

Pyrrolidine Analogues of Lobelane: Relationship of Affinity for the Dihydratetabenazine Binding Site with Function of the Vesicular Monoamine Transporter 2 (VMAT2)[†]

Ashish P. Vartak, Justin R. Nickell, Jaturaporn Chagkutip, Linda P. Dwoskin, and Peter A. Crooks*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 725 Rose Street, Lexington, Kentucky 40536

Received May 31, 2009

Ring size reduction of the central piperidine ring of lobelane yielded pyrrolidine analogues that showed marked inconsistencies in their ability to bind to the dihydratetabenazine (DTBZ) binding site on the vesicular monoamine transporter-2 (VMAT2) and their ability to inhibit VMAT2 function. The structure–activity relationships indicate that structural modification within the pyrrolidine series resulted in analogues that interact with two different sites, i.e., the DTBZ binding site and an alternative site on VMAT2 to inhibit transporter function.

Introduction

The vesicular monoamine transporter-2 (VMAT2^a) transports monoamines from the neuronal cytosol into the synaptic vesicle, a process that prevents metabolism by monoamine oxidase (MAO) and stores monoamines, such as dopamine (DA), for eventual release by exocytosis.¹ Psychostimulants, such as methamphetamine (METH), inhibit the function of VMAT2, thereby increasing cytosolic levels of DA.² METH also inhibits MAO and reverses the DA transporter (DAT), which together with the increased cytosolic levels of DA results in a substantial efflux of neurotransmitter into the synaptic cleft. Thus, the interaction of METH with VMAT2 is the first step in a series of events that leads ultimately to its pharmacological effects, including the psychological high that results from METH administration. The design and development of VMAT2-selective inhibitors is therefore a worthwhile pursuit toward the broader goal of developing efficacious therapeutics as a means to clinically intervene in the abuse of psychostimulants such as METH.³

The piperidine alkaloid and principal constituent of *Lobelia inflata*, lobelane (**1**), was found to inhibit the uptake of DA into synaptic vesicles.⁴ Because lobelane and its analogues competitively inhibit the binding of [³H]dihydratetabenazine (DTBZ), a known VMAT2 inhibitor, this binding site was identified as a target for the development of potential therapeutic agents for the treatment METH abuse. Thus, the DTBZ binding site on VMAT2 was utilized as a probe in preliminary screening of lobelane analogues for potency and selectivity for VMAT2 interaction. Large increases in potency were achieved through complete deoxygenation and reduction of the lobelane molecule, which resulted in the identification of a lead compound, *cis-N*-methyl-2,6-diphenethylpiperidine (**2**, lobelane).^{5–7}

Compound **2**, which is also a minor alkaloid of *Lobelia inflata*, was found to be more selective than **1** as a VMAT2 ligand,

possessing little affinity for $\alpha 4\beta 2^*$ nicotinic acetylcholine receptors (nAChRs), to which **1** binds with high potency.⁸ The *cis*-2,6-diethylarylpiperidine structural motif in **2** was therefore regarded as an initial pharmacophore on which to develop subsequent generations of VMAT2 inhibitors. Initial structure–activity studies on **2** were focused on the incorporation of substituents into the aromatic rings, variation in the number of methylenes between the central piperidine ring and the phenyl rings, and the positioning of the two phenethyl moieties at different sites around the central piperidine ring. Modest increases in affinity for the DTBZ binding site on VMAT2 were obtained through substitution of anisyl and naphthyl moieties for phenyl moieties in the phenethyl side chains,⁷ while departure from the 2,6-diphenethyl substitution pattern around the piperidine ring was found to be generally deleterious.⁹ A minimum of two methylene units in the alkane linker moiety was found to be essential for retention of binding affinity exhibited by **2** at the DTBZ binding site on VMAT2. Last, the *trans*-configured enantiomeric analogues of **2** (*trans*-**2s**, **4**, and **5**) exhibited diminished affinity for the DTBZ binding site, as did *N*-demethylated analogues, including *nor*-**2** (**3**) (Figure 1).

Design Rationale. Available SAR suggests that the intramolecular distance between the two aromatic rings in **2**, and the relative orientation of the phenethyl moieties, are determinants for affinity at the DTBZ binding site on VMAT2. To improve affinity, we investigated the effect of restricting the flexibility of the central piperidine ring to afford more conformationally defined analogues. In this respect, the current study investigates the effect of replacing the central piperidine ring in **2** with a more conformationally restricted pyrrolidine ring. A series of six pyrrolidine analogues **6–11** were chosen as the initial target molecules (Figure 2). Compound **7** is a pyrrolidino analogue of **2**, and the *trans*-configured analogues **9** and **11** represent the pyrrolidino-analogues of *trans*-**2s** **4**, and **5**. Analogues **6**, **8**, and **10** were also included as analogues of *nor*-**2** (**3**) to determine the importance of the *N*-methyl group in the SAR of these pyrrolidine analogues.

Results

Scheme 1 shows the synthesis of the initial 2,5-diphenethylpyrrolidine analogues. The absolute and relative stereochemistries were achieved utilizing Katritzky's elegant benzotriazole-oxazolidine approach.¹⁰ Briefly, L- and D-phenylglycinol were

[†] We express profound gratitude to the ACS Division of Medicinal Chemistry for its relentless efforts toward fostering research and training in the discipline of Medicinal Chemistry over the past 100 years.

