135.85, 148.32, 148.35, 148.45, 152.36, 154.62, 162.94 and 167.00 (C═N), 194.57 (C═O); MS *m*/*z* 311 (MH^+). Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

5-(Benz [1,3]-dioxolo-5-yl)-2,3-dihydro-5H-imidazo-[2,1-a]-isoindol-5-ol and 5-(Benz [1,3]-dioxolo-5-yl)-[2-(4,5-dihydro-1H-imidazol-2-yl) phenyl]-methanone (18). 36%, mp 203−203.5 °C dec (DMF/i-PrOH); ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 2.95 (q, 0.6H), 3.18 (t, 0.9H) 3.31 (q, 0.8H), 3.41 (t, 0.7H), 4.18 (m, 1.6H), 6.02 (d, 1.0H), 6.14 (s, 0.8H), 6.75 (s, 0.5H), 6.80−6.93 (m, 1.6H), 7.01 (d, 0.5H), 7.14 (s, 0.4H), 7.29 (d, 0.4H), 7.37 (d, 0.4H), 7.49 (m, 1.0H), 7.60 (m, 0.8H), 7.69 (d, 0.5H), 7.73 (d, 0.4H); ^{13}C NMR ($CDCl_3$/DMSO-d_6) δ 42.2, 45.2, 56.3, 60.7, 88.7, (C-5), 102.0, 102.8, 107.4, 108.2, 120.3, 122.8, 124.6, 126.0, 127.8, 128.6, 129.5, 130.4, 130.8, 132.1, 133.5, 135.9, 140.7, 147.6, 148.2, 148.4, 151.6, 155.4, 163.8 and 167.8 (C═N), 195.5 (C═O); MS *m*/*z* 295 (MH^+). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

5-(2,3-Dihydrobenz-[1,4]-dioxin-6-yl)-2,3-dihydro-5H-imidazo-[2,1-a]-isoindol-5-ol and (2,3-Dihydrobenz-[1,4]-dioxin-6-yl)-[2-(4,5-dihydro-1H-imidazol-2-yl)-phenyl]-methanone (20). 31%, mp 186−187 °C (DMF/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.73 (q, 0.6H), 3.03 (t, 0.8H), 3.14 (q, 0.7H), 3.25 (t, 0.7H), 3.95 (q, 1.2H), 4.08 (s, 2.5H), 4.10 (d, 0.9H), 4.15 (0.9H), 6.53 (s, 0.6H), 6.57 (d, 0.6H), 6.67 (d, 1.2H), 6.72 (d, 1.8H), 6.88 (d, 0.9H), 7.10 (d, 0.6H), 7.16 (d, 0.4H), 7.32 (m, 0.8H), 7.50 (d, 0.5H), 7.55 (d, 0.4H); ^{13}C NMR (DMSO-d_6) δ 41.31, 44.26, 55.40, 59.78, 63.87, 64.00, 64.44, 87.65 (C-5), 114.72, 116.65, 116.75, 117.24, 118.78, 121.85, 122.58, 123.70, 126.91, 127.40, 127.60, 128.56, 129.34, 129.71, 129.80, 131.18, 131.31, 133.93, 139.80, 142.77, 142.85, 142.93, 147.07, 154.51,

162.79 and 166.87 (C═N), 194.36 (C═O); MS *m*/*z* 309 (MH^+). Anal. ($C_{18}H_{16}N_2O_3$) C, H, N.

5-(3,4,5-Trimethoxyphenyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol and 3,4,5-Trimethoxyphenyl-[2-(4,5-dihydro-1H-imidazol-2-yl) phenyl]-methanone (21). 42%, mp 200−201 °C dec (DMF/MeOH); ^{1}H NMR (DMSO-d_6) δ 3.05 (m, 0.8H), 3.16 (t, 0.8H), 3.33 (m, 0.9H), 3.68 (s, 2.0H), 3.73 (s, 3.0H), 3.76 (s, 4.0H), 4.21 (m, 1.5H), 6.69 (s, 1.4H), 6.74 (s, 0.7H), 6.84 (s, 0.5H), 6.96 (s, 0.2H), 7.33 (d, 0.7H), 7.41 (d, 0.3H), 7.47 (m, 1.8H), 7.60 (quin, 0.4H), 7.68 (d, 0.6H), 7.71 (d, 0.3H); ^{13}C NMR (DMSO-d_6) δ 42.1, 45.3, 56.2, 56.7, 56.8, 60.8, 61.0, 88.9, (C-5), 100.0, 104.1, 106.8, 122.9, 124.7, 127.7, 128.2, 129.0, 129.6, 130.7, 130.8, 131.2, 132.1, 133.9, 137.7, 137.9, 140.2, 142.0, 153.3, 153.6, 155.1, 163.7 and 168.0 (C═N), 195.6 (C═O); MS *m*/*z* 341 (MH^+). Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

Method B: Preparation of 5-(3-Pyridyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (23) and 5-(4-Pyridyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (24). A stirred solution of 2-phenylimidazoline (1.75 g, 0.012 mol) in THF (20 mL) under a N_2 atmosphere was treated dropwise with 1.6 M *n*-BuLi in hexanes (22.5 mL, 0.036 mol) over a 0.5 h period. The stirred suspension was heated at 50 °C for ca. 3 h and then treated dropwise with a solution of methyl nicotinate (3.29 g, 0.024 mol) in THF (20 mL). The mixture was maintained at 50 °C for an additional 6 h, then cooled in an icebath to 15 °C, and treated dropwise with saturated NH_4Cl solution (15 mL). After standing overnight at room temperature, the resultant solid was filtered and washed with H_2O (20 mL) to give 0.88 g (29%) of **23**, mp 210 °C dec (EtOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 2.98 (q, 1H), 3.34 (q, 1H), 4.18 (m, 2H), 6.99 (s, 1H), 7.31 (d, 1H), 7.41 (q, 1H), 7.52 (m, 2H), 8.05 (d, 1H), 8.16 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 41.98, 60.86, 87.81, (C-5), 123.06, 124.70, 127.86, 129.91, 132.36, 134.76, 137.49, 148.32, 149.91, 154.57, 169.70 (C═N); MS *m*/*z* 252 (MH^+). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

In a similar manner, there was obtained **24** (40.6%), mp 226 °C dec (MeOH/C_6H_6); ^{1}H NMR (DMSO-d_6) δ 2.95 (q, 1H), 3.33 (q, 1H), 4.18 (m, 2H), 7.07 (s, 1H), 7.30 (d, 1H), 7.38 (d, 2H), 7.52 (t, 2H), 7.73 (d, 1H), 8.58 (d, 2H); ^{13}C NMR (DMSO-d_6) δ 42.04, 60.88, 88.01 (C-5), 121.89, 123.08, 124.69, 130.04, 132.38, 150.53, 150.81, 154.21, 167.68 (C═N); MS *m*/*z* 252 (MH^+). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

Method C. Preparation of 6-Aryl-2,3,4,6-terahydropyrimido-[2, 1-a]-isoindol-6-ols. General Procedure. A stirred solution of 2-phenyl-1,4,5,6-tetrahydropyrimidine (0.006 mol) in dry THF (15 mL) under a N_2 atmosphere was treated dropwise with 1.6 M *n*-BuLi in hexanes (0.018 mol) over a 0.5 h period. The suspension was heated at 35° for 4 h and then treated dropwise with a solution of substituted methyl benzoate (0.012 mol) in THF (10 mL) over ca. 20 min. The mixture was stirred at 50 °C for an additional 4 h, cooled to 10 °C in an icebath, and treated dropwise with saturated NH_4Cl solution (10 mL). After standing overnight at room temperature, the resulting solid was filtered, washed with H_2O (ca. 15 mL), and crystallized from the appropriate solvent.

