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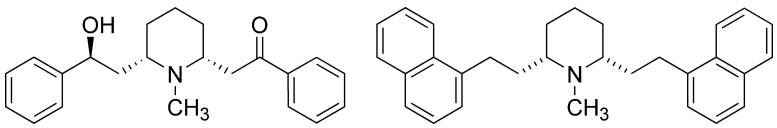

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Defunctionalized Lobeline Analogues: Structure−Activity of Novel Ligands for the Vesicular Monoamine Transporter

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(−)-Lobeline (2*R*,6*S*,10*S*; **1a**), an antagonist at nicotinic acetylcholine receptors (nAChRs), inhibits the neurochemical and behavioral effects of methamphetamine and inhibits dopamine transporter (DAT) and vesicular monoamine transporter (VMAT2) function. VMAT2 is a target for the development of treatments for methamphetamine abuse. Structural modification of lobeline affords the defunctionalized analogues *meso*-transdiene (MTD) and lobelane, which have high potency and selectivity for VMAT2. To establish the structure−activity relationships within this novel class of VMAT2 ligands, specific stereochemical forms of MTD, lobelane, and other structurally related analogues have been synthesized. These compounds have been evaluated for inhibition of [^{3}H]nicotine ([^{3}H]NIC) binding (α4β2* nAChR), [^{3}H]methyllycaconitine ([^{3}H]MLA) binding (α7* nAChR), and [^{3}H]dihydrotetrabenazine ([^{3}H]DTBZ) binding (VMAT2). Generally, all of these analogues had lower affinities at α4β2* and α7* nAChRs compared to lobeline, thereby increasing selectivity for VMAT2. The following structural modifications resulted in only modest changes in affinity for VMAT2, affording analogues that were less potent than the lead compound, lobelane: (1) altering the stereochemistry at the C-2 and C-6 positions of the piperidino ring, (2) varying unsaturation in the piperidino C-2 and C-6 substituents, (3) introducing unsaturation into the piperidine ring, (4) ring-opening or eliminating the piperidine ring, and (5) removing the piperidino *N*-methyl group. Furthermore, incorporating a quaternary ammonium group into defunctionalized lobeline molecules in the cis-series resulted in significant loss of affinity for VMAT2, whereas only a modest change in affinity was obtained in the trans-series. The most potent (K_i = 630 nM) and VMAT2-selective compound evaluated was the *N*-methyl-2,6-*cis*-bis(naphthaleneethyl)piperidine analogue **28b** (1-NAP-lobelane), in which the phenyl groups of lobelane were replaced with 1-naphthyl moieties. Thus, initial structure−activity relationship studies reveal that the most promising structural changes to the lobeline molecule that lead to enhancement of VMAT2 affinity and selectivity are defunctionalization, affording lobelane and MTD, and replacement of the phenyl rings of lobelane with other aromatic moieties that have a π-extended structure.

Introduction

Psychostimulant-induced behavioral activation and reinforcement are mediated, at least in part, via interaction with neurotransmitter transporters, which regulate synaptic dopamine (DA) concentrations.[1,2] Recent studies have demonstrated that psychostimulants alter vesicular monoamine transporter (VMAT2) function.[3] Methamphetamine decreases VMAT2 function[4,5] and is also a substrate for the DA transporter (DAT).[6,7] VMAT2 heterologous knockout mice exhibit reduced amphetamine conditioned reward,[8] enhanced amphetamine locomotion,[8] and enhanced sensitivity to cocaine and amphetamine,[9] indicating that VMAT2 plays an important role in mediating the behavioral effects of psychostimulants. These results support the idea that VMAT2 should be considered as a target for the development of pharmacotherapies to treat psychostimulant abuse. Other evidence supporting the role of VMAT2 in psychostimulant pharmacology is the finding that benzoquinolizine derivatives, such as tetrabenazine, have high affinity for VMAT2, decrease locomotor activity and aggressiveness in monkeys, and, moreover, depress methamphetamine-induced hyperactivity in rats and mice.[13]

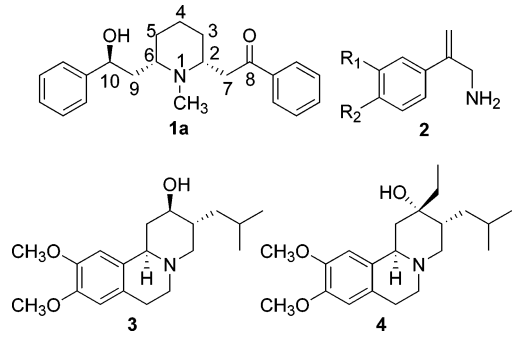

Figure 1.

(−)-Lobeline (2*R*,6*S*,10*S*; **1a**) (Figure 1), a weakly basic lipophilic alkaloid from *Lobelia inflata*, interacts with VMAT2 and DAT via a novel mechanism of action.[14] Lobeline potently inhibits [^{3}H]dihydroxytetrabenazine ([^{3}H]DTBZ) binding to VMAT2 with an IC_{50} of 0.90 μM and inhibits [^{3}H]DA uptake into rat striatal vesicle preparations with an IC_{50} of 0.88 μM.[15,16] Furthermore, lobeline also inhibits (IC_{50} = 80 μM) [^{3}H]DA uptake into rat striatal synaptosomes via DAT, but with

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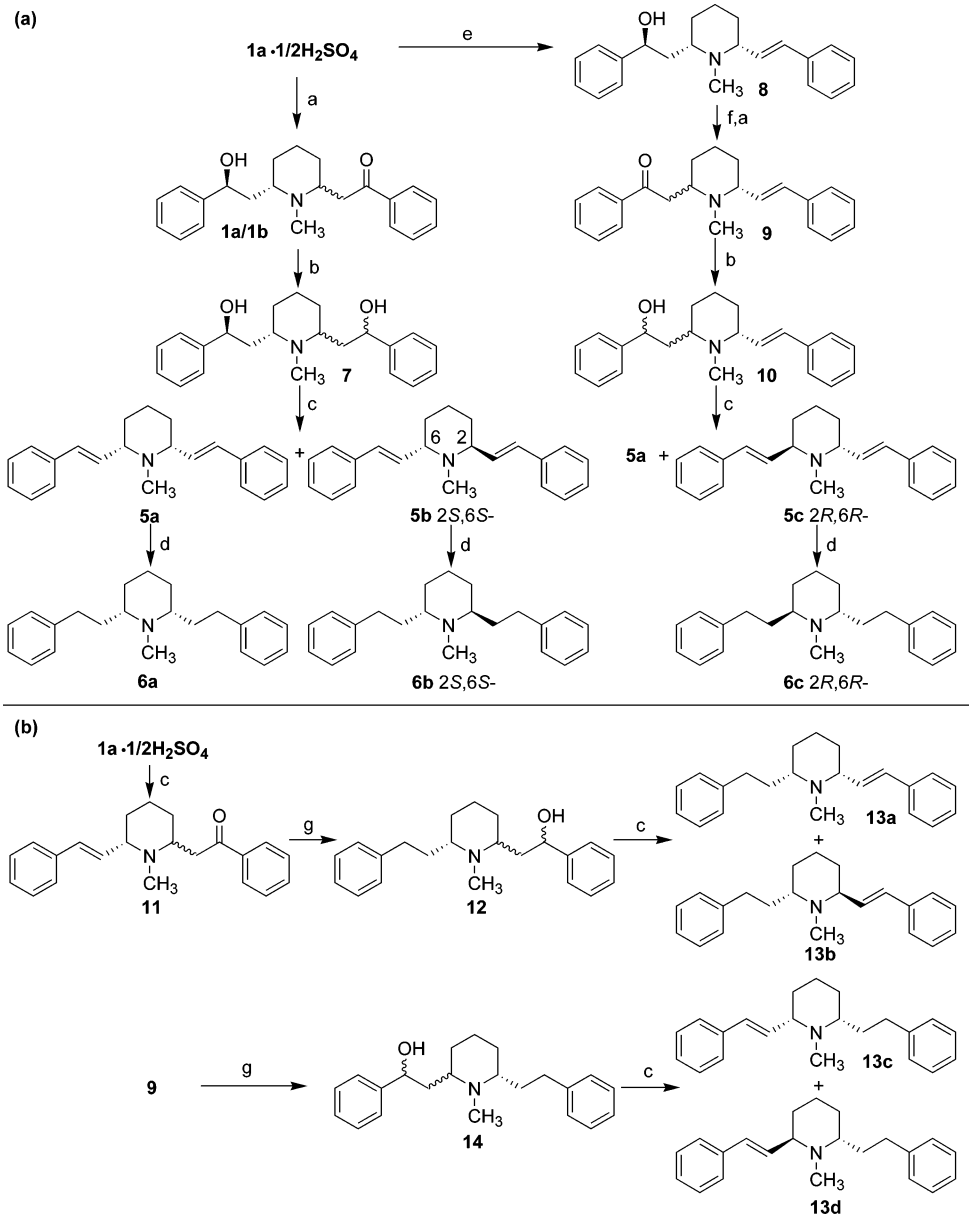
Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH; (b) $NaBH_4$, EtOH, rt; (c) 85% H_3PO_4, 60 °C; (d) H_2, 10% Pd/C, MeOH, 45 psi; (e) Zn/Hg, HCl (5%), reflux; (f) CrO_3, H_2SO_4, acetone, 0 °C; (g) H_2, 10% Pd/C, 10% HOAc/MeOH, 45 psi.