*To whom correspondence should be addressed. Phone: 859-257-1718. Fax: 859-257-7585. E-mail: pcrooks@email.uky.edu.

^a Abbreviations: VMAT2, vesicular monoamine transporter 2; MAO, monoamine oxidase; DA, dopamine; METH, methamphetamine; DTBZ, dihydratetabenazine; TBZ, tetraabenazine; nAChRs, nicotinic acetylcholine receptors.

both obtained through NaBH_4 reduction of the corresponding phenylglycine methyl esters. A three-component condensation of L-phenylglycinol with benzotriazole and succinaldehyde afforded the key synthon **12** in useful quantities. Addition of a 6-fold excess of phenethyl magnesium bromide to synthon **12** at room temperature led to a slightly exothermic reaction, affording a 2:1 mixture of the *cis*- and *trans*-configured diastereomers of **13** within a few hours; these diastereomers were separated by silica gel chromatography. The *2R,5S*- and *2R,5R*-diastereomers, **14** and **15**, were hydrogenolyzed separately by catalytic-transfer hydrogenation with palladium hydroxide-over carbon, employing ammonium formate as the hydrogen source in refluxing methanol. These conditions afforded quantitative conversion to the respective products, **6** and **8**, within 20 min, as opposed to 12 h utilizing alternate conditions (i.e., $\text{H}_2/\text{Pd}-\text{C}$).⁵ The resulting *meso*- and (+)-*trans-2R,5R* pyrrolidines **6** and **8** were then each *N*-methylated with paraformaldehyde and sodium triacetoxyborohydride/acetic acid in acetonitrile followed by treatment with HCl in diethyl ether to afford the *meso* isomer **7** and the (+)-*trans-2R,5R* isomer **9**, respectively. Utilization of D-phenylglycinol in place of L-phenylglycinol in the above synthetic scheme

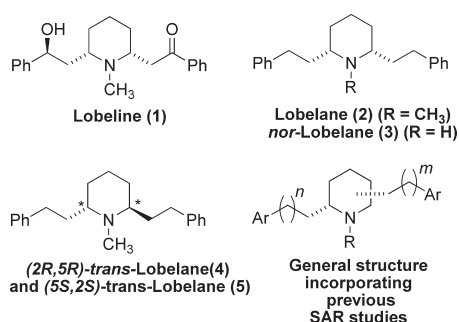


Figure 1. Structures of **1**, **2**, and synthetic analogues.

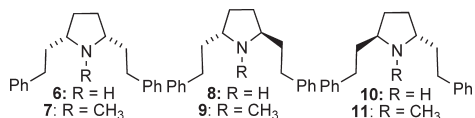
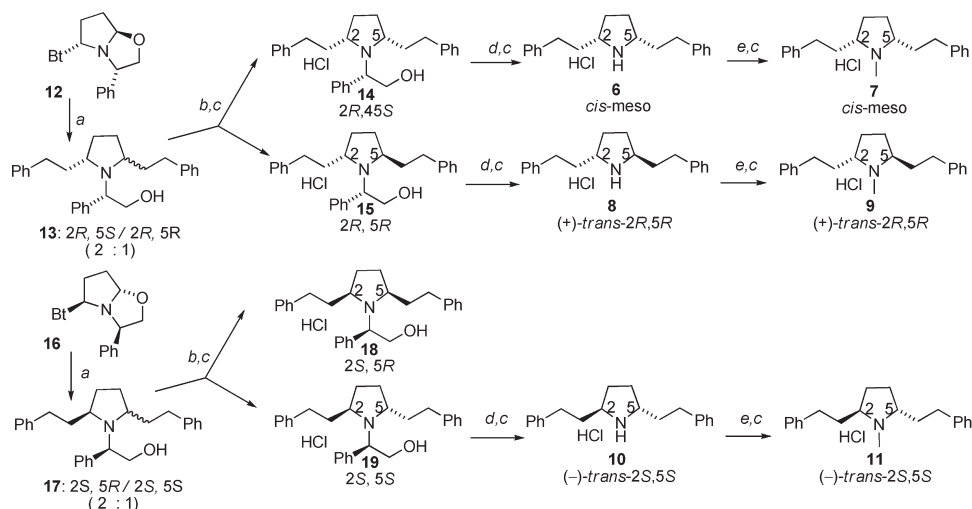


Figure 2. Structures of initial pyrrolidine analogues of **2** and *nor-2*.

Scheme 1. Synthesis of 2,5-Diphenethylpyrrolidines



provided synthon **16**, which could be converted into **18** and **19**. Hydrogenolysis of **19** followed by hydrochloride salt formation afforded **10** ((-)-*trans-2S,5S*), which in turn was *N*-methylated to **11** ((-)-*trans-2S,5S*). Note that compound **18** also forms the *cis-meso* isomer **6** upon hydrogenolysis, making further elaboration of this compound redundant.