6-(4-Benzyloxyphenyl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol (15). 29%, mp 204 °C dec (CH_2Cl_2/pentane); ^{1}H NMR (DMSO-d_6) δ 1.76 (m, 2H), 3.28 (m, 1H), 3.42 (m, 2H), 5.08 (s, 2H), 6.62 (s, 1H), 6.93 (d, 2H), 7.18 (m, 1H), 7.25 (d, 2H), 7.34 (m, 1H), 7.42 (m, 4H), 7.45 (d, 1H), 7.63 (dd, 1H); ^{13}C NMR (DMSO-d_6) δ 20.73, 36.29, 43.59, 69.18, 91.37 (C-6), 114.48, 120.67, 122.55, 127.66, 127.80, 128.26, 128.40, 130.25, 132.92, 133.28, 137.02, 147.98, 153.17, 157.86 (C═N); MS *m*/*z* 371 (MH^+). Anal. ($C_{24}H_{22}N_2O_2$) C, H, N.

6-(3,4-Dimethoxyphenyl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol (17). 30%, mp 224 °C dec (CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 1.73 (t, 2H), 2.91 (quin, 1H), 3.27 (quin, 1H), 3.40 (t, 2H), 3.69 (s, 6H), 6.58 (s, 1H), 6.72 (dd, 1H), 6.87 (d, 1H), 6.97 (s, 1H), 7.18 (dd, 1H), 7.33 (t, 2H), 7.57 (dd, 1H), 6.87 (d, 1H), 6.97 (s, 1H), 7.18 (dd, 1H), 7.33 (t, 2H), 7.57 (dd, 1H); ^{13}C NMR (DMSO-d_6) δ 21.38, 36.94, 44.28, 59.36, 56.40, 91.99 (C-6), 111.29, 113.03, 118.96, 121.12, 122.94, 128.58, 130.48, 133.66, 134.53, 148.48, 149.44, 153.75 (C═N); MS *m*/*z* 325 (MH^+). Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

Method D. Preparation of 6-Aryl-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ols. General Procedure. To a stirred mixture of Mg (99.95%, 0.053 mol) and THF (25 mL) under a N_2 atmosphere there was added dropwise a solution of substituted bromobenzene (0.05 mol) in THF (20 mL). The mixture was refluxed for ca. 2 h, cooled to room temperature, and then treated dropwise with a solution of 3,4-dihydropyrimido-[2,1-a]-isoindole-6(2H) one[34] (0.033 mol) in THF (25 mL) and then refluxed for ca. 5 h. After stirring overnight at room temperature, the mixture was treated dropwise with saturated NH_4Cl solution (25 mL) and CH_2Cl_2 (20 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resultant solid was crystallized from the appropriate solvent.

6-(4-Methoxyphenyl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol (12). 57%, mp 219−220 °C (DMF); ^{1}H NMR (DMSO-d_6) δ 1.98 (m, 1H), 2.11 (m, 1H), 3.12 (m, 1H), 3.52 (q, 1H), 3.61 (m, 2H), 3.75 (s, 3H), 6.91 (d, 1H), 6.96 (d, 1H), 7.08 (s, 1H; OH), 7.30 (t, 1H), 7.41 (d, 1H), 7.64 (t, 1H), 7.69 (t, 1H), 7.83 (s, 1H), 8.47 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 19.23, 37.30, 38.48, 56.06, 96.04 (C-6), 112.52, 114.99, 118.49, 124.22, 124.81, 126.57, 130.74, 130.98, 135.11, 139.46, 149.10, 157.79, 160.51 (C═N); MS *m*/*z* 295 (MH^+). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

6-(3-Allyloxyphenyl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol hydrochloride (13). 23%, mp 202−203 °C (DMF/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.08 (2H, t), 3.18 (1H, m), 4.68 (2H, m), 4.59 (2H, d), 5.31 (1H, dt), 5.40 (1H, dt), 6.00 (1H, m), 4.68 (2H, m), 4.59 (2H, d), 5.31 (1H, dt), 5.40 (1H, dt), 6.00 (1H, m), 6.99 (3H, m), 7.34 (1H, t), 7.43 (1H, d), 7.67 (2H, m), 7.81 (s, 1H), 8.25 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 18.35, 36.41, 38.13, 68.26, 95.19 (C-6), 112.32, 114.89, 117.57, 117.79, 123.40, 123.50, 125.57, 129.96, 130.08, 133.51, 134.31, 138.44, 148.15, 156.93, 158.54 (C═N); MS *m*/*z* 321 (MH^+). Anal. ($C_{20}H_{20}N_2O_2$·HCl) C, H, N.

6-(4-Allyloxyphenyl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol (14). 27%, mp 198−200 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 1.91 (2H, t), 2.90 (1H, m), 3.26 (1H, m), 3.43 (2H, t), 4.55 (2H, d), 5.25 (1H, dd), 5.40 (1H, dt), 6.05 (1H, m), 6.60 (1H, s, OH), 6.90 (2H, d), 7.18 (1H, t), 7.25 (2H, d), 7.40 (2H, m), 7.60 (1H, d); ^{13}C NMR (DMSO-d_6) δ 20.73, 36.27, 43.61, 68.11, 91.34 (C-6), 114.37, 117.36, 120.64, 122.53, 127.03, 128.23, 130.21, 132.94, 133.18, 133.69, 147.97, 153.13, 157.64 (C═N); MS *m*/*z* 321 (MH^+). Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

6-(1, 3-Benzodioxol-5-yl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol hydrochloride (19). 41%, mp 224−226 °C (DMF/MeOH); ^{1}H NMR ($CDCl_3$) δ 1.79 (2H, m), 2.94 (1H, m), 3.33 (1H, m), 3.46 (2H, m), 6.02 (2H, d), 6.72 (1H, s, OH), 6.84 (1H, d), 6.88 (1H, d), 7.20 (1H, t), 7.42 (2H, m), 7.65 (1H, t); ^{13}C NMR (DMSO-d_6) δ 20.7, 36.3, 43.6, 93.1 (C-6), 101.1, 106.3, 108.0, 199.2, 120.7, 122.5, 128.3, 130.3, 132.9, 147.4, 147.8, 153.1, 165.7 (C═N); MS *m*/*z* 309 (MH^+). Anal. ($C_{18}H_{16}N_2O_3$·HCl) C, H, N.

6-(4-Hydroxyphenyl)-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindol-6-ol (10). A mixture of **15** (5.00 g, 0.0135 mol), 5% Pd/C (0.50 g), and HOAc (50 mL) in a hydrogenation bottle under H_2 pressure (50 psi) was agitated on a Parr hydrogenation apparatus at room temperature until H_2 uptake ceased (ca. 4 h). The catalyst was filtered off through Celite and washed with HOAc (20 mL), and the combined filtrates were concentrated in vacuo. The residue was treated with 2N NaOH (50 mL), extracted with CH_2Cl_2 (50 mL), and then with HOAc until pH 8. After 3 h at room temperature the resultant solid was filtered to give 2.51 g (62%) of **10**, mp 219 °C dec (MeOH/DMSO); ^{1}H NMR (DMSO-d_6) δ 1.75 (m, 2H), 2.90 (m, 1H), 3.27 (m, 1H), 3.44 (m, 2H), 6.51 (s, 1H), 6.71 (d, 2H), 7.12 (d, 2H), 7.14 (m, 3H), 7.60 (dd, 1H), 9.51 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 20.72, 36.27, 43.56, 91.52 (C-6), 114.99, 120.64, 122.54, 127.01, 128.14, 130.19, 131.17, 132.85, 148.19, 153.27, 156.89, 159.65 (C═N); MS *m*/*z* 281 (MH^+). Anal. ($C_{17}H_{16}N_2O_2$·$2H_2O$) C, H, N.

