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Synthesis and evaluation of a series of tropane analogues as novel vesicular monoamine transporter-2 ligands

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Abstract—A series of tropane derivatives has been synthesized as lobelane analogues and evaluated for their binding affinity at the vesicular monoamine transporter-2 (VMAT2), and at α4β2* and α7* nicotinic acetylcholine receptors. The trop-2-ene analogues **4a** and **4b** exhibited good affinity and high selectivity for VMAT2.

The brain vesicular monoamine transporter (VMAT2) is a vital component in the regulation of synaptic dopamine (DA) concentrations.[1] Recent studies have suggested that VMAT2 plays an important role in mediating the behavioral effects of psychostimulants.[2] The abuse liability of psychostimulants is thought to result from modulation of the dopaminergic system in brain, which is generally accepted as being responsible for the rewarding effects of these abused drugs.[3]

Amphetamine and methamphetamine, increasingly abused drugs worldwide, promote DA release from the synaptic vesicles into the cytosol of the dopaminergic presynaptic terminals through interaction with VMAT2.[4] (−)-Lobeline (the 2*R*,6*S*,10*S*-stereoisomer, **1**; Fig. 1), the major alkaloid in *Lobelia inflata*, decreases both the stimulant and rewarding effects of methamphetamine, and does not act as a substitute reinforcer.[5] The mechanism underlying the lobeline-induced inhibition of these effects of methamphetamine has been suggested to be due to a noncompetitive inhibition of VMAT2 function.[6] In addition, the observation that lobeline is not self-administered is consistent with the findings that lobeline does not evoke DA release.[5c,6,7] Furthermore, the observation that lobeline inhibits methamphetamine-evoked DA release from superfused rat striatal slices[5a] is consistent with its ability to decrease methamphetamine self-administration.[5b] These studies clearly suggest the significance of VMAT2 as a potential target for the development of agents to treat methamphetamine abuse. To date, there are very few VMAT2 ligands reported in the literature.[8] Thus, lobeline analogues with selectivity for VMAT2 would provide a novel structural class of ligands representing new tools for probing the VMAT2 pharmacophore and in developing potential leads for therapeutic development as treatments for methamphetamine abuse.

Due to the high affinity of lobeline (**1**) for several neuronal nicotinic acetylcholine receptor (nAChR) subtypes,[9] studies have been conducted in our laboratory, which have focused on structural modification of the lobeline molecule to increase affinity and selectivity for VMAT2.[9c,10] Systematic structural modification of lobeline afforded lobelane (**2**) (Fig. 1), a chemically defunctionalized, saturated lobeline analogue. Lobelane has higher affinity than lobeline and good selectivity for the [^{3}H]dihydrotetrabenazine ([^{3}H]DTBZ) binding site on VMAT2 compared with lobeline.[9c,10] An extensive SAR study on lobelane (**2**) has been carried out, mainly focusing on structural modifications of the two phenyl rings, changing the chirality of C-2 and C-6 chiral centers, or changing the positions of the two side chains around the piperidine ring.[10,11] As part of continuing project to discover novel ligands for VMAT2, and to gain more information on the structural requirement for ligand binding to VMAT2, SAR studies focusing on structural modification of the piperidine ring of the lobelane molecule were considered to be worthwhile. In the present study, our efforts were focused on