The pyrrolidine analogues in Scheme 1 were evaluated for affinity at the DTBZ binding site on VMAT2 (Table 1). When the 2,5-diphenethyl substituents are in the *meso cis*-configuration, overall binding affinity clearly decreases upon replacement of the piperidine ring with the pyrrolidine ring, because **7** is about 10 times less potent than **2** (**2**). When the phenethyl substituents are in the 2,5-*trans* configuration (analogues **9** and **11**), the binding affinity does not appreciably change with azacyclic ring-size reduction when compared to the piperidine (**2**) counterparts **4** and **5**, respectively. Interestingly, the (*S,S*)-*trans-nor-N*-methyl analogue **10** is about 10-fold more potent than its *N*-methyl derivative **11**, whereas replacement of the *N*-methyl group in both the *cis*- and *trans*-analogues with an *N*-phenethanolyl substituent afforded no significant change in binding affinity, with analogues **14**, **15**, **18**, and **19** possessing affinities comparable to the *nor*- and *N*-methyl pyrrolidine analogues.

The ultimate goal of this SAR study was to identify chemical entities that would inhibit VMAT2 function. It is known that there are multiple binding sites on VMAT2 that may influence transporter function, i.e., a ketanserin binding site near the *N*-terminus, a tetrabenazine (TBZ) binding site near the *C*-terminus, and two reserpine binding sites (a high affinity site and a low affinity site).¹¹ In this respect, the affinity of a ligand toward the DTBZ binding site on VMAT2 may not necessarily be a sufficient indicator of the activity of the ligand in altering function of VMAT2. In light of these data and the possibility that other, as yet unidentified, binding sites on VMAT2 may exist, the ability of the current analogues to inhibit the uptake of [³H]-DA into isolated synaptic vesicles was also evaluated (Table 1).

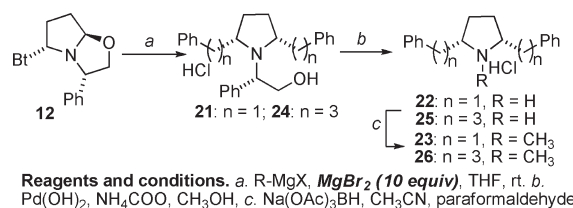
While the affinities of the pyrrolidine ligands for the DTBZ binding site did not vary much with respect to structural change (K_i values were in the range of 0.3–14 μM), their ability to inhibit the uptake of [³H]-DA into synaptic vesicles varied greatly with subtle structural changes, with K_i values ranging from 9 nM to 1.94 μM . Ring-size reduction of the piperidine moiety in **2** afforded a 10-fold decrease in the

Table 1. Interaction of **2** and Pyrrolidine Analogues with VMAT2

analogue	[³ H]DTBZ; $K_i \pm \text{SEM}$ (μM)	VMAT2 [³ H]-DA uptake $K_i \pm \text{SEM}$ (μM)	ratio [³ H]DTBZ binding/[³ H]- DA uptake
TBZ	0.013 ± 0.001	0.054 ± 0.015	0.24
1	2.76 ± 0.64	0.470 ± 0.045	5.87
2	0.97 ± 0.19	0.033 ± 0.041	29.4
3	2.31 ± 0.21	0.023 ± 0.004	100
4	5.32 ± 0.45	4.05 ± 0.612	1.31
5	6.46 ± 1.70	2.11 ± 0.50	3.06
6	3.06 ± 0.17	0.029 ± 0.007	106
7	8.80 ± 2.30	0.270 ± 0.035	32.6
8	3.40 ± 0.030	0.054 ± 0.017	63.0
9	3.97 ± 0.64	0.120 ± 0.015	33.1
10	1.33 ± 0.19	0.035 ± 0.006	38.0
11	14.45 ± 2.13	0.170 ± 0.030	85.0
14	4.34 ± 0.71	1.290 ± 0.240	3.36
15	5.05 ± 1.05	1.940 ± 0.470	2.60
18	2.75 ± 0.63	0.140 ± 0.049	19.6
19	5.61 ± 0.91	0.270 ± 0.053	20.7
22	1.64 ± 0.12	0.0093 ± 0.00075	176
23	2.46 ± 0.42	0.019 ± 0.00091	130
25	0.27 ± 0.0067	0.014 ± 0.0011	19.3
26	2.78 ± 0.63	0.12 ± 0.011	23.2

potency to inhibit [³H]-DA uptake into vesicles when compared to **2** (*meso cis*-analogues **7** and **2**) However, a similar ring-size reduction in *nor*-**2** had no effect on inhibition of [³H]-DA uptake because **3** and **6** were equipotent. The most striking difference in [³H]-DA uptake inhibition was observed between the *trans*-configured piperidine- and pyrrolidine-analogues. Thus, (*S,S*)-*trans*-**2** (**4**) ($K_i = 4.05 \mu\text{M}$) and (*R,R*)-*trans*-**2** ($K_i = 2.11 \mu\text{M}$) (**5**) are 33- and 12-fold, respectively, less potent than their corresponding pyrrolidine analogues **9** ($K_i = 120 \text{ nM}$) and **11** ($K_i = 170 \text{ nM}$), respectively. Within the pyrrolidine series, the presence of the *N*-methyl group is clearly deleterious toward potency of inhibition of [³H]-DA uptake because the *N*-methyl pyrrolidines **7**, **9**, and **11** are all less potent than their *nor-N*-methyl counterparts **6**, **8**, and **10**, with the effect of *N*-methyl substitution being most prominent in the *cis*-configured analogues **6** and **7**. Also, within the *N*-phenethanolyl pyrrolidine series of analogues, the configuration of the pendant phenethanolyl stereocenter appears to be a factor that contributes to the potency for [³H]-DA uptake inhibition because the *R*-configured analogues **18** and **19** are about 10-fold more potent than the *S*-configured analogues **14** and **15**.