5-(4-Chlorophenyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindole (26). A mixture of 2-(4-chlorobenzoyl)-benzaldehyde (19.57 g, 0.08 mol), 1, 2-diaminoethane (24.04 g, 0.40 mol) and

xylenes (400 mL) was stirred and refluxed in a flask under N_2 and equipped with a Dean−Stark tube. After the "H_2O layer" remained constant (ca. 4 h), the orange-yellow solution was allowed to come to room temperature. A solid (1.65 g) identified as **2** by MS [285 (MH^+)], was quickly filtered off, and the orange-red filtrate was concentrated in vacuo at 100 °C to give 21.75 g of a viscous yellow-orange material. The substance was treated with EtAc (80 mL) and 100% EtOH (80 mL) and warmed under N_2 until a clear yellow solution occurred. After cooling to room temperature, the solution was stirred, treated with a solution of concd. H_2SO_4 (4 mL) in EtOH (40 mL) and stirred for ca. 3 h. The resultant solid was filtered, washed with EtOH (ca. 15 mL) and dried in vacuo to give 13.04 g (44.4%) of **26**·H_2SO_4, mp 228−229 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (q, 1H), 3.96 (q, 1H), 4.42 (m, 2H), 6.08 (s, 1H), 7.43 (d, 2H), 7.45 (d, 1H), 7.51 (d, 2H), 7.68 (t, 1H), 7.75 (t, 1H), 8.05 (d, 1H), 11.01 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 44.03, 50.51, 64.38 (C-5), 121.47, 124.60, 129.22, 129.32, 129.43, 133.16, 133.71, 134.60, 152.58, 168.76 (C═N), MS *m*/*z* 269 (MH^+). Anal. ($C_{16}H_{13}ClN_2$·H_2SO_4), C, H, N, S.

1-(*p*-Chlorophenyl)-1-methoxymethyl-3-ethoxy-1H-isoindole (28b). To a stirred suspension of 60% NaH (0.88 g, 0.022 mol in mineral oil) in DMF (25 mL), under a N_2 atmosphere, was added dropwise at room temperature a solution of **27**[35] (5.43 g, 0.02 mol) in DMF (60 mL). After 2 h, the mixture was treated dropwise with a solution of bromethyl methyl ether (3.75 g, 2.54 mL, 0.03 mol) in DMF (10 mL) and allowed to stir overnight. The reaction was poured onto H_2O (175 mL) and extracted with Et_2O (2×, 100 mL), and the Et_2O layer was washed with H_2O (100 mL), brine (100 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo to give 5.35 g of an oil. The oil was chromotographed on silica gel (62 g) and eluted with hexane/EtOAc (85:15) to give 1.93 g (31%) of **28b** as an oil (Rf 0.65); ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H), 3.25 (s, 3H), 3.59 (d, 1H), 3.95 (d, 1H), 4.51 (m, 2H), 7.17 (d, 2H), 7.33 (t, 2H), 7.43 (d, 2H), 7.49 (d, 1H), 7.53 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 14.91, 60.16, 64.61, 78.71, 121.42, 124.12, 127.60, 128.33, 128.66, 129.36, 130.76, 133.35, 139.65, 154.73, 169.42 (C═N); MS *m*/*z* 316 (MH^+). Anal. ($C_{18}H_{18}ClNO_2$) C, H, N.

1-(*p*-Chlorophenyl)-1-methyl-3-(3-hydroxypropylamino)-1H-isoindole (29a). A stirred mixture of 1-(*p*-chlorophenyl)-1-methyl-3-ethoxy-1H-isoindole[35] (**28a**: 0.286 g, 0.01 mol) and 3-aminopropanol (5 mL, 4.91 g, 0.065 mol) was heated at 180° for 3 h and then poured onto ca. 40 mL ice/H_2O and stirred for ca. 0.25 h. The resultant solid was filtered off, dissolved in CH_2Cl_2/MeOH (100 mL, 9:1), washed with satd. NaCl (20 mL), dried with anhyd. $MgSO_4$, filtered, and evaporated in vacuo to give 0.182 g (58%) of **29a**, mp 152−152.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.67 (s, 3H), 1.78 (m, 2H), 3.51 (m, 4H), 5.13 (bs, 1H), 7.21 (bs, 1H), 7.28−7.78 (m, 8H); MS *m*/*z* 315 (MH^+).

1-(*p*-Chlorophenyl)-1-methoxymethyl-3-(hydroxypropylamino)-1H-isoindole (29b). Following the procedure used to prepare **29a**, and reacting **28b**, there was obtained **29b** (49%) as a light yellow oil (R_f 0.25); ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H), 1.71 (bs, 1H), 2.31 (bs, 1H), 3.31 (s, 3H), 3.72 (m, 4H), 3.91 (d, 1H), 4.05 (d, 1H), 7.30 (d, 2H), 7.39 (d, 1H), 7.45 (d, 2H), 7.58 (m, 2H), 7.71 (t, 1H); MS *m*/*z* 344.9 (MH^+).

6-(4-Chlorophenyl)-6-methyl-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindole hydroiodide (30). To a stirred solution of **29a** (0.085 g, 0.0003 mol), triphenylphosphine (0.136 g, 0.0009 mol), imidazole (0.062 g, 0.0012 mol) in DMF (6 mL) cooled to 0 °C and protected from moisture was added I_2 (0.204 g, 0.0008 mol). The mixture was allowed to warm to room temperature for 1 h and then treated with hexane (20 mL). The DMF layer was separated, treated with satd. $NaHCO_3$ (10 mL), CH_2Cl_2 (20 mL), and small chips of I_2 until a light orange color was obtained. The organic phase was then treated with satd. $Na_2S_2O_3$ until colorless, washed with satd. NaCl solution (10 mL), dried with anydr. Na_2SO_4, filtered, and concentrated in vacuo to give 195 mg of colorless oil (R_f 0.48, 0.56 and 0.98; MeOH/CH_2Cl_2: 5:95). The oil (190 mg) was dissolved in MeOH/CH_2Cl_2 (3:97), chromatographed on 4 Analtech silica plates (20 cm × 20 cm × 1 cm). The main band (Rf 0.48) was eluted with CH_2Cl_2/CH_3OH (90:10) to give 0.085 g (64%) of **30**·HI as a hygroscopic light yellow foam, mp 142°; ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 3H), 2.09 (m, 2H), 3.17 (m, 1H), 3.64 (m, 3H), 7.35 (d, 2H), 7.45 (d, 2H), 7.51 (d, 1H), 7.64 (t, 1H), 7.72 (t, 1H), 8.17 (d, 1H), 10.81 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 18.24, 21.31, 37.58, 37.85, 73.26 (C-6), 122.80, 123.08, 123.32, 125.27, 128.06, 129.08, 133.36, 134.23, 136.47, 150.82, 157.37 (C═N); MS *m*/*z* 297 (MH^+). Anal. ($C_{18}H_{17}ClN_2$·HI) C, H, N.

6-(4-Chlorophenyl)-6-methoxymethyl-2,3,4,6-tetrahydropyrimido-[2, 1-a]-isoindole (31). A stirred mixture of **29b** (1.07 g, 0.0031 mol), triphenylphosphine (2.44 g, 0.0093 mol), and imidazole (0.632 g, 0.0093 mol) in DMF (45 mL) and protected from moisture was treated portionwise with I_2 (1.58 g, 0.0062 mol) at room temperature. After ca. 0.5 h at room temperature, the mixture was heated at 50 °C for 1 h, stirred overnight at room temperature, poured onto H_2O (80 mL), and then extracted with Et_2O (3× 50 mL). The aqueous layer was separated, made basic (pH 10) by the addition of NaOH pellets, and extracted with Et_2O (2×, 50 mL). The Et_2O layer was washed with satd. brine (2 × 50 mL), dried with anydr. $MgSO_4$, filtered, and evaporated in vacuo to give 0.350 g (35%) of **31**, mp 152−154 °C (C_6H_6); ^{1}H NMR (DMSO-d_6) δ 1.95 (m, 2H), 3.18 (m, 1H), 3.39 (s, 3H), 3.45 (m, 1H), 3.60 (m, 2H), 4.25 (d, 2H), 7.43 (d, 3H), 7.55 (t, 2H), 7.59 (d, 2H), 7.75 (d, 2H); ^{13}C NMR (DMSO-d_6) δ 20.90, 38.53, 43.25, 58.69, 71.30, 73.78 (C-6), 121.00, 122.38, 127.86, 128.05, 128.72, 129.92, 132.14, 134.00, 138.74, 146.18, 154.58 (C═N); MS *m*/*z* 327 (MH^+). Anal. ($C_{19}H_{19}ClN_2O$) C, H, N.