100-fold lower affinity.[15] Importantly, lobeline has been shown to inhibit both the neurochemical and behavioral effects of amphetamine in rodents.[17−19] Specifically, methamphetamine-evoked DA release from rat striatal slices is inhibited by lobeline.[17] Furthermore, lobeline attenuates *d*-amphetamine-, methamphetamine-, and nicotine-induced hyperactivity in rodents.[17,20] Importantly, lobeline is not self-administered but decreases methamphetamine self-administration in rats.[18,19] The lobeline-induced decrease in methamphetamine self-administration was not surmounted by increasing unit doses of methamphetamine.[18,19] Taken together, these results suggest that lobeline lacks abuse liability, while decreasing the stimulant and rewarding effects of methamphetamine via a noncompetitive interaction with VMAT2. Consistent with the observation that lobeline is not self-administered,[19] lobeline does not evoke DA release but stimulates dihydroxyphenylacetic acid overflow,[15] which likely results from alterations in presynaptic DA storage via an interaction with VMAT2.[14] Furthermore, the observation that lobeline inhibits *d*-amphetamine- and methamphetamine-evoked DA release from superfused rat striatal slices[17] is consistent with its inhibition of methamphetamine self-administration.[18] Thus, VMAT2 appears to be a novel target for development of therapeutic candidates to treat methamphetamine abuse.

In addition to its activity at VMAT2, lobeline acts as an antagonist at nicotinic acetylcholine receptors (nAChRs).[21] Lobeline inhibits nicotine-evoked [^{3}H]DA overflow from rat striatal slices with an IC_{50} of 1 μM, suggesting that lobeline acts as an antagonist at $\alpha6\beta2\beta3$* nAChRs that mediate DA release.[21] Lobeline also inhibits nicotine-evoked $^{86}Rb^+$ efflux from rat thalamic synaptosomes with an IC_{50} of 0.7 μM and inhibits [^{3}H]nicotine ([^{3}H]NIC) binding to rat striatal membranes with a K_i of 4.7 nM, indicating that lobeline is also an antagonist at $\alpha4\beta2$* nAChRs.[21] Moreover, lo-

beline also inhibits [^{3}H]methyllycaconitine ([^{3}H]MLA) binding to rat brain membranes with a K_i of 6.26 μM, indicating an interaction with the α7* nAChR subtype.[22] Lobeline has also been reported to be an antagonist at human α7* nAChRs expressed in *Xenopus* oocytes with an IC_{50} of 8.5 μM.[23]

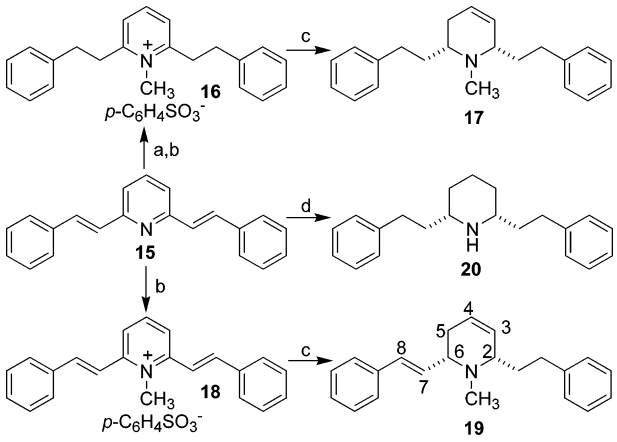
Due to the multiple pharmacological actions of lobeline, it is important to examine structural analogues of lobeline to begin to define the receptor pharmacophores and develop compounds with enhanced selectivity. Our preliminary studies[22,24] show that structural changes to the lobeline molecule, such as removal of one or both of the oxygen functionalities, result in a decrease in α4β2* nAChR affinity, which is consistent with the findings of others.[25] Thus, it is possible that analogues of lobeline can be developed with selectivity for VMAT2. Drug discovery selectively targeting VMAT2 may provide a unique opportunity to further probe the underlying neurochemical mechanisms responsible for psychostimulant abuse and may provide a novel approach for its treatment. To date, there are very few VMAT2 ligands reported in the literature; these include low affinity ligands, such as 3-amino-2-phenylpropene derivatives (**2**),[26] and high affinity tetrabenazine analogues, such as dihydrotetrabenazine (**3**) and Ro4-1284 (**4**)[27,28] (Figure 1). Thus, lobeline analogues with selectivity for VMAT2 provide a novel structural class of VMAT2 ligand as potential leads for therapeutic development.

Selective targeting of VMAT2 by systematic structural modification of lobeline provided two non-oxygen containing lobeline analogues: *N*-methyl-2,6-bis(*cis*-phenylethenyl)piperidine (*meso*-transdiene, MTD, **5a**) and *N*-methyl-2,6-bis(*cis*-phenylethyl)piperidine (lobelane, **6a**) (Scheme 1). The latter two analogues showed good affinity for VMAT2 and DAT, with negligible affinity for α4β2* and α7* nAChRs.[22] Of note, both **5a** and **6a** are optically inactive meso-compounds. These interesting results prompted us to carry out a more detailed investigation of the structure−activity of the various isomeric forms of **5a** and **6a**, as well as structurally related defunctionalized analogues, to identify selective ligands for VMAT2 that have potential as therapeutic interventions in psychostimulant abuse.

Chemistry

The synthetic routes to the defunctionalized analogues **5a**−**5c**, **6a**−**6c**, and **13a**−**13d** all emanate from lobeline (**1a**) and are illustrated in Scheme 1. C-2 epimerization of lobeline (**1a**) in K_2CO_3/methanol for 48 h at room temperature afforded an equal mixture of the two C-2 epimers, **1a** and **1b**,[29,30] which were transformed into compound **7** via reduction with $NaBH_4$ in absolute ethanol. Utilizing previously described dehydration methodologies,[25,31] compound **7** was treated with 85% phosphoric acid to yield a mixture of the *cis*- and *trans*-distyryl compounds **5a**[31] (MTD) and **5b**[31] ((−)-TTD), which were obtained in their pure isomeric forms via silica gel column chromatographic separation. Catalytic hydrogenation of **5a** afforded lobelane (**6a**),[25,32] and similar treatment of **5b** afforded (−)-2*S*,6*S* *trans*-lobelane [(−)-*trans*-lobelane, **6b**]. The unsaturated lobeline analogue, **8**, which was prepared by Clemmensen reduction of lobeline (**1a**),[25] was converted into a mixture

Scheme 2[a]



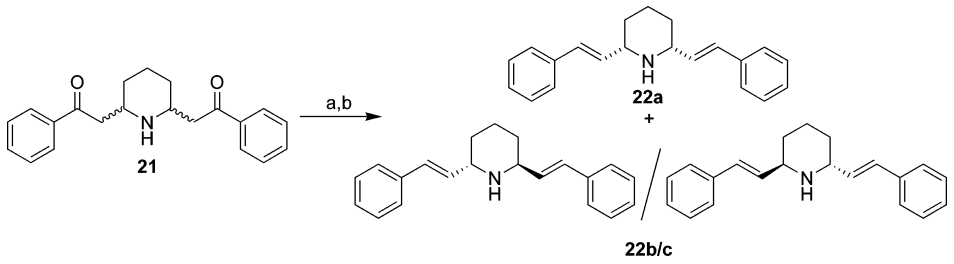
[a] Reagents and conditions: (a) H_2, 10% Pd/C, MeOH, 45 psi; (b) p-$C_6H_4SO_3CH_3$, 170−180 °C; (c) $NaBH_4$, EtOH, 0 °C; (d) H_2, PtO_2, HOAc, 45 psi.

of the C-6 epimers of **9** through Jones oxidation, followed by treatment with K_2CO_3/methanol. The mixture of the epimers of **9** was reduced with $NaBH_4$/EtOH to afford **10**, which was a mixture of the four possible diastereomers. Treatment of this isomeric mixture with 85% H_3PO_4 afforded epimers **5a** (MTD) and **5c** [(+)-TTD], which were separated by silca gel chromatography. Subsequently, catalytic hydrogenation of **5c** gave (+)-2*R*,6*R* *trans*-lobelane [(+)-*trans*-lobelane, **6c**] (Scheme 1a).