A marked disparity exists in the abilities of the above series of analogues to bind to the DTBZ binding site on VMAT2 and to inhibit [³H]-DA uptake into vesicles, as can be seen in the ratios of the K_i values, as well as in the rank order of potency of the compounds in the two assays; this is evident for both the piperidine and pyrrolidine analogues (Table 1, right column). Compound **3** and its pyrrolidine analogue **6** are both about 100-fold more potent in inhibiting [³H]-DA uptake into isolated synaptic vesicles than in binding to the DTBZ-binding site, while their *N*-methyl counterparts **2** and **7** are both about 30 times more potent in this regard. While the *N*-methyl *trans*-configured pyrrolidines **9** and **11** are both significantly more potent in inhibiting [³H]-DA uptake than in binding to the DTBZ binding site, their piperidine counterparts (i.e., the *trans*-**2** enantiomers **4** and **5**) are relatively weak [³H]-DA uptake inhibitors ($K_i > 1 \mu\text{M}$) and show relatively low potency in the DTBZ binding assay (K_i s 5.32 and 6.46 μM , respectively). In the *N*-phenethanolyl series, the 2'*R*-analogues **18** and **19** exhibit significant differences in their ability

Scheme 2. Synthesis of Second-Generation Pyrrolidine Analogues

to bind to the DTBZ binding site on VMAT2 (K_i 2.75 and 5.61 μM , respectively) and their ability to inhibit [³H]-DA uptake into vesicles (K_i 140 and 270 nM, respectively). Pyrrolidines **18** and **19** are more potent as [³H]-DA uptake inhibitors, compared to their corresponding 2'*S*-configured analogues **14** (K_i (binding) = 4.34 μM , K_i (uptake) = 1.29 μM) and **15** (K_i (binding) = 5.05 μM , K_i (uptake) = 1.94 μM).

cis-2,5-Diphenethylpyrrolidine (**6**) was identified as the most potent pyrrolidine analogue for the inhibition of VMAT2 function, and structural optimization of this lead molecule was further investigated. Thus, compound **22**, in which the methylene linker moieties have been truncated to a single methylene unit, as well as compound **25**, in which the methylene units have been increased from two to three, were synthesized. The synthesis of pyrrolidine **22** was approached by utilizing the method illustrated in Scheme 2. Treatment of **12** with benzyl magnesium chloride led to an inseparable ~3:1 mixture of diastereomers with the 2,5-*cis*-configured diastereomer **21** being the predominant isomer. Because diastereomer **21** was required for further elaboration, conditions were identified to increase the proportion of this diastereomer in the reaction product. During the synthesis of the 2,5-diphenethyl pyrrolidines, it was noted that the presence of MgBr_2 in the reaction mixture (originating from the activation of Mg turnings by dibromoethane) resulted in diastereomeric ratios that favored the 2,5-*cis* diastereomers (**14** and **18**) over the 2,5-*trans* diastereomers (**15** and **19**). A solution of **12** was treated with progressively increasing equivalents of MgBr_2 (generated in situ from Mg and dibromoethane) prior to the addition of benzylmagnesium chloride. When 10 equiv of MgBr_2 had been utilized, the product mixture consisted solely of the free-base of **21** (the *trans*-configured diastereomer could not be detected by ¹H NMR or by gas chromatography), however, this favorable exclusive formation of the desired diastereomer was achieved at the expense of a significantly lower yield (42%), as compared to the 80% yield obtained in the reaction without MgBr_2 . Hydrogenolysis of **21** led to **22**, which was *N*-methylated and treated with HCl in diethylether to afford **23**. The diastereomerically pure propyl homologue **25** and its *N*-methylated derivative **26** were prepared in a similar manner by utilizing the aforementioned procedure employing phenpropyl magnesium bromide.

The methylene truncated analogues **22** and **23** bound to the DTBZ-binding site of VMAT2 with affinities comparable to that of the parent pyrrolidines **6** and **7**, whereas the methylene elongated *nor*-analogue **25**, bound with high affinity ($K_i = 270 \text{ nM}$), making it one of the most potent **2**-derived ligands known for this binding site. *N*-Methylation of **25** afforded **26**, which was 10 times less potent than **25**.

Pyrrolidine **22** was the most potent [³H]-DA uptake inhibitor in this series of analogues, with a K_i of 9.3 nM. Its *N*-methylated derivative **23** was also a good uptake inhibitor ($K_i = 19 \text{ nM}$). Pyrrolidine **25** was also a potent uptake inhibitor ($K_i = 14 \text{ nM}$); its *N*-methylated analogue **26** was about 10 times less potent.

As seen in Table 1, the disparity between [^3H]-DTBZ binding and [^3H]-DA uptake inhibition is more prominent for the methylene truncated pyrrolidines **22** and **23**, which inhibit [^3H]-DA uptake 176- and 129-fold more potently than they inhibit [^3H]-DTBZ binding to VMAT2. The methylene elongated pyrrolidines **25** and **26** inhibit [^3H]-DA uptake about 20 times more potently than they inhibit DTBZ binding, irrespective of their relative potencies in the individual assays.