4,5-Dihydro-4, 4-dimethyl-2-aryl-oxazoles (33a−33c). To a stirred solution of acid chloride (0.054 mol) in CH_2Cl_2 (50 mL) under N_2, and protected from moisture was added dropwise at room temperature a solution of 2-amino-2-methyl-1-propanol (0.216 mol) in CH_2Cl_2 (50 mL). After stirring at room temperature for ca. 24 h, the mixture was treated with H_2O (75 mL) and CH_2Cl_2 (75 mL). The CH_2Cl_2 layer was separated, dried ($MgSO_4$), filtered, and evaporated in vacuo to give the amide alcohols **32a** (74%, mp 90−91°, MS *m*/*z* 238 (MH^+)), **32b** (93%, oil, MS *m*/*z* 222 (MH^+)), and **32c** (68%, mp 63−65 °C, MS *m*/*z* 270 (MH^+)).

A stirred solution of amide alcohol (0.039 mol) in toluene (60 mL) was cooled in an icebath and treated dropwise with a solution of $SOCl_2$ (0.155 mol) in toluene (40 mL) and allowed to stir at room temperature for ca. 48 h. The mixture was poured onto ice (300 mL) and CH_2Cl_2 (300 mL) and stirred for ca. 1 h. The CH_2Cl_2 layer was separated, washed with satd. NaCl (100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give **33a**, **33b**, or **33c**.

5-(4,5-Dihydro-4,4-dimethyl-oxazol-2-yl)-1,3-benzodioxole (33a). 97%, oil, R_f 0.65 (EtOAc/hexane 30:70); ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 6H), 4.00 (s, 2H), 5.93 (s, 2H), 6.74 (d, 1H), 7.35 (s, 1H), 7.42 (d, 1H); MS *m*/*z* 220 (MH^+).

4,5-Dihydro-4,4-dimethyl-2-(3,5-dimethylphenyl)-oxazole (33b). 74%, oil, R_f 0.80 (EtOAc/hexane 35:65); ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 6H), 2.19 (s, 6H), 3.95 (s, 2H), 6.96 (s, 1H), 7.42 (s, 2H); MS *m*/*z* 204 (MH^+).

4,5-Dihydro-4,4-dimethyl-2-(2-phenylphenyl)-oxazole (33c). 78%, oil, R_f 0.65 (MeOH/CH_2Cl_2 5:95); ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 6H), 3.80 (s, 2H), 7.30−7.43 (m, 7H), 7.47 (q, 1H), 7.72 (d, 1H); MS *m*/*z* 252 (MH^+).

2-Aryl-4,5-dihydro-1H-imidazoles (35a−35 g). Compounds **35a**,[33] **35b**,[34] **35c**,[35] and **35d**[36] were prepared by the cited literature procedures.

Preparation of 35e, 37f, and 37 g. A solution of **33a**, **33b**, or **33c** (0.0083 mol) and CH_3I (0.083 mol), protected from moisture, was stirred at room temperature for ca. 72 h. The resultant solid was filtered off and washed with dry acetone (ca. 5 mL) to give **34a** (82%, mp 200−210 °C; MS *m*/*z* 234), **34b** (64%, 180−181 °C; MS *m*/*z* 218 (MH^+) and **34c** (77%, mp 196−198 °C; MS *m*/*z* 267 (MH^+).

A stirred solution of **34a**, **34b**, or **34c** (0.0066 mol) and 1,2-diaminoethane (0.0067 mol) in dry acetonitrile (25 mL) was refluxed for ca. 10 h. The solvent was removed in vacuo and

the residue dissolved in CH_2Cl_2 (100 mL), washed with 2N KOH (20 mL), and H_2O (50 mL). The CH_2Cl_2 layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give a solid.

5-(4,5-Dihydro-1H-imidazol-2-yl)-1,3-benzodioxle (35e). 61%, mp 178 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 3.58 (s, 4H), 6.08 (s, 2H), 6.78 (s, 1H), 6.96 (d, 1H), 7.35 (s, 1H), 7.38 (d, 1H); MS *m*/*z* 191 (MH^+). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

2-(3,5-Dimethylphenyl)-4,5-dihydro-1H-imidazole (35f). 59%, mp 196−198 °C dec (MeOH-DMF); ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 6H), 4.00 (s, 4H), 7.43 (s, 1H), 7.57 (s, 2H), 12.57 (bs, 2H); MS *m*/*z* 175 (MH^+). Anal. ($C_{11}H_{14}N_2$·HI) C, H, N.

2-[(2-Phenyl)-phenyl]-4,5-dihydro-imidazole (35 g). 69%, mp 101−101.5 °C (MeOH/DMF); ^{1}H NMR (DMSO-d_6) δ 3.42 (s, 4H), 7.37 (d, 1H), 7.39−7.46 (m, 7H), 7.48 (d, 1H), 7.52 (d, 1H); MS *m*/*z* 223 (MH^+). Anal. ($C_{15}H_{14}N_2$) C, H, N.

Method D. Preparation of Ring C Substituted-5-(4-Chlorophenyl)-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ols. General Procedure. A stirred solution of 2-(R-phenyl)-4,5-dihydro-1H-imidazole (0.005 mol) in dry THF (10 mL) under a N_2 atmosphere was treated dropwise at room temperature or 15 °C (**35e**) with 1.6 Mn-BuLi in hexanes (7.0 mL, 0.011 mol) over a 0.5 h period. The mixture was stirred an additional 2−3 h and then treated dropwise with a solution of methyl 4-chlorobenzoate (0.01 mol) in THF (15 mL) over ca. 15−20 min. The mixture was stirred an additional 3−4 h, cooled in an icebath, treated dropwise with saturated NH_4Cl solution (7.5 mL), and allowed to stand overnight at room temperature. The resulting solid was filtered, washed with H_2O (ca. 10 mL), and then recrystallized from the appropriate solvent.

5-(4-Chlorophenyl)-9-fluoro-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (39). 21%, mp 184−186 °C (CH_2Cl_2/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.89 (q, 1H), 3.28 (q, 1H), 4.18 (m, 2H), 6.98 (s, 1H), 7.08 (d, 1H), 7.29 (t, 1H), 7.43 (s, 4H), 7.53 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 40.93, 52.98, 87.16 (C-5), 109.60, 114.06, 117.02, 118.97, 128.12, 130.02, 132.73, 137.02, 139.20, 157.15, 167.12 (C═N); MS *m*/*z* 303 (MH^+). Anal. ($C_{16}H_{12}ClFN_2O$) C, H, N.

5-(4-Chlorophenyl)-6-methoxy-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol and 4-Chlorophenyl-[2-(4,5-dihydro-1H-imidazol-2-yl)-6-methoxy) phenyl]--methanone (40). 15%, mp 194−196 °C (MeOH/DMF); ^{1}H NMR (DMSO-d_6) δ 2.78 (q, 1H), 3.38 (q, 1H), 3.51 (s, 2.25H), 3.67 (s, 0.75H), 4.08 (m, 2H), 6.68 (s, 0.75H), 7.10 (d, 0.75H), 7.28 (m, 2.50H), 7.32 (d, 1,75H), 7.48 (q, 1.25H), 7.57 (t, 0.75H); ^{13}C NMR (DMSO-d_6) δ 41.07, 55.25, 55.92, 59.70, 87.12 (C-5), 113.50, 113.93, 114.48, 119.76, 127.72, 127.92, 128.19, 128.44, 128.96, 129.80, 129.88, 130.22, 131.04, 131.89, 136.84, 137.06, 139.49, 139.77, 154.83, 156.32, 162.28, and 166.56 (C═N), 192.50 (C═O); MS *m*/*z* 315 (MH^+). Anal. ($C_{17}H_{15}ClN_2O_2$).