Dehydration of **1a** with 85% H_3PO_4, followed by epimerization at C-2, afforded **11** as a mixture of C-2 epimers. Concomitant reduction of the olefinic bond and the carbonyl group in **11** was achieved by catalytic hydrogenation to afford **12**, which was a mixture of all four possible diastereomers. Dehydration of **12** with H_3PO_4 afforded **13a** and **13b**, which were obtained in isomerically pure form by silica gel chromatography. Compound **13c** and **13d** were obtained from **9** by utilizing the same procedure for the synthesis of **13a** and **13b** from **11** (vide supra) (Scheme 1b).

The synthesis of compounds **17** and **19** is illustrated in Scheme 2. Compound **15** was prepared by condensation of 2,6-lutidine with benzaldehyde.[32] Hydrogenation and then methylation of **15** afforded the quaternary ammonium compound **16**,[32] which upon reduction with $NaBH_4$/EtOH afforded compound **17**. A similar procedure was applied to the synthesis of compound **19** from **15**. Both **17** and **19** can be transformed into lobelane (**6a**) under Pd−C catalytic hydrogenation conditions, which indicates that the C2, C6 stereochemistry in **17** and **19** is cis. The COSY NMR spectrum of **19** showed correlations of H-6 (δ 3.00) with H-5 (δ 2.22) and H-7 (δ 6.21) and correlations of H-5′ (δ 2.04) with H-4 (δ 5.82) and H-5 (δ 2.22). Thus, as a result of these data, the structure of **19** was assigned as shown in Scheme 2. Norlobelane (**20**) (Scheme 2) was prepared by catalytic hydrogenation of compound **15** by utilizing PtO_2 as the catalyst.[32] The preparation of compound **22a**, the *N*-demethylated analogue of MTD (**5a**), is illustrated in Scheme 3. A mixture of *cis*- and *trans*-norlobelanine (**21**) was prepared.[31] Utilizing a similar procedure for the preparation of **5a** and **5b** from the mixture of lobeline isomers **1a** and **1b**, the meso-compound **22a** was prepared from **21** and obtained in an isomerically pure

Scheme 3[a]



[a] Reagents and conditions: (a) $NaBH_4$, EtOH, rt; (b) 85% H_3PO_4, 60 °C.

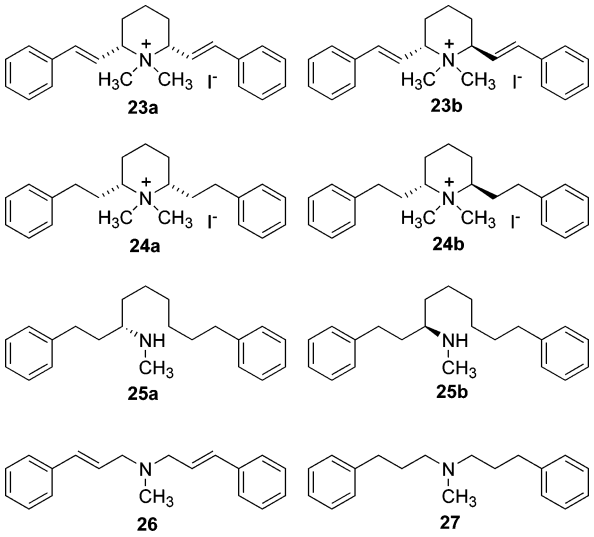
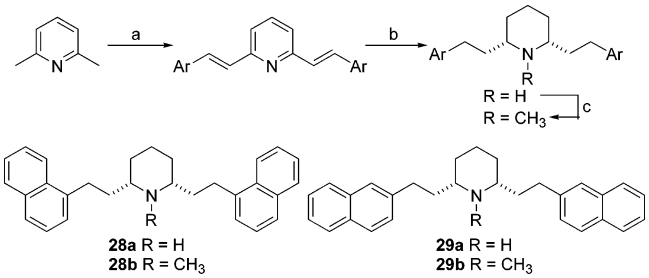


Figure 2.

Scheme 4[a]



[a] Reagents and conditions: (a) 1- or 2-naphthaldehyde, Ac_2O, reflux; (b) H_2, PtO_2, 10% HOAc/MeOH, 45 psi; (c) $NaCNBH_3$, $(CH_2O)_n$, MeOH, rt.

form; the racemic compound **22b/c** was also isolated from this reaction.

The quaternary ammonium compounds **23a**, **23b**, **24a**, and **24b** (Figure 2) were prepared by treatment of **5a**, **5b**, **6a**, and **6b**, respectively, with excess iodomethane in acetone. Ring-opened compounds **25a** and **25b** (Figure 2) were obtained during the hydrogenation of **5b** to **6b** and of **5c** to **6c**, respectively, as byproducts. Acyclic compound **26**[33] and **27**[34] (Figure 2) were prepared as previously reported. The nor-1-naphthyl and nor-2-naphthyl compounds **28a** and **29a** were prepared according to the procedure utilized to prepare norlobelane (**20**),[32] except that 1- or 2-naphthaldehyde was used instead of benzaldehyde (Scheme 4). *N*-Methylation was then carried out using $NaCNBH_3/(CH_2O)_n$ to afford the corresponding *N*-methyl compounds, **28b** (1-NAP-lobelane) and **29b** (2-NAP-lobelane).

Results and Discussion

Table 1 shows that lobeline (**1a**) potently inhibited [^{3}H]NIC binding to rat striatal membranes with a K_i value of 4 nM, had low affinity (K_i = 6.26 μM) for α7* nAChRs, and, importantly, inhibited [^{3}H]DTBZ binding at VMAT2 (K_i = 2.76 μM). Partially defunctionalized lobeline analogues, retaining a single oxygen functionality in the molecule, have low affinity at α4β2* [22,25] but are equipotent or exhibit higher affinity at α7*.[22] More importantly, these compounds retained affinity at VMAT2, comparable with lobeline.[22] Further structural modification of the lobeline molecule revealed that the fully deoxygenated, unsaturated meso-compound, MTD (**5a**), had no affinity at either α4β2* or α7* nAChRs but had comparable but slightly lower affinity (K_i = 9.88 μM) compared with lobeline at VMAT2. Thus, MTD was more selective, but slightly less potent at VMAT2 than lobeline. Reduction of the trans double bonds in MTD afforded lobelane (**6a**, K_i value = 0.97 μM at VMAT2), which was more selective and 3-fold more potent than lobeline at VMAT2.

The trans analogues of MTD, **5b** and **5c**, and the trans analogues of lobelane, **6b** and **6c**, were synthesized to assess the importance of the C-2 and C-6 stereochemistry of the piperidine ring on VMAT2 affinity and selectivity. Enantiomers **5b** and **5c** had no affinity at either α4β2* or α7* nAChRs. Interestingly, **5c** was equipotent with its meso-isomer, MTD, but **5b** was slightly less potent (2−3-fold) than MTD. Surprisingly, only a small difference in affinity between these two trans enantiomers was observed at VMAT2. These results indicate a surprising lack of stereochemical sensitivity at the ligand recognition site at VMAT2, in that a change in the stereochemistry of the piperidino ring at the C2 and C6 positions from cis to trans within the MTD series (i.e., from **5a** to **5b** and **5c**) does not affect interaction with VMAT2. More surprisingly, the observation that there was little difference in affinity for VMAT2 for the trans enantiomers **5b** and **5c** suggests that the specific configuration at C2 and C6 in these compounds is not recognized by the VMAT2 binding site. Within the lobelane series of compounds (i.e., **6a**, **6b**, and **6c**), a change of C2, C6 stereochemistry from cis to trans afforded a modest reduction (5−6-fold) in affinity at VMAT2. Again, the trans enantiomers **6b** and **6c** exhibited comparable affinities at VMAT2. Taken together, these data indicate that the VMAT2 binding site does not recognize major stereochemical changes to the MTD and lobelane molecules at the C2 and C6 piperidino ring carbons.