Discussion

In summary, ring size reduction of the central piperidine ring of **2** yielded pyrrolidine analogues that showed marked disparities in their ability to bind to the [^3H]-DTBZ binding site on VMAT2 and their ability to inhibit VMAT2 function. Unlike TBZ, the compounds in this series of pyrrolidine analogues of **2** were more potent in inhibiting VMAT2 function than they were in inhibiting the binding of [^3H]-DTBZ to VMAT2. The SAR indicate that structural modification within the pyrrolidine series results in analogues that likely interact with two different sites on VMAT2, i.e., the DTBZ binding site, and perhaps an alternative site on VMAT2, to inhibit transporter function. While the affinity of the pyrrolidine analogues toward the DTBZ binding site is not significantly sensitive to structural changes, the potency for [^3H]-DA uptake inhibition is sensitive to subtle structural changes, such as alteration in methylene linker length, *N*-substitution, and stereochemistry in the case of the *N*-phenethanolyl analogues. Such sensitivity suggests that the putative binding site on VMAT2 whose interaction with the pyrrolidine analogues leads to functional inhibition, is topographically distinct from the DTBZ ligand binding site. It should be noted that compound **22**, which has the greatest potency to inhibit [^3H]-DA uptake into synaptic vesicles, can be considered a structural dimer of methamphetamine (Figure 3), which is itself known to inhibit VMAT2 function.¹² In this respect, **2** (**2**) could also be considered to be a “dimeric” methamphetamine analogue; however, this compound does not itself support self-administration but rather decreases methamphetamine self-administration in animal models.¹³

The results of this study are expected to have a significant impact on the future design of lobelane-derived VMAT2 inhibitors. Due caution needs to be exercised in utilizing the SAR from [^3H]-DTBZ-binding assays for the design of lobelane-derived VMAT2 inhibitors because such data may not be functionally relevant. The pyrrolidine ring has been identified as a valid replacement for the piperidine ring in **2**, and *nor*-analogues (i.e., *N*-demethylated homologues of **2**) appear to be optimal for both, binding to the DTBZ binding site on VMAT2 and inhibition of VMAT2 function. A potent VMAT2 ligand, **25**, and a potent water-soluble inhibitor of VMAT2 function (**22**) were also identified during this study, which appear to be suitable for further development and advancement into behavioral studies.

Experimental Section

(2'*S*)-2-[(2*R*,5*S*)-2,5-Di-(2-phenethyl)-tetrahydro-1*H*-1-pyrrolyl]-2-phenylethan-1-ol (**14**) and (2'*S*)-2-[(2*R*,5*R*)-2,5-Di-(2-phenethyl)-tetrahydro-1*H*-1-pyrrolyl]-2-phenylethan-1-ol (**15**). To 306 mg (1 mmol) of **12** suspended in 20 mL of THF was added a solution of phenethylmagnesium bromide in THF (6 equiv) over a period of 10 min at 0 °C. The solution was warmed to ambient temperature over 8 h, 10 mL of 2*N* NaOH were added, and the biphasic mixture was extracted with diethyl ether (3 × 100 mL). The ethereal layers were combined, diluted with 50 mL of CH₂Cl₂, dried with anhydrous Na₂SO₄ and evaporated under reduced pressure to afford a

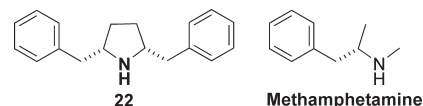


Figure 3. Pyrrolidine **22** may be regarded structurally a “dimer” of methamphetamine.

yellow oil. The oil was dissolved in 20 mL of dry diethyl ether through which HCl gas had been bubbled with stirring for 10 min. The supernatant was decanted, and the resulting gummy sediment was triturated with diethyl ether (5 × 50 mL). Aqueous NaOH solution (2*N*, 20 mL) was then added, and the suspension extracted with diethyl ether (2 × 100 mL). The organic layers were washed with brine, dried, and the oily residue was applied to a silica gel column (10 g). The column was eluted with 15:1 (hexanes/diethyl ether) to afford pure fractions of compounds **14** (240 mg, 60%) and **15** (60 mg, 15%) as colorless oils. **14** *R_f* = 0.4 (8:1, hexanes/EtOAc). ¹H NMR (300 MHz, CDCl₃) δ ppm 7.45–7.03 (m, 15H, Ar), 4.02–3.83 (m, 2H), 3.78–3.60 (m, 1H), 3.35–3.20 (br, s, 1H), 3.15–2.95 (m, 2H), 2.80–2.45 (m, 4H), 1.95–1.20 (m, 10H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 143.7, 137.2, 129.1, 128.5, 128.5, 127.9, 125.9, 64.8, 63.6, 61.9, 57.6, 38.1, 36.4, 29.8, 29.2, 28.9. [α]_D²⁰ = –13.2° (*c* 1.0, CH₂Cl₂). Anal. C₂₈H₃₃NO (C,H,N) **15** *R_f* = 0.2 (8:1, hexanes/EtOAc). ¹H NMR (300 MHz, CDCl₃) δ ppm 7.50–6.98 (m, 15H), 4.30–3.92 (m, 2H), 3.84–3.79 (m, 1H), 3.32–3.16 (br, s, 1H), 3.17–2.92 (m, 2H), 2.73–2.56 (m, 4H), 2.03–1.20 (m, 10H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 146.3, 138.2, 125.4, 127.9, 128.8, 126.1, 125.4, 122.1, 66.2, 61.6, 57.6, 38.1, 29.2, 28.9. [α]_D²⁰ = –2.0° (*c* 1.0, CH₂Cl₂). Anal. C₂₈H₃₃NO (C,H,N).