5-(4-Chlorophenyl)-7-methoxy-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (41). 70%, mp 176−177 °C dec (MeOH/DMF); ^{1}H NMR (DMSO-d_6) δ 2.88 (q, 1H), 3.32 (q, 1H), 3.73 (s, 3H), 4.12 (m, 2H), 6.78 (s, 1H), 6.89 (d, 1H), 7.08 (s, 1H), 7.43 (m, 4H), 7.68 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 41.10, 55.62, 59.08, 87.71 (C-5), 108.66, 115.23, 119.29, 123.60, 128.03, 128.33, 132.45, 139.53, 156.71, 162.07, 167.21 (C═N); MS *m*/*z* 315 (MH^+). Anal. ($C_{17}H_{15}ClN_2O_2$) C, H, N.

5-(4-Chlorophenyl)-9-methoxy-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (42). 62%, mp 186−187 °C dec (MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 3.00 (q, 1H), 3.32 (q, 1H), 3.91 (s, 3H), 4.30 (m, 2H), 6.68 (s, 1H), 6.95 (d, 1H), 7.20 (d, 1H), 7.40 (d, 2H), 7.50 (d, 2H), 7.70 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 41.80, 47.79, 53.56, 87.87 (C-5), 108.79, 113.08, 116.18, 118.68, 127.85, 128.85, 132.05, 135.96, 138.95, 156.53, 165.57 (C═N); MS *m*/*z* 315 (MH^+). Anal. ($C_{17}H_{15}ClN_2O_2$) C, H, N.

10-(4-Chlorophenyl)-7,10-dihydro-8H-1,3-dioxolo-[4, 5-e]-imidazo-[2, 1-a]-isoindol-10-ol and 4-Chlorophenyl-[6-(4,5-dihydro-1H-imidazol-2-yl)-benz-[1, 3]--dioxolo-5-yl]-methanone (43). 15%, mp 194−195 °C dec (CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 2.93 (q, 1H), 3.31 (q, 1H), 4.12 (m, 2H), 6.04 (s, 1H), 6.11 (s, 1H), 7.00 (s, 0.75H), 7.08 (d, 0.85H), 7.18 (d, 0.15H), 7.28 (d, 0.85H), 7.35 (d, 0.15H), 7.46 (s, 3.35H), 7.58 (d, 0.42H); ^{13}C NMR (DMSO-d_6) δ 41.03, 44.20, 55.05, 59.82, 86.10 (C-5), 102.16, 108.53, 108.99, 116.00, 121.69, 121.83, 123.11, 128.13, 128.56, 129.83, 132.47, 133.34, 136.45, 139.01, 141.41, 145.16, 148.97, 150.44, 166.03 (C═N), 190.28 (C═O). Anal. ($C_{17}H_{13}ClN_2O_3$) C, H, N.

5-(4-Chlorophenyl)-6,8-dimethyl-5H-imidazo-[2, 1-a]-isoindol-5-ol (44). 18%, mp 196−198 °C dec (MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.98 (s, 3H), 2.35 (s, 3H), 2.56 (q, 1H), 3.23 (q, 1H), 4.05 (m, 2H), 6.82 (s, 1H), 7.08 (s, 1H), 7.18 (d, 2H), 7.34 (s, 1H), 7.39 (d, 2H); ^{13}C NMR (DMSO-d_6) δ 41.51, 54.88, 59.52, 87.67 (C-5), 119.72, 127.68, 127.99, 128.18, 132.08, 133.97, 138.75, 138.90, 148.61, 166.48 (C═N); MS *m*/*z* 313 (MH^+). Anal. ($C_{18}H_{17}ClN_2O$) C, H, N.

5-(4-Chlorophenyl)-9-phenyl-2,3-dihydro-5H-imidazo-[2, 1-a]-isoindol-5-ol (45). 30%, mp 155−156 °C (MeOH/DMF); ^{1}H NMR (DMSO-d_6) δ 2.86 (q, 1H), 3.28 (q, 1H), 4.08 (m, 2H), 6.90 (bs, 1H), 7.23 (d, 1H), 7.28−7.48 (m, 8H), 7.55 (t, 1H), 7.69 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 41.54, 60.84, 87.71 (C-5), 123.67, 124.54, 128.61, 128.99, 129.19, 130.45, 130.94, 132.37, 133.32, 138.49, 139.18, 141.06, 156.13, 167.55 (C═N); MS *m*/*z* 343 (MH^+-H_2O). Anal. ($C_{22}H_{17}ClN_2O$) C, H, N.

[^{3}H]-WIN 35,428 Binding Assays A. Rat. Brains from male Sprague−Dawley rats (Harlan Labs) weighing 200−225 g were removed, striatum dissected, and quickly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (W/V) of ice cold modified sucrose phosphate buffer (0.32M sucrose, 7.74 mM Na_2HPO_4, 2.26 mM NaH_2PO_4, pH adjusted to 7.4) using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 20 000*g* for 10 min at 4 °C. The resulting pellet was suspended in buffer, recentrifuged, and resuspended in buffer to a concentration of 10 mg/mL. Ligand binding experiments were conducted in assay tubes containing 0.5 mL sucrose phosphate buffer for 120 min on ice. Each tube contained 0.5 nM [^{3}H] WIN 35,428 (specific activity 84 Ci/mmol) and 1.0 mg striatal tissue (original wet weight). Nonspecific binding was determined using 30 μM cocaine HCl. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% PEI (polyethylenimine), using a Brandel R48 filtering manifold (Brandel Instruments, Gaithersburg, Maryland). The filters were washed twice with 5 mL cold buffer and transferred to scintillation vials. Beckman formula 964 (4.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, California). Data were analyzed by using EBDA software.

B. Guinea Pig. Compounds listed in Table 1 and marked with an asterisk (*) were tested at NOVASCREEN, a division of Oceanix Biosciences Corporation, Hanover, MD. In brief, guinea pig striatal membranes, [^{3}H] WIN 35,428 and the test compound at concentrations of 10^{-10} to 10^{-6} M in DMSO/H_2O) were incubated with 50 mM TRIS−NaCl (pH 7.4) containing 100 mM NaCl at 25 °C for 2 h. The reaction assay was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the fibers is determined and compared to control values in order to ascertain any interaction of test compound with the uptake site.

[^{125}I] RTI-55 Binding Assay. All test compounds were prepared as 10 mM stock solution in DMSO. Subsequent dilutions were made in assay buffer, achieving a final concentration of 0.1%. Pipetting was conducted using a Biomek 2000 robotic work station. HEK293 cells expressing hDAT, hSERT, or hNET inserts were grown to 80% confluence on 150 mm diameter tissue culture dishes and serve as the tissue source. The medium was poured off the plate, and the plate was washed with 10 mL of calcium- and magnesium-free phosphate-buffered saline and lysis buffer (10 mL; 2 mM HEPES with 1 mM EDTA) was added. After 10 min, cells were scraped from the plates, poured into centrifuge tubes, and centrifuged 30,000 x g for 20 min. The supernatant fluid was removed, and the pellet was resuspended in 12−32 mL of 0.32 M sucrose using a Polytron at setting 7 for 10 s. The resuspension volume depends on the density of binding sites within a cell line and is chosen to reflect binding of 10% or less of the total radioactivity. Each assay tube contained 50 μL of membrane prepara-

tion (about 10−15 μg of protein), 25 μL of test compound or buffer (Krebs-HEPES, pH 7.4; 122 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 μM pargyline, 100 μM tropolone, 0.2% glucose and 0.02% ascorbic acid, buffered with 25 mM HEPES), 25 μL of [^{125}I] RTI-55 (40−80 pM final concentration) and additional buffer sufficient to bring the final volume to 250 μL. The membranes are preincubated with unknowns for 10 min prior to the addition of [^{125}I] RTI-55. The assay tubes were incubated at 25 °C for 90 min, and the binding was terminated by filtration over GF/C filters using a Tomtec 96-well cell harvester. Filters are washed for 6 s with ice-cold saline. Scintillation fluid was added to each square and radioactivity remaining on the filter was determined using a Wallac μ- or β-plate reader. Specific binding was defined as the difference in binding observed in the presence and absence of 5 μM mazindol (HEK-hDAT and HEK-hNET) or 5 μM imipramine (HEK-hSERT). Two or three independent competition experiments were conducted with duplicate determinations. GraphPAD Prism was used to analyze the ensuing data, with IC_{50} values converted to K_i values using the Cheng-Prusoff equation. Additional details about this assay have been published.[40]