Reduction of only one double bond in MTD affords enantiomers **13a** (*cis*-lob-7*E*-ene) and **13c** (*cis*-lob-9*E*-

Table 1. K_i Values for Lobeline Analogue Inhibition of [^{3}H]NIC ($\alpha4\beta2$* nAChR) and [^{3}H]MLA ($\alpha7$* nAChR) Binding to Rat Brain Membranes and Inhibition of [^{3}H]DTBZ Binding (VMAT2) to Rat Vesicle Membranes

	K_i, μM, (SEM[a]			K_i ratio	
compd	[^{3}H]NIC binding ($\alpha4\alpha2$*)	[^{3}H]MLA binding ($\alpha7$*)	[^{3}H]DTBZ binding (VMAT2)	$\alpha4\alpha2$*/ VMAT2	$\alpha7$*/ VMAT2
1a	0.004 ± 0.000	6.26 ± 1.30	2.76 ± 0.64	0.0014/1	2.3/1
5a	11.6 ± 2.01	>100	9.88 ± 2.22	1.2/1	>10.1/1
5b	>100	>100	19.4 ± 1.25	>5.2/1	>5.2/1
5c	>100	>100	7.09 ± 2.42	>14.1/1	>14.1/1
6a	14.9 ± 1.67	26.0 ± 6.57	0.97 ± 0.19	15.4/1	26.8/1
6b	>100	25.3 ± 4.27	5.32 ± 0.45	>18.8/1	4.8/1
6c	>100	40.0 ± 14.1	6.46 ± 1.70	>15.5/1	6.2/1
13a	>100	>100	2.50 ± 0.23	>40.0/1	>40.0/1
13b	>100	55.1 ± 13.3	5.27 ± 1.32	>19.0/1	10.5/1
13c	>100	>100	2.67 ± 0.56	>37.4/1	>37.4/1
13d	>100	25.2 ± 3.00	> 100	–	<0.2/1
17	>100	>100	3.02 ± 0.41	>33.1/1	>33.1/1
19	>100	>100	3.84 ± 1.20	>26.0/1	>26.0/1
20	>100	>100	2.31 ± 0.21	>43.3/1	>43.3/1
22a	>100	>100	2.08 ± 0.08	>48.1/1	>48.1/1
22b/c	>100	>100	5.19 ± 2.45	>19.3/1	>19.3/1
23a	>100	33.8 ± 9.79	>100	–	<0.3/1
23b	>100	15.1 ± 4.31	9.24 ± 2.14	>10.8/1	1.6/1
24a	24.3 ± 7.20	9.63 ± 0.81	16.5 ± 9.26	1.5/1	0.6/1
24b	16.2 ± 2.05	2.39 ± 0.16	24.6 ± 14.0	0.7/1	0.1/1
25a	>100	>100	5.21 ± 1.23	>19.2/1	>19.2/1
25b	>100	>100	3.96 ± 0.80	>25.2/1	>25.2/1
26	>100	>100	2.37 ± 0.55	>42.2/1	>42.2/1
27	>100	>100	3.07 ± 1.72	>32.3/1	>32.3/1
28a	>100	>100	4.68 ± 0.70	>21.4/1	>21.4/1
28b	>100	>100	0.63 ± 0.16	>158.7/1	>158.7/1
29a	>100	>100	>100	--	--
29b	>100	>100	2.03 ± 0.45	>49.3/1	>49.3/1

[a] Each K_i value represents data from four independent experiments, each performed in duplicate.

ene), both of which had no affinity at either $\alpha4\beta2$* or $\alpha7$* nAChRs and, importantly, had significantly higher (4-fold) affinity than MTD at VMAT2, indicating that reduction of just one of the double bonds in MTD results in an increase in affinity and selectivity for VMAT2. Again, it is surprising that there are no differences in affinity between these enantiomers at VMAT2. Similarly, the enantiomers **13b** (*trans*-lob-7*E*-ene) and **13d** (*trans*-lob-9*E*-ene) showed no affinity at either $\alpha4\beta2$* or $\alpha7$* nAChRs but interestingly did show very different affinities for VMAT2. Specifically, compound **13b** had similar affinity to MTD at VMAT2, whereas **13d** exhibited no affinity for VMAT2. These results suggest a somewhat complex structure–activity relationship within these structurally related analogues, in that the VMAT2 recognition site appears to accommodate three *trans*-lobelene isomers, with different stereochemistry at C2 and C6, but does not recognized one specific stereochemical form **13d**, which has the 2*R*,6*R* stereochemical configuration.

Increasing conformational rigidity in the piperidine ring by introducing a C-3, C-4 double-bond afforded two racemic cis-analogues, **17** and **19**. Compound **19** is a mixture of enantiomers that are structural analogues of the chirally pure compounds **13a** and **13c**. The racemic compound **17** is structurally related to the meso-compound lobelane (**6a**). As expected, these defunctionalized analogues [(±)-**17** and (±)-**19**] exhibited no affinity at $\alpha4\beta2$* and $\alpha7$* nAChRs, were equipotent with compounds **13a** and **13c** at VMAT2, and exhibited 4-fold lower affinity than lobelane (**6a**) at VMAT2. Although **17** and **19** are racemic, making the structure–activity relationships more difficult to elucidate, it would appear that introduction of unsaturation into the piperidine ring does not markedly affect affinity at VMAT2, since only a modest decrease in affinity is observed in these compounds when compared with the corresponding saturated piperidino ring analogues.

Two conformationally flexible, ring-opened compounds, **25a** and **25b**, were evaluated. Compound **25a** and **25b** exhibited a 4–5-fold lower affinity compared to lobelane (**6a**) at VMAT2. Additionally, the two acyclic compounds, **26** and **27** (K_i = 2.37 and 3.07 μM, respectively), exhibited lower affinity for VMAT2 compared to lobelane but were comparable to **25a** and **25b**. Thus, ring-opening or complete removal of the piperidine ring results in only a modest reduction in affinity at VMAT2 compared to lobelane (**6a**).

The effect of removing the piperidine *N*-methyl substituent of lobelane and its analogues was also investigated. Accordingly, compounds **20**, **22a**, and **22b/c**, which are the corresponding nor-compounds of lobelane (**6a**), **5a**, and **5b/5c**, respectively, were prepared and evaluated. None of these nor-compounds exhibited affinity at either $\alpha4\beta2$* or $\alpha7$* nAChRs. With respect to VMAT2, removal of the *N*-methyl group did not have a dramatic effect when compared to the corresponding parent *N*-methyl compound. These results suggest that the presence of the *N*-methyl group is not critical for interaction with VMAT2.

1-Methyl-4-phenylpyridinium (MPP^+), a quaternary ammonium compound, is a well-known ligand for VMAT2.[35–38] Thus, incorporating a quaternary ammonium group into the above lobeline analogues was considered a structural modification worthy of investigation with regard to enhancing VMAT2 affinity. Four

quaternary ammonium analogues of MTD and lobelane, i.e., **23a**, **23b**, **24a**, and **24b**, were prepared and evaluated. All of these analogues had negligible affinity for α4β2* nAChRs, although **24a** and **24b** exhibited low affinity ($K_i = 2.4-9.6\ \mu M$) at α7* nAChRs. Interestingly, compound **23a** exhibited no affinity for VMAT2, indicating that *N*-quaternization of MTD eliminates the interaction with this transporter. Similarly, compound **24a**, the *N*-quaternized form of lobelane, exhibited a near 20-fold decrease in affinity for VMAT2, compared with lobelane (**6a**). In the *trans*-MTD and *trans*-lobelane series, *N*-quaternization to afford **23b** and **24b** afforded only modest changes in affinity for VMAT2, when compared to the corresponding nonquaternized parent compounds **5b** and **6b**, respectively. In summary, incorporating a quaternary ammonium group into defunctionalized lobeline molecules in the cis-series resulted in significant loss of affinity for VMAT2, whereas only a modest effect was obtained in the trans-series.

Four analogues of lobelane (compounds **28a**, **28b**, **29a**, and **29b**), in which the phenyl moieties were replaced with 1-naphthyl or 2-naphthyl moieties to determine the relative contribution of $\pi\angle\pi$ or hydrophobic interactions at the VMAT2 binding site, were prepared. The 1-naphthyl (**28a**, 1-NAP-norlobelane, $K_i = 4.68\ \mu M$) and 2-naphthyl (**29a**, 2-NAP-norlobelane, $K_i > 100\ \mu M$) norcompounds were both less potent than lobelane; however, a dramatic difference in VMAT2 affinity was observed between these meso-analogues. The corresponding *N*-methyl analogues (**28b**, 1-NAP-lobelane, $K_i = 0.63\ \mu M$; **29b**, 2-NAP-lobelane, $K_i = 2.03\ \mu M$) exhibited a more modest difference in VMAT2 affinity. More importantly, 1-NAP-lobelane (**28b**), which incorporates aromatic moieties with a greater π-surface area, was found to be the most potent and selective lobelane analogue evaluated in this series, suggesting that structural modification of the aromatic moieties of lobelane, and specifically those that increase $\pi\angle\pi$ interactions and/or hydrophobic interactions at the recognition site of VMAT2, are worth pursuing further.

Summary

In summary, based upon structural modification of the lobeline molecule, a novel class of VMAT2-selective ligands has been identified. Initial structure−activity relationship studies reveal that the most promising structural changes that enhance VMAT2 affinity and selectivity are defunctionalization, affording lobelane and MTD, and replacement of the phenyl rings with other aromatic moieties that have a π-extended structure, i.e., a 1-naphthyl moiety.