(2'*R*)-2-(2*S*,5*R*)-2,5-Di-(2-phenethyl)-tetrahydro-1*H*-1-pyrrolyl-2-phenylethan-1-ol (**18**). Compound **18** was synthesized from **16** and was the major diastereomer (55% yield). ¹H and ¹³C NMR spectra were identical to **3**. [α]_D²³ = +12.1° (*c* 1.0, CH₂Cl₂). Anal. C₂₈H₃₃NO (C,H,N).

(2'*R*)-2-(2*S*,5*S*)-2,5-Di-(2-phenethyl)-tetrahydro-1*H*-1-pyrrolyl-2-phenylethan-1-ol (**19**). Compound **19** was synthesized from **16** as the minor diastereomer (20% yield). ¹H and ¹³C NMR spectra were identical to **4**. [α]_D²⁰ = +2.2° (*c* 1.0, CH₂Cl₂). Anal. C₂₈H₃₃NO (C,H,N).

cis-2,5-Di-(2-phenethyl)-pyrrolidine Hydrochloride (**6**). To a solution of **14** (100 mg, 0.25 mmol) in MeOH (5 mL) was added Pd(OH)₂C (10 mg, 10 wt %) and ammonium formate (100 mg, 100 wt %). The mixture was refluxed for 20 min before being diluted with CH₂Cl₂ (30 mL) and filtered through celite. The filtrate was treated with 1 mL conc HCl and evaporated to a crusty white solid under reduced pressure. The solid was triturated with diethyl ether and precipitated from a mixture of CH₂Cl₂ and hexanes by addition of hexanes, to yield **6** (60 mg, 77%) as a white solid, mp = 155 °C. ¹H NMR (300 MHz, CDCl₃) δ ppm 10.22 (br, s, 1H), 9.03 (br, s, 1H), 7.32–7.08 (m, 10H), 3.62–3.43 (apparent s, br, 2H), 2.63–2.47 (m, 4H), 1.83–1.62 (m, 8H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 141.7, 128.6, 128.6, 126.2, 60.4, 41.1, 35.8, 29.0. Anal. C₂₀H₂₆ClN (C,H,N).

(2*R*,5*R*)-Di-(2-phenethyl)-pyrrolidine Hydrochloride (**8**). Compound **15** (100 mg, 0.25 mmol) was prepared as described for the preparation of **6**, to yield 38 mg (48%) of **8** as a white solid, mp = 95 °C (deliquescent). ¹H NMR (300 MHz, CDCl₃) δ ppm 9.80 (br, s, 1H), 9.72 (br, s, 1H), 7.40–7.00 (m, 10H), 3.52–3.30 (s, br, 2H), 2.40–1.99 (m, 4H), 1.70–1.53 (m, 8H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 140.0, 129.6, 128.5, 126.1, 58.3, 43.8, 33.0, 29.4. [α]_D²⁰ = +7.5° (*c* = 1.0, CH₂Cl₂). Anal. C₂₀H₂₆ClN (C,H,N).

(2*S*,5*S*)-di-(2-phenethyl)-pyrrolidine Hydrochloride (**10**). Compound **10** was synthesized from **19** in a manner similar to that described for **8** (73% yield). Spectral characteristics were identical to those of **8**. [α]_D²⁰ = –8.1° (*c* = 1.0, CH₂Cl₂). Anal. C₂₀H₂₆ClN (C,H,N).

1-Methyl-*cis*-2,5-di-(2-phenethyl)-pyrrolidine Hydrochloride (**7**). To a solution of **6** (50 mg, 0.14 mmol) in CH₃CN (5 mL) and acetic acid (1 mL) was added NaBH₄ (50 mg, excess) and paraformaldehyde (50 mg, excess). The initial suspension afforded a clear solution upon stirring for 12 h. The solution was then treated with

conc HCl (0.5 mL), stirred for 2 h, poured into 50% aqueous NaOH (10 mL), and extracted with CH₂Cl₂ (6 × 20 mL). The CH₂Cl₂ extracts were combined, dried (anhydrous MgSO₄), filtered, and the solvent evaporated to yield an oil that was treated with conc HCl (1 mL) and azeotroped twice with toluene. Trituration of the residue with diethyl ether afforded a white solid **7** (43 mg, 83%), mp = 94 °C (deliquescent). ¹H NMR (300 MHz, CDCl₃) δ ppm 9.48 (br, s, 1H), 7.25–7.02 (m, 10H), 3.73–3.48 (m, 2H), 2.63–2.47 (m, 4H), 2.34 (s, 3H), 1.74–1.38 (m, 8H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 144.8, 127.2, 127.1, 126.5, 65.2, 48.3, 38.3, 32.2, 30.1. Anal. C₂₁H₂₈ClN·0.5H₂O (C,H,N).