Inhibition of [^{3}H] Neurotransmitter Uptake in HEK293 cells expressing Recombinant Biogenic Amine Transporters. HEK293 cells expressing hDAT, hSERT, or hNET were grown to confluence as described above. The medium was removed, and the cells were washed twice with phosphate buffered saline (PBS) at room temperature. Following the addition of Krebs- HEPES buffer (3 mL), the plates were warmed in a 25 °C water bath for 5 min. The cells were gently scraped and then triturated with a pipet. Cells from multiple plates were combined. One plate provides enough cells for 48 wells, which is required to generate data on two complete curves for the test compounds. Krebs-HEPES (350 μL) and test compounds (50 μL) were added to 1-mL vials and placed in a 25 °C water bath. Specific uptake is defined as the difference in uptake observed in the presence and absence of 5 μM imipramine (HEK-hSERT). Cells (50 μL) are added and preincubated with the unknowns for 10 min. The assay is initiated by the addition of [^{3}H] dopamine, [^{3}H] serotonin, or [^{3}H] norepinephrine (50 μL, 20 nM final concentration). Filtration through Whatman GF/C filters presoaked in 0.05% polyethylenimine is used to terminate uptake after 10 min. The IC_{50}s are calculated applying the GraphPAD Prism program to triplicate curves made up of 6 drug concentrations each. Two or three independent determinations of each curve are made.

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References

(1) (a) Zafar, H.; Vaz, A.; Carlson, R. W. Acute Complications of Cocaine Intoxication. *Hospital Practice* **1997**, 167−181. (b) Altman, J.; Everitt, B. J.; Glautier, S.; Markou, A.; Nutt, D.; Oretti, R.; Phillips, G. D.; Robbins, T. W. The Biological, Social, and Clinical Bases of Drug Addiction: Commentary and Debate. *Psychopharmacology* **1996**, *125*, 285−345.

(2) (a) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The Dopamine Hypothesis of the Reinforcing Properties of Cocaine. *Trends Neurosci.* **1991**, *14*, 299−302. (b) Kuhar, M. J.; Ritz, M. C.; Sharkey, J. In *Cocaine Receptors on Dopamine Transporters Mediate Cocaine-Reinforced Behavior in Mechanisms of Cocaine Abuse and Toxicity*; Clovet, D., Agshar, K. and Brown, R., Eds.; U.S. Government Printing Office: Washington, DC, 1998; pp 14−22. (c) Giros, B.; Jaber, M.; Jones, S. R.; Wrightman, R. M.; Caron, M. G. Hyperlocomotion and Indifference to Cocaine and Amphetamine in Mice Lacking the Dopamine Transporter. *Nature* **1996**, *379*, 606−612.

(3) (a) Rocah, G. A.; Sceare-Levie, K.; Lucas, J. J.; Hiroi, N.; Castanon, N.; Crabbe, J. C.; Nestler, D. J.; Hen, R. Increased Vulnerability to Cocaine in Mice Lacking the Serotonin-1B Receptor. *Nature* **1998**, *393*, 175−178. (b) Buydens-Branchey, L.; Branchey, M.; Fergeson, P.; Hudson, J.; McKernin, C. Craving for Cocaine in Addicted Users, Role of Serotonergic Mechanisms. *Am. J. Addict.* **1997**, *6*, 65−73.

(4) (a) Singh, S. Chemistry, Design and Structure−Activity Relationship of Cocaine Antagonists. *Chem. Rev.* **2000**, *100*, 925−1024. (b) Carroll, F. I.; Howell, L. L.; Kuhar, M. J. Pharmacotherapies for Treatment of Cocaine Abuse: Preclinical Aspects. *J. Med. Chem.* **1999**, *42*, 2721−2736. (c) Smith, M. P.; Hoepping, A.; Johnson, K. M.; Trzcinska, M.; Kozikowski, A. P. Dopaminergic Agents for the Treatment of Cocaine Abuse. *Drug Discuss. Today* **1999**, *4*, 322−332. (d) Panikkar, G. P. Cocaine Addiction: Neurobiology and Related Current Research in Pharmacotherapy. *Substance Abuse* **1999**, *20*, 149−166.

(5) Avor, K. S.; Singh, S.; Seale, T. W.; Pouw, B.; Basmadjian, G. P. 2β-Substituted Analogues of 4′-Iodococaine: Synthesis and Dopamine Transporter Binding Potencies. *J. Med. Chem.* **1998**, *41*, 2380−2389.

(6) (a) Kozikowski, A. P.; Araldi, G. L.; Prakash, K. R. C.; Zhang, M.; Johnson, K. M. Synthesis and Biological Properties of New 2β-Alkyl- and 2β-Aryl-3-(substituted phenyl) Tropane Derivatives: Stereochemical Effect of C-3 on Affinity and Selectivity for Neuronal Dopamine and Serotonin Transporters. *J. Med. Chem.* **1998**, *41*, 4973−4982. (b) Zhang, C.; Izenwasser, S.; Katz, J. L.; Terry, P. D.; Trudell, M. L. Synthesis and Dopamine Transporter Affinity of the Four Stereoisomers of (±)-2-(Methoxycarbonyl)-7-methyl-3-phenyl-7-azabicyclo [2.2.1] heptane. *J. Med. Chem.* **1998**, *41*, 2430−2435. (c) Lieske, S. F.; Yang, B.; Eldefrawi, M. E.; MacKerell, Jr., A. D.; Wright, J. (−)-3β-Substituted Ecgonine Methylesters as Inhibitors for Cocaine Binding and Dopamine Uptake. *J. Med. Chem.* **1998**, *41*, 864−876. (d) Melzer, P. C.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Chen, Z.; George, C.; Madras, B. K. 2-Carbomethoxy-3-aryl-8-oxabicyclo [3.2.1] Octanes: Potent Non-Nitrogen Inhibitors of Monoamine Transporters. *J. Med. Chem.* **1997**, *40*, 2661−2673. (e) Zhao, L.; Johnson, K. M.; Zhang, M.; Flippen-Anderson, J.; Kozikowski, A. P. Chemical Synthesis and Pharmacology of 6- and 7-Hydoxylated-2-carbomethoxy-3-(p-tolyl) tropanes: Antagonism of Cocaines' Locomotor Stimulant Effects. *J. Med. Chem.* **2000**, *43*, 3283−3294. (f) Hoepping, A.; Johnson, K. M.; George, C.; Flippen-Anderson, J.; Kozikowski. Novel Conformationally Constrained Tropane Analogues by 6-endo-trig Radical Cyclization and Stille Coupling − Switch of Activity Toward the Serotonin and/or Norepinephrine Transporter. *J. Med. Chem.* **2000**, *43*, 2064−2071. (g) Robarge, M. J.; Agoston, G. E.; Izenwasser, S.; Kopajtic, T.; George, C.; Katz, J. L., Newman, A. H. Highly Selective Chiral N-Substituted 3α-[Bis (4′-fluorophenyl)methoxy] tropane Analogues for the Dopamine Transporter: Synthesis and Comparative Molecular Field Analysis. *J. Med. Chem.* **2000**, *43*, 1085−1093. (h) Xu, L.; Iszenwasser, S.; Katz, J. L.; Kopajtic, T.; Klein-Stevens, C.; Zhu, N.; Lomenzo, S. A.; Winfield, L.; Trudell, M. L. Synthesis and Biological Evaluation of 2-Substituted 3β-Tolyltropane Derivatives at Dopamine, Serotonin and Norepinephrine Transporters. *J. Med. Chem.* **2002**, *45*, 1203−1210.