Experimental Section

Chemistry. All purchased reagents were used without further purification. Flash column chromatography was carried out using ICN SilicTech 32−63, 60 Å silica gel. TLC analysis was carried out on EMD Chemicals Inc. glass plates precoated with 250 μm silica gel 60 F_{254}. Melting points were determined on a Fisher Scientific melting point apparatus and are uncorrected. NMR spectra were recorded in $CDCl_3$ on a Varian 300 MHz instrument and are reported in ppm relative to TMS as internal standard. Mass spectra were recorded on a JEOL JMS-700T MStation or on a Bruker Autoflex MALDI-TOF MS. GC−mass spectra were recorded on an Agilent 6890 GC incorporating an Agilent 7683 autosampler and an Agilent 5973 MSD. Optical rotation data were obtained on a Perkin-Elmer 241 polarimeter. All of the final amine compounds were converted to their hydrochloride salts with 2 N HCl in Et_2O. Elemental analyses were carried out on a COSTECH elemental combustion system and are within ±0.4% of theory. Compounds **20**,[32] **26**,[33] and **27**[34] were prepared according to previously reported methods.

***N*-Methyl-2,6-*cis*-di-(*E*)-styrylpiperidine (5a) and *N*-Methyl-2*S*,6*S*-*trans*-di-(*E*)-styryl piperidine (5b).** (−)-Lobeline semisulfate salt (1.85 g) was dissolved in methanol (100 mL) and treated with excess K_2CO_3 for 48 h at room temperature, concentrated, brought into water, extracted with $CHCl_3$, dried over Na_2SO_4, filtered, and concentrated to afford a mixture of 2*R*,6*S*- and 2*S*,6*S*-lobeline free base in nearly equal ratio (determined by NMR) as a white solid (1.35 g, 4.00 mmol). This product was suspended in absolute ethanol (40 mL), and $NaBH_4$ (300 mg, 8.00 mmol) was added at room temperature. The mixture was stirred for 1 h and then quenched with acetone. The solvents were evaporated under reduced pressure, and the residue was suspended in water (60 mL) and extracted with $CHCl_3$ (50 mL × 3). The combined organic extract was dried (Na_2SO_4), filtered, and concentrated to give a mixture of lobelanidine and its isomers (**7**) as a white solid, which was used directly in the next reaction. The crude product **7** was dissolved in 85% H_3PO_4 (40 mL) and the solution allowed to stir at 60 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with water (150 mL), and basified with solid K_2CO_3 and then NaOH (pH ∼10). The aqueous solution was then extracted with EtOAc (80 mL × 3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (10:1 to 1:1 hexanes:EtOAc gradient) to give title compounds **5a** (390 mg, 32%) and **5b** (341 mg, 28%), each as white solids. Compound **5a**: mp 149−150 °C (lit.[31] mp 149−150 °C); ^{1}H NMR δ 1.42−1.88 (m, 6H), 2.25 (s, 3H), 2.63 (m, 2H), 6.21 (dd, J = 15.9, 9.0 Hz, 2H), 6.51 (d, J = 15.9 Hz, 2H), 7.19−7.41 (m, 10H) ppm; ^{13}C NMR δ 24.1, 33.9, 42.6, 68.6, 126.3, 127.5, 128.7, 130.5, 134.0, 137.2 ppm; MS *m/z* 303 (M^+). Anal. ($C_{22}H_{25}N\cdot HCl\cdot 0.5H_2O$) C, H, N. Compound **5b**: $[\alpha]^{25}_D$ −180.2 (*c* 1.0, $CHCl_3$); mp 93−94 °C (lit.[31] mp 96−97 °C); ^{1}H NMR δ 1.60−1.75 (m, 4H), 1.82−1.97 (m, 2H), 2.28 (s, 3H), 3.38 (m, 2H), 6.38 (dd, J = 15.9, 8.7 Hz, 2H), 6.52 (d, J = 15.9 Hz, 2H), 7.17−7.42 (m, 10H) ppm; ^{13}C NMR δ 19.6, 32.9, 42.0, 62.3, 126.4, 127.5, 128.7, 130.5, 131.8, 137.2 ppm; MS *m/z* 303 (M^+). Anal. ($C_{22}H_{25}N\cdot HCl\cdot {}^1/_3H_2O$) C, H, N.

***N*-Methyl-2,6-*cis*-bis(2-phenethyl)piperidine (6a).** Compound **5a** (420 mg, 1.38 mmol) was dissolved in methanol (50 mL) and 10% Pd/C (40 mg) was added. The mixture was hydrogenated on a Parr hydrogenation apparatus (45 psi) for 18 h. The catalyst was removed by filtration through a Celite pad. The filter cake was rinsed with methanol, and the combined organic portions were concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography ($CHCl_3$:MeOH 30:1) to afford **6a** (223 mg, 53%) as a white solid: mp 171−172 °C; ^{1}H NMR δ 1.30−1.42 (m, 4H), 1.60−1.85 (m, 6H), 2.19 (s, 3H), 2.32−2.44 (m, 2H), 2.60−2.77 (m, 4H), 7.12−7.35 (m, 10H) ppm; ^{13}C NMR δ 25.4, 27.1, 31.0, 32.5, 36.6, 62.7, 125.7, 128.4, 128.5, 143.0 ppm; MS *m/z* 307 (M^+). Anal. ($C_{22}H_{29}N\cdot HCl\cdot 0.2H_2O$) C, H, N.

***N*-Methyl-2*S*,6*S*-*trans*-bis(2-phenethyl)piperidine (6b) and *N*-Methyl-1,9-diphenyl-3*S*-nonanamine (25a).** Compound **6b** was prepared by utilizing a similar procedure to that described for **6a,** except that the starting material was **5b** (3.0 g, 9.90 mmol), to give 1.32 g (43%) of product as a colorless oil, along with the ring opened product **25a** (1.35 g, 44%) as a colorless oil. Compound **6b**: $[\alpha]^{25}_D$ −35.4 (*c* 0.5, $CHCl_3$); mp 152−153 °C (HCl salt); ^{1}H NMR δ 1.37−1.52 (m, 2H), 1.55−1.75 (m, 6H), 1.81−1.95 (m, 2H), 2.36 (s, 3H), 2.55−2.67 (m, 4H), 2.67−2.78 (m, 2H), 7.14−7.35 (m, 10H) ppm; ^{13}C NMR δ 20.1, 26.6, 32.4, 33.1, 37.9, 57.6, 125.8, 128.4, 128.5, 142.9 ppm; MS *m/z* 307 (M^+). Anal. ($C_{22}H_{29}N\cdot HCl\cdot 0.2H_2O$) C, H, N. Compound **25a**: $[\alpha]^{25}_D$ −4.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.24−1.45 (m, 6H), 1.53−1.71 (m, 4H), 1.94 (m, 2H), 2.52 (s, 3H),

2.59 (t, J = 7.5 Hz, 2H), 2.66−2.84 (m, 3H), 6.51 (brs, 1H), 7.09−7.37 (m, 10H) ppm; ^{13}C NMR δ 25.5, 29.3, 29.7, 31.3, 31.5, 31.6, 31.8, 33.2, 36.2, 59.1, 125.7, 126.2, 128.3, 128.5 (2C), 128.6, 141.1, 142.8 ppm; MS *m/z* 309 (M^+). Anal. ($C_{22}H_{31}N$·HCl) C, H, N.

***N*-Methyl-2*R*,6*R*-*trans*-di-(*E*)-styrylpiperidine (5c).** Jones reagent (prepared by dissolving 26.72 g of CrO_3 in 23 mL of concentrated H_2SO_4, followed by dilution with water to 100 mL) was added dropwise to a solution of compound **8**[25] (1.22 g, 3.80 mmol) in acetone (60 mL) at 0 °C until an orange-colored reaction mixture was formed. The mixture was stirred for another 30 min, and then methanol was added to quench the reaction. The mixture was then filtered and washed with acetone. The filter cake was dissolved in water (50 mL) and extracted with $CHCl_3$ (25 mL × 3). The filtrate was concentrated under vacuum and diluted with water (30 mL). The aqueous solution was extracted with $CHCl_3$ (25 mL × 3). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated to afford a yellow solid. Following a similar procedure previously described for the preparation of compound **5a** and **5b** from LOB sulfate, compound **5c** was prepared to give 3.45 g (23%) as a white solid, along with compound **5a** (3.22 g, 22%): $[\alpha]^{25}_D$ 182.8 (*c* 1.0, $CHCl_3$); mp 92−93 °C; ^{1}H NMR δ 1.62−1.75 (m, 4H), 1.80−1.96 (m, 2H), 2.28 (s, 3H), 3.38 (m, 2H), 6.38 (dd, J = 15.9, 8.7 Hz, 2H), 6.52 (d, J = 15.9 Hz, 2H), 7.20−7.42 (m, 10H) ppm; ^{13}C NMR δ 19.6, 32.9, 41.9, 62.3, 126.4, 127.5, 128.7, 130.5, 131.7, 137.2 ppm; MS *m/z* 303 (M^+). Anal. ($C_{22}H_{25}N$·HCl·H_2O) C, H, N.