1-Methyl-(2*R*,5*R*)-di-(2-phenethyl)-pyrrolidine Hydrochloride (9). Compound **8** was subjected to identical conditions to those described for the preparation of **7** to yield 30 mg (58%) of **9** as a white solid, mp = 113 °C. ¹H NMR (300 MHz, CDCl₃) δ ppm 9.90 (br, s, 1H), 7.30–6.99 (m, 10H), 3.84–3.65 (m, 2H), 2.50–2.23 (m, 4H), 2.10 (s, 3H), 1.66–1.20 (m, 8H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 144.3, 127.06, 127.00, 126.9, 63.1, 45.3, 37.2, 31.6, 31.1. [α]_D²⁰ = +46.2° (c = 1.5, MeOH). Anal. C₂₁H₂₈ClN·H₂O (C,H,N).

1-Methyl-(2*S*,5*S*)-di-(2-phenethyl)-pyrrolidine Hydrochloride (11). Compound **10** was subjected to conditions similar to those described for the preparation of **9**. Spectral characteristics were identical to those of **7**. [α]_D²⁰ = –48.7° (c = 1.0, MeOH). Anal. C₂₁H₂₈ClN (C,H,N).

cis-2,5-Di(2-benzyl)-pyrrolidine Hydrochloride (22). To a solution of **21** (200 mg, 0.50 mmol) in MeOH (5 mL) was added Pd(OH)₂-C (10 mg, 10 wt %) and ammonium formate (100 mg, 50 wt %). The mixture was refluxed for 20 min, diluted with CH₂Cl₂ (60 mL), and filtered through celite. The filtrate was treated with 5 mL of conc HCl and evaporated to a crusty white solid under reduced pressure. The solid was triturated with diethyl ether and precipitated from a mixture of CH₂Cl₂ and hexanes by addition of hexanes, to yield **22** (108 mg, 74%) as a white solid, mp = 173 °C. ¹H NMR (300 MHz, CDCl₃) δ ppm 9.4 (s, 1H), 9.2 (br, s, 1H), 7.45–7.00 (m, 10H), 4.00–3.64 (s, br, 2H), 2.48–2.14 (m, 4H), 1.70–1.52 (m, 6H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 139.0, 126.3, 124.6, 120.8, 58.4, 37.1, 34.3, 33.1. Anal. C₁₈H₂₂ClN (C,H,N).

1-Methyl-cis-2,5-di(2-benzyl)-pyrrolidine Hydrochloride (23). To a solution of **22** (100 mg, 0.32 mmol) in MeOH (3 mL) was added NaCNBH₃ (50 mg, excess) and paraformaldehyde (50 mg, excess). The initial suspension became a clear solution upon stirring for 6 h. The solution was diluted with conc HCl (10 mL) and stirred for 2 h, during which time all of the cyanoborohydride and hydrogen cyanide was assumed to be decomposed. The mixture was poured into 20% aq NaOH (10 mL) and extracted with CH₂Cl₂ (100 mL). Drying and evaporation of the organic layer yielded an oil that was treated with conc HCl (5 mL) and coevaporated with toluene. Trituration of the residue afforded a white solid **23** (80 mg, 81%). mp = 126 °C (deliquescent). ¹H NMR (300 MHz, CDCl₃) δ ppm 9.98 (br, s, 1H), 7.33–7.00 (m, 10H), 3.63–3.53 (m, 2H), 2.46–2.23 (m, 4H), 2.42 (s, 3H), 1.51–1.22 (m, 6H). ¹³C NMR (75 MHz, CDCl₃) 139.1, 128.9, 127.4, 125.1, 65.2, 48.3, 39.0, 30.1. Anal. C₁₉H₂₄ClN·H₂O (C,H,N).

cis-2,5-Di(3-phenylpropyl)-pyrrolidine Hydrochloride (25). To compound **24** (100 mg, 0.21 mmol) was added Pd(OH)₂-on-carbon (10 wt %), MeOH (10 mL) and ammonium formate (500 mg). After refluxing for 2 h, the solution was poured into CH₂Cl₂ (50 mL), filtered, and evaporated to obtain a white solid (70 mg, 94%). ¹H NMR (300 MHz, CDCl₃) δ ppm 10.31 (s, 1H), 9.03 (s, 1H), 7.27–7.13 (m, 15H, Ar), 3.50 (s, 1H), 2.62–2.14 (m, 4H), 2.12–1.68 (m, 14H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 140.2, 129.7, 127.2, 126.2, 65.66, 37.38, 29.5, 28.2, 27.85. Anal. C₂₂H₃₀ClN (C,H,N).