(7) (a) Kozikowski, A. P.; Araldi, G. L.; Boja, J.; Meil, W. M.; Johnson, K. M.; Flippen-Anderson, J. L.; George, C.; Saiah, E. Chemistry and Pharmacology of the Piperdine-based Analogues of Cocaine. Identificaiton of Potent DAT Inhibitors Lacking the Tropane Skeleton. *J. Med. Chem.* **1998**, *41*, 1962−1969. (b) Tamiz, A. P.; Zhang, J.; Flippen-Anderson, J. L.; Zhang, M.; Johnson, K. M.; Deschaux, O.; Tellah, S.; Kozikowski, A. P. Further SAR Studies of Piperdine-Based Analogues of Cocaine. Potent Dopamine and Serotonin Reuptake Inhibitors. *J. Med. Chem.* **2000**, *43*, 1215−1222. (c) Wang, S.; Sakamuai, S.; Enyedy, I. J.; Kozikowski, A. P.; Deschaux, O.; Bandyopadhyay, B. C.; Tella, S. R.; Zaman, W. A.; Johnson, K. M. Discovery of a Novel Dopamine Transporter Inhibitor, 4-Hydroxy-1-methyl-4-(4-methylphenyl)-3-piperidyl-4-methyl ketone, as a Potential Cocaine Antagonist through 3D-Database Searching. Molecular Modeling, Structureactivity Relationships, and Behavioral Pharmacological Studies. *J. Med. Chem.* **2000**, *43*, 351−360. (d) Choi, S.-W.; Elmaleh, D. R.; Hanson, R. N.; Fischman, A. J. Novel 3-Aminomethyl- and 4-Aminopiperdine Analogues of 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl) piperazines: Synthesis and Evaluation as Dopamine Transporter Ligands. *J. Med. Chem.* **2000**, *43*, 205−213. (e) Tamiz, A. P.; Bandyopadhyay, B. C.; Zhang, J.; Flippen-Anderson, J. L.; Zhang, M.; Wang, C. Z.; Johnson, K. M.; Tella, S.; Kozikowski, A. P. Pharmacological and Behavioral Analysis of the Effects of Some Bivalent Ligand-Based Monamine Uptake Inhibitor. *J. Med. Chem.* **2001**, *44*, 1615−1622.

(8) Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J.; Kline, R. H.; Hauck Newman, A. Novel N-Substituted 3α-[bis (4′-fluorophenyl)methoxy] tropane Analogues: Selective Ligands for the Dopamine Transporter. *J. Med. Chem.* **1997**, *40*, 4329−4339.

(9) (a) Dutta, A. K.; Davis, M. C.; Fei, X.-S.; Beardsley, P. M.; Cook, C. D.; Reith, M. E. A. Expansion of Structure − Activity Studies of Piperidine Analogues of 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935) Compounds by Altering Substitutions in the Benzyl Moiety and Behavioral Pharmacology of Selected Molecules. *J. Med. Chem.* **2002**, *45*, 654−662. (b) Dutta, A. K.; Xu, C.; Reith, M. E. A. Tolerance in the Replacement of the Benzhydrylic O atom in 4-[2-(Diphenylmethoxy)ethyl]-1-benzylpiperdine Derivatives by an N atom: Development of New Generation Potent and Selective N-Analogue Molecules for the Dopamine Transporter. *J. Med. Chem.* **1998**, *41*, 3293−3297. (c) Zhang, Y.; Rothman, R. B.; Dersch, C. M.; de Costa, B. R.; Jacobsen, A. E.; Rice, K. C. Synthesis and Transporter Binding Properties of Bridged Piperazine Analogues of 1-{2-[Bis-(4-fluorophenyl)methoxyethyl}-4-(3-phenylpropyl)-piperazine (GBR12909). *J. Med. Chem.* **2000**, *43*, 4840−4849. (d) Lewis, D. B.; Matecka, D.; Zhang, Y.; Hsin, L.-W.; Dersch, C. M.; Stafford, D.; Glowa, J. R.; Rothman, R. B.; Rice, K. C. Oxygenated Analogues of 1-[2-[Bis-(4-fluorophenyl)methoxy]-ethyl]-4-(3-Phenylpropyl) piperazines (GBR12935 and GBR12909) as Potential Extended-Action Cocaine-Abuse Therapeutic Agents. *J. Med. Chem.* **1999**, *42*, 5029−5042. (e) Zhang, Y.; Joseph, D. B.; Bowen, W. D.; Flippen-Anderson, J. L.; Dersch, C. M.; Rothman, R. B.; Jacobson, A. E.; Rice, K. C.; Synthesis and Biological Evaluation of Tropane-like 1-{2-[Bis(4-fluorophenyl)-methoxy]ethyl}-4-(3-phenylpropyl)piperazine (GBR 12909) Analogues. *J. Med. Chem.* **2001** *44*, 3937−3945. (f) Hsin, L. W.; Dersch, C. M.; Baumann, M. H.; Stafford, D.; Glowa, J. R.; Rothman, R. B.; Jacobson, A. E.; Rice, K. C. Development of Long-Acting Dopamine Transporter Ligands as Potential Cocaine Abuse Therapeutic Agents: Chiral Hydroxyl-Containing Derivatives of 1-[2-[Bis (4-fluorophenyl) methoxy] ethyl]-4-(3-phenylpropyl)-piperazine and 1-[2-(Diphenylmethoxy) ethyl]-4-(3-phenylpropyl) piperazine. *J. Med. Chem.* **2002**, *45*, 1321−1329.

(10) (a) Thai, D. L.; Sapko, M. T.; Reiter, C. T.; Bierer, D. E.; Perel, J. M. Asymmetric Synthesis and Pharmacology of Methylphenidate and its para-substituted Derivatives. *J. Med. Chem.* **1998**, *41*, 591−601. (b) Deutsch, H.; Shi, Q.; Gruszecka-Kowalik, Z.; Schweri, M.; Synthesis and Pharmacology of Potential Cocaine Antagonists. Structure−Activity Relationship Studies of Aromatic Ring-Substituted Methylphenidate Analogues. *J. Med. Chem.* **1996**, *39*, 1201−1209.

(11) (a) Javanmard, S.; Deutsch, H. M.; Collard, D. M.; Kuhar, M. J.; Schweri, M. M. Synthesis and Pharmacology of Site-Specific Cocaine Abuse Treatment Agents: 2-Substituted-6-amino-5-phenylbicyclo [2.2.2] octanes. *J. Med. Chem.* **1999**, *42*, 4836−4843. (b) Deutsch, H. M.; Collard, D. M.; Zhang, L.; Burnham, K. S.; Deshpande, A. K.; Holtzman, S. G.; Schweri, M. M. Synthesis and Pharmacology of Site-Specific Cocaine Abuse Treatment Agents: 2-(Aminomethyl)-3-phenylbicyclo [2.2.2]-and-[2.2.1] alkane Dopamine Uptake Inhibitors. *J. Med. Chem.* **1999**, *42*, 882−895.

(12) (a) Froimowitz, M.; Wu, K.-M.; Moussa, A.; Haidar, R. M.; Jurayj, J.; George, C.; Gardner, E. L. Slow-Onset, Long Duration 3-(3′,4′-Dichlorophenyl)-1-indanamine Monoamine Reuptake Blockers as Potential Medications to Treat Cocaine Abuse. *J. Med. Chem.* **2000**, *43*, 4981−4992. (b) Gu, X.-H.; Yu, H.; Jacobson, A. E.; Rothman, R. B.; Dersch, C. M.; George, C.; Flippen-Anderson, J. L.; Rice, K. C. Design, Synthesis and Monoamine Transporter Binding Site Affinities of Methoxy Derivatives of Indatraline. *J. Med. Chem.* **2000**, *43*, 4868−4876.

(13) Meltzer, P. C.; Blundell, P.; Yong, Y. F.; Chen, Z.; George, C.; Gonzalez, M. D.; Madras, B. K. 2-Carbomethoxy-3-aryl-8-bicyclo [3.2.1] octanes: Potent Non-Nitrogen Inhibitors of Monamine Transporters. *J. Med. Chem.* **2000**, *43*, 2982−2991.