***N*-Methyl-2*R*,6*R*-*trans*-bis(2-phenethyl)piperidine (6c) and *N*-Methyl-1,9-diphenyl-3*R*-nonanamine (25b).** Compound **6c** was prepared utilizing a similar procedure to that described for **6a,** except the starting material was **5c** (2.20 g, 7.26 mmol) to give 1.06 g (48%) of product as a colorless oil, along with the ring-opened product **25b** (990 mg, 44%) as a colorless oil. Compound **6c**: $[\alpha]^{25}_D$ 36.0 (*c* 1.0, $CHCl_3$); mp 151−152 °C (HCl salt); ^{1}H NMR δ 1.39−1.55 (m, 2H), 1.50−1.75 (m, 6H), 1.81−1.95 (m, 2H), 2.39 (s, 3H), 2.58−2.70 (m, 4H), 2.63−2.78 (m, 2H), 7.15−7.38 (m, 10H) ppm; ^{13}C NMR δ 20.0, 26.6, 32.3, 33.0, 38.0, 57.8, 125.6, 128.5, 128.6, 142.8 ppm; MS *m/z* 307 (M^+). Anal. ($C_{22}H_{29}N$·HCl·0.2H_2O) C, H, N. Compound **25b**: $[\alpha]^{25}_D$ 4.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.24−1.50 (m, 8H), 1.55−1.77 (m, 4H), 2.39 (s, 3H), 2.47 (m, 1H), 2.56−2.68 (m, 4H), 7.10−7.34 (m, 10H) ppm; ^{13}C NMR δ 25.8, 29.5, 30.1, 31.7, 32.3, 33.5, 33.6, 35.4, 36.2, 58.9, 125.7, 125.8, 128.3, 128.4 (2C), 128.5, 142.7, 142.9 ppm; MS *m/z* 309 (M^+). Anal. ($C_{22}H_{31}N$·HCl·0.5H_2O) C, H, N.

***N*-Methyl-2*R*-(*E*)-styryl-6*S*-(2-phenethyl)piperidine (13a) and *N*-Methyl-2*S*-(*E*)-styryl-6*S*-(2-phenethyl)piperidine (13b).** Compound **11** (1.20 g, 3.76 mmol), which was prepared by dehydration of **1a** and then epimerization in methanol, was dissolved in 10% HOAc/CH_3OH (50 mL), and 10% Pd/C (120 mg) was added. The mixture was hydrogenated on a Parr hydrogenation apparatus (45 psi) for 24 h. The catalyst was removed by filtration through a Celite pad. The filter cake was rinsed with methanol, and the combined organic portion was concentrated under reduced pressure. The crude product **12** was dissolved in 85% H_3PO_4 (30 mL) and allowed to stir at 60 °C for 24 h. Work up was as describe for **5a** and **5b** above. The dehydration product was purified by column chromatography (4:1 to 1:1 hexanes:EtOAc gradient) to give the title compound **13a** (188 mg, 16%), as a white solid, and **13b** (167 mg, 15%), as a colorless oil. Compound **13a**: $[\alpha]^{25}_D$ 53.8 (*c* 1.0, $CHCl_3$); mp 75−76 °C; ^{1}H NMR δ 1.30−1.89 (m, 7H), 1.92−2.09 (m, 2H), 2.27 (s, 3H), 2.51−2.69 (m, 2H), 2.70−2.83 (m, 1H), 6.21 (dd, J = 15.9, 8.4 Hz, 1H), 6.47 (d, J = 15.9 Hz, 1H), 7.15−7.40 (m, 10H) ppm; ^{13}C NMR δ 24.5, 31.3, 31.9, 33.7, 36.2, 40.3, 63.9, 68.9, 125.8, 126.3, 127.4, 128.4, 128.5, 128.7, 130.2, 134.9, 137.3, 142.9 ppm; MS *m/z* 305 (M^+). Anal. ($C_{22}H_{27}N$·HCl·$^1/_3H_2O$) C, H, N. Compound **13b**: $[\alpha]^{25}_D$ −103.6 (*c* 1.0, $CHCl_3$); mp 106−107 °C (HCl salt); ^{1}H NMR δ 1.50−1.82 (m, 7H), 1.84−1.98 (m, 1H), 2.32 (s, 3H), 2.51 (ddd, J = 13.5, 10.8, 5.7 Hz, 1H), 2.68 (ddd, J = 13.5, 10.8, 5.4 Hz, 1H), 2.83 (m, 1H), 3.23 (m, 1H), 6.28 (dd, J = 15.9, 8.4 Hz, 1H), 6.47 (d, J = 15.9 Hz, 1H), 7.13−7.39 (m, 10H) ppm; ^{13}C NMR δ 19.4, 28.9, 29.1, 32.2, 33.2, 40.7, 58.6, 61.8, 125.9, 126.4, 127.4, 128.4, 128.5, 128.7, 131.0, 132.0, 137.3, 142.7 ppm; MS *m/z* 305 (M^+). Anal. ($C_{22}H_{25}N$·HCl) C, H, N.

***N*-Methyl-2*R*-(2-phenylethyl)-6*S*-(*E*)-styrylpiperidine (13c) and *N*-Methyl-2*R*-(2-phenylethyl)-6*R*-(*E*)-styrylpiperidine (13d).** Compounds **13c** and **13d** were prepared by utilizing a similar procedure to that described above for compounds **13a** and **13b**, except the starting material was compound **8** (1.20 g 3.74 mmol), to give the title compound **13c** (160 mg, 14%), as a white solid, and **13d** (113 mg, 10%), as a colorless oil. Compound **13c**: $[\alpha]^{25}_D$ −51.5 (*c* 1.0, $CHCl_3$); mp 74−75 °C; ^{1}H NMR δ 1.30−1.84 (m, 7H), 1.92−2.09 (m, 2H), 2.27 (s, 3H), 2.51−2.69 (m, 2H), 2.70−2.83 (m, 1H), 6.21 (dd, J = 15.9, 8.4 Hz, 1H), 6.47 (d, J = 15.9 Hz, 1H), 7.15−7.40 (m, 10H) ppm; ^{13}C NMR δ 24.5, 31.2, 31.9, 33.7, 36.2, 40.2, 63.9, 68.9, 125.8, 126.3, 127.4, 128.4, 128.5, 128.7, 130.2, 134.8, 137.3, 142.9 ppm; MS *m/z* 305 (M^+). Anal. ($C_{22}H_{27}N$·HCl) C, H, N. Compound **13d**: $[\alpha]^{25}_D$ 103.5 (*c* 1.0, $CHCl_3$); mp 105−106 °C (HCl salt); ^{1}H NMR δ 1.52−1.85 (m, 7H), 1.85−1.90 (m, 1H), 2.32 (s, 3H), 2.51 (ddd, J = 13.5, 10.8, 5.7 Hz, 1H), 2.68 (ddd, J = 13.5, 10.8, 5.7 Hz, 1H), 2.85 (m, 1H), 3.25 (m, 1H), 6.28 (dd, J = 15.9, 8.4 Hz, 1H), 6.47 (d, J = 15.9 Hz, 1H), 7.11−7.41 (m, 10H) ppm; ^{13}C NMR δ 19.2, 28.7, 29.0, 32.0, 33.1, 40.5, 58.6, 61.9, 125.9, 126.3, 127.5, 128.3, 128.5, 128.6, 131.3, 131.4, 137.1, 142.5 ppm; MS *m/z* 305 (M^+). Anal. ($C_{22}H_{25}N$·HCl) C, H, N.

(±)-*N*-Methyl-2,6-*cis*-diphenethyl-1,2,3,6-tetrahydropyridine (17). Compound **15** (colorless crystals recrystallized from benzene, mp 167−168 °C) was synthesized from the condensation reaction of 2,6-lutidine with benzaldehyde utilizing the reported procedure.[32] The double bonds of the 2,6-side chains of compound **15** were then reduced by Pd−C catalytic hydrogenation, and the resulting product was treated with methyl *p*-toluenesulfonate following the reported procedure,[32] to afford the *N*-methylated compound **16** (yellow crystals recrystallized from methanol, mp 238−239 °C). $NaBH_4$ (160 mg, 4.22 mmol) was added to a suspension of compound **16** (2.00 g, 4.22 mmol) in absolute ethanol (100 mL) at 0 °C. The mixture was stirred for 2 h and then quenched with acetone. Solvent was removed under reduced pressure and the residue was taken up in water (50 mL) and extracted with $CHCl_3$ (50 mL × 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (1:1 hexanes: EtOAc) to give the title compound **17** (245 mg, 19%) as a white solid: mp 162−163 °C (HCl salt); ^{1}H NMR δ 1.62−1.97 (m, 4H), 1.99 (m, 2H), 2.19 (s, 3H), 2.60−2.81 (m, 5H), 3.09 (m, 1H), 5.52 (ddd, J = 10.2, 3.6, 1.8 Hz, 1H), 5.78 (ddd, J = 10.2, 3.9, 3.0 Hz, 1H), 7.13−7.32 (m, 10H) ppm; ^{13}C NMR δ 28.4, 32.2, 32.3, 32.9, 35.6, 36.0, 58.5, 61.2, 125.6, 125.8, 128.4, 128.5, 128.6, 128.8, 142.76, 142.82 ppm; MS *m/z* 305 (M^+). Anal. ($C_{22}H_{27}N$·HCl·0.5H_2O) C, H, N.