1-Methyl-cis-2,5-di(3-phenylpropyl)-pyrrolidine Hydrochloride (26). To a solution of **25** (20 mg, 0.08 mmol) in MeOH (3 mL) was added NaCNBH₃ (20 mg, excess) and paraformaldehyde (20 mg, excess). The initial suspension became a clear solution upon stirring for 12 h. The solution was diluted with conc HCl

(1 mL) and stirred for 2 h, during which time all of the cyanoborohydride and hydrogen cyanide was assumed to be decomposed. The mixture was then poured into 20% aq NaOH (3 mL) and extracted with CH₂Cl₂ (10 mL). Drying and evaporation of the organic layer yielded an oil that was treated with conc HCl (1 mL) and coevaporated with toluene. Trituration of the residue afforded a white solid (18 mg, 87%), mp = 78 °C. ¹H NMR (300 MHz, CDCl₃) δ ppm 9.48 (s, 1H), 7.45–7.03 (m, 10H), 3.47–3.18 (m, 2H), 2.58–2.47 (m, 4H), 2.19 (s, 3H), 1.91–1.36 (m, 6H). ¹³C NMR (75 MHz, CDCl₃) δ ppm 140.6, 128.2, 128.13, 128.02, 64.1, 37.6, 36.4, 29.5, 29.2, 28.3. Anal. C₂₃H₃₂ClN·H₂O (C,H,N).

Acknowledgment. We thank Agripina G. Deaciuc for technical assistance and the National Institutes of Health, NIDA grant DA013519, for financial support.

Supporting Information Available: Combustion analysis data for all compounds, preparation and characterization data for **21** and **24** and procedures for the determination of [3H]-DTBZ binding and [3H]-DA uptake inhibition. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- Eiden, L. E. The vesicular neurotransmitter transporters: current perspectives and future prospects. *FASEB J.* **2000**, *14*, 2396–2400.
- Ascher, J. A.; Cole, J. O.; Colin, J. N.; Feighner, R. M.; Ferris, R. M.; Fibiger, H. C.; Golden, R. N.; Martin, P.; Potter, W. Z.; Richelson, E.; Sulser, F. Bupropion: a review of its mechanism of antidepressant activity. *J. Clin. Psychiatry* **1995**, *56*, 395–401.
- Zheng, G.; Dwoskin, L. P.; Crooks, P. A. Vesicular monoamine transporter 2: role as a novel target for drug development. *AAPS J.* **2006**, *8*, E682–E692.
- Dwoskin, L. P.; Crooks, P. A. A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. *Biochem. Pharmacol.* **2002**, *63*, 89–98.
- Terry, L.; Williamson, R.; Gattu, M.; Beach, J. W.; McCurdy, C. R.; Sparks, J. A.; Pauly, J. R. Lobeline and structurally simplified analogs exhibit differential agonist activity and sensitivity to antagonist blockade when compared to nicotine. *Neuropharmacology* **1998**, *37*, 93–102.
- Flammia, D.; Dukat, M.; Damaj, M. I.; Martin, B.; Glennon, R. A. Lobeline: Structure–Activity Investigation of Nicotinic Acetylcholinergic Receptor Binding. *J. Med. Chem.* **1999**, *42*, 3726–3731.
- Zheng, G.; Dwoskin, L. P.; Deaciuc, A. G.; Norrholm, S. D.; Crooks, P. A. Defunctionalized lobeline analogues: structure–affinity of novel ligands for the vesicular monoamine transporter. *J. Med. Chem.* **2005**, *48*, 5551–5560.
- Court, J. A.; Perry, E. K.; Spurdin, D.; Lloyd, S.; Gillespie, J. I.; Whiting, P.; Barlow, R. Comparison of the binding of nicotinic agonists to receptors from human and rat cerebral cortex and from chick brain (α₂β₂) transfected into mouse fibroblasts with ion channel activity. *Brain Res.* **1994**, *667*, 118–122.
- Zheng, G.; Dwoskin, L. P.; Deaciuc, A. G.; Crooks, P. A. Synthesis and evaluation of a series of homologues of lobeline at the vesicular monoamine transporter-2. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6509–6512.
- Katritzky, A. R.; Cui, X.; Baozhen, Y.; Steel, P. J. Asymmetric syntheses of 2-substituted and 2,5-disubstituted pyrrolidines from (3*S*,5*R*,7*aR*)-5-(benzotriazol-1-yl)-3-phenyl[2,1-*b*]oxazolopyrrolidine. *J. Org. Chem.* **1999**, *64*, 1979–1985.
- Gopalakrishnan, A.; Sievert, M.; Ruoho, A. E. Identification of the substrate binding region of vesicular monoamine transporter-2 (VMAT-2) using iodoaminoflupolol as a novel photoprobe. *Mol. Pharmacol.* **2007**, *72*, 1567–1575.
- Ugarte, Y. V.; Rau, K. S.; Riddle, E. L.; Hanson, G. R.; Fleckenstein, A. E. Methamphetamine rapidly decreases mouse vesicular dopamine uptake: role of hyperthermia and dopamine D2 receptors. *Eur. J. Pharmacol.* **2003**, *472*, 165–171.
- Neugebauer, N. M.; Harrod, S. B.; Stairs, D. J.; Crooks, P. A.; Dwoskin, L. P.; Bardo, M. T. Lobeline decreases methamphetamine self-administration in rats. *Eur. J. Pharmacol.* **2007**, *571*, 33–38.
- Teng, L.; Crooks, P. A.; Sonsalla, P. K.; Dwoskin, L. P. Lobeline and nicotine evoked [³H] overflow from rat striatal slices preloaded with [³H]dopamine: differential inhibition of synaptosomal and vesicular [³H]dopamine uptake. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 1432–1444.