(14) Aeberli, P.; Eden, P.; Gogerty, J. H.; Houlihan, W. J.; Penberthy, C. 5-Aryl-2, 3-dihydro-5H-imidazo [2, 1-a] isoindol-5-ols. A Novel Class of Anoretic Agents. *J. Med. Chem.* **1975**, *18*, 177−182.

(15) (a) Javitch, J. A.; Blaustein, R. O.; Snyder, S. H. [^{3}H] Mazindol Binding Associates with Neural Dopamine and Norepinehrine Uptake Sites. *Mol. Pharmacol.* **1984**, *26*, 35−44. (b) Kozikowski, A. P.; Liang, L.; Tanaka, J.; Bergmann, J.; Johnson, K. M. Use of Nitrile Oxide Cyclic Addition (NOC) Chemistry in the Synthesis of Cocaine Analogues; Mazindol Binding and Dopamine Uptake Studies. *Med. Chem. Res.* **1991**, *1*, 312−321.

(16) Chait, L. D.; Uhlenhuth, E. H.; Johanson, C. E. Reinforcing and Subjective Effects of Several Anorectics in Normal Human Volunteers. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 777−783.

(17) Margolin, A.; Avants, S. K.; Kosten, T. R. Mazindol for Relapse Prevention to Cocaine Abuse in Methadone-Maintained Patients. *Am. J. Drug Alcohol Abuse* **1995**, *21*, 469−481.

(18) Houlihan, W. J.; Boja, J. W.; Parrino, V. A.; Kopajtic, T. A.; Kuhar, M. J. Halogenated Mazindol Analogues as Potential Inhibitors of the Cocaine Binding Site at the Dopamine Transporter. *J. Med. Chem.* **1996**, *39*, 4935−4941.

(19) Houlihan, W. J.; Boja, J. W.; Kopajtic, T. A.; Kuhar, M. J.; Degrado, S. J.; Toledo, L. Positional Isomers and Analogues of Mazindol as Potential Inhibitors of the Cocaine Binding Site on the Dopamine Transporter Site. *Med. Chem. Res.* **1998**, *8*, 77−90.

(20) Houlihan, W. J.; Parrino, V. A.: Directed Lithiation of 2-Phenyl- and 2-(o-Methylphenyl)imidazoline. *J. Org. Chem.* **1982**, *47*, 5177−5180.

(21) Metlesics, W.; Anton, T.; Chaykovsky, M.; Toome, V.; Sternbach, L. H. The Condensation of o-Benzoylbenzaldehyde with Aliphatic Diamines. *J. Org. Chem.* **1968**, *33*, 2874−2877. (b) Metlesics, W.; Sternbach, L. H. U.S. Patent 4,018,765, 1977.

(22) Eberle, M. K.; Brzechffa, L.; Houlihan, W. J. Alkylations of 1-(4-Chlorophenyl)-3-ethoxy-1H-isoindole. *J. Org. Chem.* **1977**, *42*, 894−897.

(23) Chadwick, D. J.; Ennis, D. S. Directed Metalation Studies on 2-(2-Heteroaryl) imidazolines: Synthesis of some 2,3-Disubstituted Thiopenes and Furans. *Tetrahedron* **1991**, *47*, 9901−9914.

(24) (a) Rodier, N.: Agafonov, V.; Cense, J. F.; Galinier, E.; Ombetta, J. E.; Frangin, Y.; Guilloteau, D. 5-(3-Iodophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo [2.1,a] isoindole. *Acta Crystallogr.* **1993**, *C49*, 841−843. (b) McKechnie, J. S.; Paul, I. C. The Molecular Structure of the Oxidised Condensation Product of o-Benzoylbenzaldehyde and Ethylenediamine. *J. Chem. Soc. (B)* **1968**, 984−988.

(25) (a) Maulding, H. V.; Turi, P.; Grossman, D.; Nazareno, J. P. Solvolysis of a Substituted Imidazoline, Mazindol. *J. Pharm. Sci.* **1975**, *64*, 829−831. (b) Barcza, S.; Houlihan, W. J. Structure Determination of the Anorexic Agent Mazindol. *J. Pharm. Sci.* **1975**, *64*, 829−831.

(26) Compounds were initially evaluated on rat striatal membranes in the laboratory of Dr. M. J. Kuhar at the NIDA Addiction Research Center in Baltimore, MD, until the laboratory was disbanded. Subsequent compounds were evaluated at NOVASCREEN in Hanover, MD, where guinea pig membranes are used as the DA receptor source.

(27) Caroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Parham, K.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis and Ligand Binding, QSAR, and CoMFA Study of 3β-(p-Substituted phenyl) tropane-2β-carboxylic acid Methyl Esters. *J. Med. Chem.* **1991**, *34*, 2719−2725.

(28) Newman, A. H.; Izenwasser, S.; Robarge, M. J.; Kline, R. H. CoMFA Study of Novel Phenyl Ring-Substituted 3α-(Diphenyl Methoxy) Tropane Analogues at the Dopamine Transporter. *J. Med. Chem.* **1999**, *42*, 3502−3509.

(29) Barker, E. L.; Perlman, M. A.; Adkins, E. A.; Houlihan, W. J.; Pristupa, Z. B.; Zdenek, B.; Niznik, H. B.; Blakely, R. D. High Affinity Recognition of Serotonin Transporter Antagonists Defined by Species-Scanning Mutagenesis. An Aromatic Residue in Transmembrane Domain I Dictates Species-Selective Recognition of Citalopram and Mazindol. *J. Biol. Chem.* **1998**, *273*, 19459−19468.

(30) Young, R. Personal communication; Chemical Design Ltd.: Oxfordshire, U.K.

(31) Houlihan, W. J.; Kelly, L. Assessment of Mazindane, a Pro-Drug Form of Mazindol, as a Potential Cocaine Treatment Agent. Unpublished results.

(32) Houlihan, W. J.; Ahmad, U. F.; Koletar, J.; Kelly, L.; Brand, L.; Kopajtic, T. A. Benzo- and Cyclohexanomazindol Analogues as Potential Inhibitors of the Cocaine Binding Site at the Dopamine Transporter. *J. Med. Chem.* **2002**, *45*, 4110.

(33) Kulkarni, S. S.; Newman, A. H.; Houlihan, W. J. Three-Dimensional Quantitative Structure−Activity Relationships of Mazindol Analogues at the Dopamine Transporter. *J. Med. Chem.* **2002**, *45*, 4119.

(34) American Home Products Corp., Belgian Patent 34417, 1968.

(35) Eberle, M. K.; Houlihan, W. J. 1-Amino-4-phenylphthalazine from 3-Ethoxyisoindoline. *Tetrahedron Lett.* **1970**, 3167−3170.

(36) Bergman, J.; Brynolf, A. Ring Interconversion Between a seven-membered Ring (2,3-Dihydro-1,4-benzodiazepine) and a 14-Membered Ring (2,3,9,10-Dibenzo-1,5,8,12-tetraazacyclotetradeca-4,11-diene. *Tetrahedron Lett.* **1989**, *30*, 2979−2982.

(37) Piskov, V. B.; Kasperovich, V. P.; Yakovleva, L. M. Synthesis of Delta. 2-Imidazolines in Ethylene Glycol. *Khim. Geterotsikl. Soedin* **1976**, *8*, 1112−1118.

(38) Oxley, P.; Short, W. F. Amidines. Part VI. Preparation of 2-Substituted 4: 5-Dihydroglyoxalines and Ring Homologues from Cyanides and Alkylenediamines. *J. Chem. Soc.* **1947**, 497−505.

(39) Beger, J.; Wagner, G. Alkylations of 2-(o-Hydroxyphenyl)-delta. 2-imidazoline. *Z. Chem.* **1981**, *21*, 184−185.

(40) Eshleman, A. J.; Carmolli, M.; Cumbay, M.; Martens, C. R.; Neve, K. A.; Janowsky, A. Characteristics of Drug Interactions with Recombinant Biogenic Amine Transporters Expressed in the Same Cell Type. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 877−885.

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