(±)-*N*-Methyl-2,6-*cis*-2-phenethyl-6-(*E*)-styryl-1,2,5,6-tetrahydropyridine (19). Compound **19** was prepared by utilizing a procedure similar to that described above for **17**, except the starting material was compound **18**[32] (1.08 g, 2.30 mmol), to give title compound **19** (265 mg, 38%) as a white solid. mp 90−91 °C; ^{1}H NMR δ 1.85−1.96 (m, 2H), 2.04 (m, 1H), 2.22 (m, 1H), 2.29 (s, 3H), 2.59 (m, 1H), 2.79 (m, 1H), 2.85 (m, 1H), 3.00 (m, 1H), 5.64 (d, J = 10.2 Hz, 1H), 5.82 (m, 1H), 6.21 (dd, J = 15.9, 8.4 Hz, 1H), 6.51 (d, J = 15.9 Hz, 1H), 7.13−7.41 (m, 10H) ppm; ^{13}C NMR δ 31.2, 33.1, 35.6, 40.3, 62.6, 64.2, 124.6, 125.7, 126.4, 127.5, 128.4, 128.7, 129.2, 130.6, 133.7, 137.1, 142.8 ppm; MS *m/z* 303 (M^+). Anal. ($C_{22}H_{25}N$·HCl) C, H, N.

2,6-*cis*-Diphenethylpiperidine (20): mp 199−200 °C (HCl salt) (lit.[32] mp 192−194 °C); ^{1}H NMR δ 1.07 (ddd, J = 24.0, 13.2, 3.9 Hz, 2H), 1.32 (ddt, J = 26.4, 12.9, 3.9 Hz, 1H), 1.62−1.74 (m, 6H), 1.78 (dq, J = 13.2, 3.0 Hz, 1H), 2.50 (m, 2H), 2.63 (t, J = 8.1 Hz, 4H), 7.12−7.33 (m, 10H) ppm; ^{13}C NMR δ 25.0, 32.7, 32.9, 39.2, 56.9, 125.9, 128.4, 128.5, 142.3 ppm; MS *m/z* 293 (M^+). Anal. ($C_{21}H_{27}N$·HCl) C, H, N.

2,6-*cis*-Di-(*E*)-styrylpiperidine (22a) and (±)-2,6-*trans*-Di-(*E*)-styrylpiperidine (22b/c). Norlobelanine (**21**) was

prepared according to a reported method.[31] Utilizing a similar procedure to that described for the preparation of **5a** and **5b** from lobeline (**1a/1b**), compounds **22a** (18%) and **22b** (13%) were obtained in pure form from norlobelanine (**21**). Compound **22a**: mp 226−227 °C (HCl salt); ^{1}H NMR δ 1.23−1.62 (m, 3H), 1.75 (dd, J = 12.3, 3.0 Hz, 2H), 1.91 (m, 1H), 3.37 (t, J = 8.1 Hz, 2H), 6.26 (dd, J = 15.9, 7.2 Hz, 2H), 6.54 (d, J = 15.9 Hz, 2H), 7.17−7.40 (m, 10H) ppm; ^{13}C NMR δ 24.7, 32.4, 59.7, 126.4, 127.4, 128.6, 129.6, 133.4, 137.2 ppm; MS *m/z* 289 (M^+). Anal. ($C_{21}H_{23}N$·HCl) C, H, N. Compound **22b/c**: mp 194−195 °C (HCl salt); ^{1}H NMR δ 1.53−1.64 (m, 2H), 1.64−1.78 (m, 2H), 1.78−1.92 (m, 2H), 3.78 (m, 2H), 6.41 (dd, J = 15.9, 6.3 Hz, 2H), 6.52 (d, J = 15.9 Hz, 2H), 7.19−7.43 (m, 10H) ppm; ^{13}C NMR δ 20.3, 31.5, 53.8, 126.4, 127.5, 128.7, 129.9, 132.8, 137.3 ppm; MS *m/z* 289 (M^+). Anal. ($C_{21}H_{23}N$·HCl·0.25H_2O) C, H, N.

***N,N*-Dimethyl-2,6-*cis*-di-(*E*)-styrylpiperidine Iodide (23a).** Iodomethane (490 mg, 3.45 mmol) was added to a stirred solution of compound **5a** (350 mg, 1.15 mmol) in acetone (8 mL), and the stirring was continued for 24 h. The solvent was removed under reduced pressure, and the residue was washed thoroughly with Et_2O to afford the title compound **23a** as a white solid (460 mg, 90%): mp 223−224 °C; ^{1}H NMR δ 1.89−2.07 (m, 3H), 2.09−2.25 (m, 3H), 2.93 (s, 3H), 3.34 (s, 3H), 5.36 (brt, J = 9.0 Hz, 2H), 6.17 (dd, J = 15.9, 9.6 Hz, 2H), 7.25 (d, J = 15.9 Hz, 2H), 7.30−7.39 (m, 6H), 7.44−7.52 (m, 4H) ppm; ^{13}C NMR δ 21.7, 27.7, 38.6, 50.5, 73.9, 118.9, 127.4, 128.9, 129.6, 134.6, 142.0 ppm; MS *m/z* maldi 318 $(M - 127)^+$. Anal. ($C_{23}H_{28}NI$) C, H, N.

***N,N*-Dimethyl-2*S*,6*S*-*trans*-di-(*E*)-styrylpiperidine Iodide (23b).** Compound **23b** was prepared by utilizing a similar procedure to that described above for **23a,** except using the starting material **5b** (50 mg, 0.16 mmol), to give 69 mg (95%) of product as a white solid: $[\alpha]^{25}_D$ −182.8 (*c* 0.5, $CHCl_3$); mp 126−127 °C; ^{1}H NMR δ 1.65−2.30 (m, 6H), 3.28 (s, 6H), 4.71 (m, 2H), 6.44 (dd, J = 15.6, 9.9 Hz, 2H), 7.21 (d, J = 15.6 Hz, 2H), 7.26−7.40 (m, 6H), 7.51−7.59 (m, 4H) ppm; ^{13}C NMR δ 17.5, 26.8, 48.7, 71.8, 118.3, 127.6, 129.0, 129.6, 134.7, 141.7 ppm; MS (maldi) *m/z* 318 $(M - 127)^+$. Anal. ($C_{23}H_{28}NI$) C, H, N.

***N,N*-Dimethyl-2,6-*cis*-bis(2-phenethyl)piperidine Iodide (24a).** Compound **24a** was prepared by utilizing a similar procedure to that described above for **23a**, except using the starting material **6a** (116 mg, 0.38 mmol) to give 166 mg (97%), to produce a white solid: mp 241−242 °C; ^{1}H NMR δ 1.69−1.95 (m, 6H), 2.04−2.18 (m, 2H), 2.28−2.43 (m, 2H), 2.67−2.86 (m, 4H), 2.85 (s, 3H), 3.21 (s, 3H), 3.73 (m, 2H), 7.19−7.35 (m, 10H) ppm; ^{13}C NMR δ 22.0, 26.8, 32.3, 33.0, 38.4, 50.1, 74.5, 126.9, 128.6, 128.9, 139.5 ppm; MS (maldi) *m/z* 322 $(M - 127)^+$. Anal. ($C_{23}H_{32}NI$·$^1/_3H_2O$) C, H, N.

***N,N*-Dimethyl-2*S*,6*S*-*trans*-bis(2-phenethyl)piperidine Iodide (24b).** Compound **24b** was prepared by utilizing a similar procedure to that described above for **23a,** except using the starting material **6b** (58 mg, 0.19 mmol), to give 82 mg (96%) of product as a white solid: $[\alpha]^{25}_D$ −37.1 (*c* 0.3, $CHCl_3$); mp 222−223 °C; ^{1}H NMR δ 1.70−2.00 (m, 6H), 2.08−2.32 (m, 4H), 2.67−2.82 (m, 4H), 3.25 (s, 6H), 3.73 (m, 2H), 7.18−7.36 (m, 10H) ppm; ^{13}C NMR δ 16.8, 24.1, 29.4, 32.6, 49.3, 70.0, 126.9, 128.6, 128.9, 139.5 ppm; MS (maldi) *m/z* 322 $(M - 127)^+$. Anal. ($C_{23}H_{32}NI$·0.5H_2O) C, H, N.

2,6-*cis*-Bis(1-naphthalenethyl)piperidine (28a). Utilizing a similar procedure[32] to that described for compound **20**, compound **28a** was prepared from 2,6-lutidine and 1-naphthaldehyde: mp 218−219 °C (HCl salt); ^{1}H NMR δ 1.16 (ddd, J = 23.4, 13.2, 3.3 Hz, 2H), 1.39 (ddt, J = 26.4, 13.2, 3.3 Hz, 1H), 1.72−1.92 (m, 7H), 2.63 (m, 2H), 3.09 (dd, J = 9.0, 6.3 Hz, 4H), 7.29−7.42 (m, 4H), 7.42−7.53 (m, 4H), 7.70 (d, J = 7.8 Hz, 2H), 7.84 (dd, J = 7.2, 2.4 Hz, 2H), 8.04 (d, J = 7.5 Hz, 2H) ppm; ^{13}C NMR δ 25.1, 29.9, 32.9, 38.6, 57.4, 124.0, 125.6, 125.7, 125.9, 126.0, 126.7, 128.9, 131.9, 134.0, 138.5 ppm; MS (maldi) *m/z* 394 $(M + 1)^+$. Anal. ($C_{29}H_{31}N$·HCl) C, H, N.

***N*-Methyl-2,6-*cis*-bis(1-naphthalenethyl)piperidine (28b).** $NaBH_3CN$ (344 mg, 5.50 mmol) was added to a mixture of **28a** (434 mg, 1.10 mmol), paraformaldehyde (330 mg, 11.00 mmol), and methanol (10 mL). The mixture was stirred at room temperature overnight. The solvent was then evaporated under reduced pressure. The residue was dissolved in water (30 mL) and the aqueous solution was extracted with $CHCl_3$ (20 mL × 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (30:1 $CHCl_3$:MeOH) to afford 425 mg (95%) of **28b** as a white solid. mp 217−218 °C (HCl salt); ^{1}H NMR δ 1.40−1.64 (m, 4H), 1.68−1.90 (m, 4H), 2.03 (m, 2H), 2.24 (s, 3H), 2.61 (m, 2H), 3.09 (dd, J = 9.0, 6.3 Hz, 4H), 3.18 (t, J = 8.1 Hz, 4H), 7.34−7.41 (m, 4H), 7.42−7.54 (m, 4H), 7.71 (dd, J = 6.3, 2.7 Hz, 2H), 7.85 (dd, J = 7.5, 1.5 Hz, 2H), 8.11 (d, J = 8.1 Hz, 2H) ppm; ^{13}C NMR δ 25.3, 26.7, 29.6, 30.5, 35.8, 63.1, 124.0, 125.5, 125.7, 125.9, 126.1, 126.6, 128.9, 132.0, 134.0, 139.1 ppm; MS (maldi) *m/z* 408 $(M + 1)^+$. Anal. ($C_{30}H_{33}N$·HCl·0.75H_2O) C, H, N.

2,6-*cis*-Bis(2-naphthalenethyl)piperidine (29a). By utilizing a similar procedure to that described for the preparation of compound **20**,[32] compound **29a** was prepared from 2,6-lutidine and 2-naphthaldehyde: mp 222−223 °C (HCl salt); ^{1}H NMR δ 1.12 (ddd, J = 23.7, 12.9, 3.0 Hz, 2H), 1.39 (ddt, J = 25.8, 12.9, 3.6 Hz, 1H), 1.60−1.86 (m, 7H), 2.52 (m, 2H), 2.77 (t, J = 7.8 Hz, 4H), 7.30 (dd, J = 8.7, 1.2 Hz, 2H), 7.36−7.47 (m, 4H), 7.58 (brs, 2H), 7.71−7.82 (m, 6H) ppm; ^{13}C NMR δ 25.0, 32.8, 32.9, 39.0, 56.9, 125.2, 126.0, 126.4, 127.4, 127.5, 127.7, 128.0, 132.1, 133.7, 139.8 ppm; MS (maldi) *m/z* 394 $(M + 1)^+$. Anal. ($C_{29}H_{31}N$·HCl·0.5H_2O) C, H, N.

***N*-Methyl-2,6-*cis*-bis(2-naphthalenethyl)piperidine (29b).** Utilizing a similar procedure to that described for the preparation of compound **28b**, compound **29b** was prepared from **29a**: mp 230−231 °C (HCl salt); ^{1}H NMR δ 1.33−1.45 (m, 4H), 1.70−1.83 (m, 4H), 2.02 (m, 2H), 2.23 (s, 3H), 2.48 (m, 2H), 2.85 (m, 4H), 7.35 (dd, J = 6.6, 1.5 Hz, 2H), 7.38−7.46 (m, 4H), 7.65 (brs, 2H), 7.74−7.82 (m, 6H) ppm; ^{13}C NMR δ 25.2, 29.7, 30.1, 32.9, 36.5, 64.1, 125.9, 126.1, 126.5, 127.6, 127.7, 127.8, 128.2, 131.9, 133.6, 139.7 ppm. MS (maldi) *m/z* 408 $(M + 1)^+$. Anal. ($C_{30}H_{33}N$·HCl·0.75H_2O) C, H, N.

[^{3}H]NIC and [^{3}H]MLA Binding Assay. Rat striatal and whole brain (excluding cerebellum) membranes were used for the [^{3}H]NIC and [^{3}H]MLA binding assays, respectively. Membrane suspensions (150−200 μg/100 μL) were added to assay tubes containing analogue (7−9 concentrations, 1 nM−1 mM) and 3 nM [^{3}H]NIC or [^{3}H]MLA for a final assay volume of 200−250 μL. For the [^{3}H]NIC and [^{3}H]MLA binding assays, tubes were incubated for 90 and 120 min, respectively. Reactions were terminated by addition of ice-cold buffer and rapid filtration onto either Whatman GF/B glass fiber filters presoaked in 0.5% polyethylenimine using a Brandel Harvester (Biomedical Research and Development Laboratory, Inc., Gaithersburg, MD) or onto Unifilter-96 GF/B 96-well filter plates also presoaked in 0.5% polyethylenimine using a Packard Filter Mate Harvester (Packard BioScience Co., Meriden, CT). Bound radioactivity was determined via liquid scintillation spectrometry. Nonspecific binding was determined in the presence of 10 μM NIC for the [^{3}H]NIC binding assay and in the presence of 10 μM MLA or 1 mM NIC for the [^{3}H]MLA binding assays.

Preparation of Rat Brain Synaptic Vesicles. Synaptic vesicles were prepared as previously described.[15] Briefly, fresh whole brain (excluding cerebellum and brain stem) was homogenized in 20 vol of ice-cold 0.32 M sucrose using a glass homogenizer (seven strokes of a Teflon pestle, clearance = 0.003 in). Homogenates were centrifuged at 1000*g* for 12 min at 4 °C. Resulting supernatants (S1) were centrifuged at 22 000*g* for 10 min. The resulting pellets (P2), containing the synaptosomes, were resuspended in 18 mL of ice-cold Milli-Q water for 5 min with seven strokes of the Teflon pestle homogenizer. Osmolarity was restored by immediate addition of 2 mL of 25 mM HEPES and 100 mM K_2-tartrate buffer (pH 7.5). Samples were centrifuged at 20 000*g* for 20 min. $MgSO_4$ (final concentration, 1 mM) was added to the resulting supernatants (S3). Final centrifugations were performed at 100 000*g* for 45 min. Pellets (P4) were resuspended im-

mediately in ice-cold buffer (see below) providing ~15 μg of protein/100 μL.

[^{3}H]DHTBZ Binding Assay. [^{3}H]DHTBZ binding to synaptic vesicles was performed according to previously described procedures.[16] Briefly, 100 μL of vesicles suspension was incubated in assay buffer (in 25 mM HEPES, 100 mM K_2 tartrate, 5 mM $MgSO_4$, 0.1 mM EDTA, and 0.05 mM EGTA, pH 7.5, 25 °C) in the presence of 5 nM [^{3}H]DHTBZ and 1 nM−1 mM lobeline analogues (final concentrations) for 30 min at room temperature. Nonspecific binding was determined in the presence of 20 μM TBZ. Assays were performed in duplicate using the Unifilter-96 96-well GF/B filter plates (presoaked in 0.5% polyethylenimine) plates and terminated by harvesting using the FilterMate harvester. After washing five times with 350 μL of the ice-cold wash buffer (in 25 mM HEPES, 100 mM K_2 tartrate, 5 mM $MgSO_4$, and 10 mM NaCl, pH 7.5), filter plates were dried, bottoms were sealed, and each well was filled with 40 μL of Packard's MicroScint 20 cocktail. Bound [^{3}H]DHTBZ was measured using a Packard TopCount NXT scintillation counter and a Packard Windows NT-based operating system.

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Supporting Information Available: Elemental analyses data. This information is available free of charge via the Internet at http://pubs.acs.org.

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