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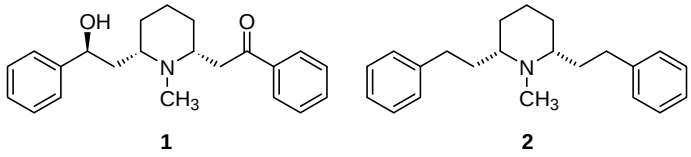
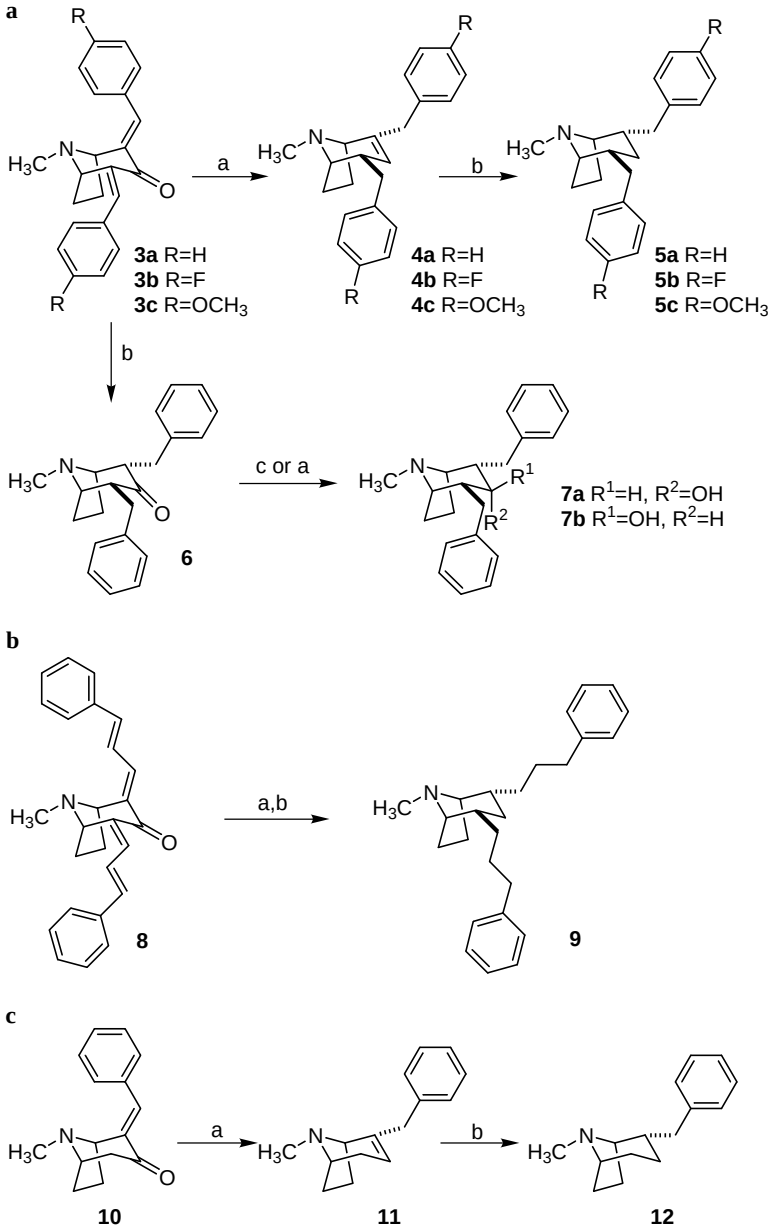


Figure 1. Structures of (−)-lobeline (**1**) and lobelane (**2**).

analogues, where a tropane ring was substituted for the piperidine ring in lobelane (**2**). The tropane ring represents a conformationally restricted form of the piperidine ring. Also, due to their interaction with monoamine transporters, derivatives of tropane (i.e., analogues of the 8-azabicyclo[3.2.1]octane ring system) have received considerable attention recently in drug discovery research.[12] Therefore, it was considered of interest to investigate tropane ring analogues of lobelane as VMAT2 ligands.

The general methodology for the preparation of compounds **4a**–**4c**[13] and **5a**–**5c**[13] is shown in Scheme 1a. Compounds **3a**–**3c** were obtained by aldol condensation



Scheme 1. Reagents and conditions: (a) Zn/Hg, 20% HCl/1,4-dioxane (1:1), reflux; (b) H_2, Pd/C, HOAc/MeOH (1:10), 45 psig, rt; (c) L-Selectride, THF, −78 °C.

of tropinone with benzaldehyde, 4-fluorobenzaldehyde, or 4-methoxybenzaldehyde.[14] Clemmensen reduction of **3a**, **3b**, or **3c** with amalgamated Zn afforded compound **4a**, **4b**, or **4c**, respectively. Compound **5a**, **5b**, or **5c** was each obtained as a single stereoisomer by catalytic hydrogenation of the corresponding precursor molecule, **4a**, **4b**, or **4c**. Compound **3a** could be reduced stereoselectively to compound **6** by catalytic hydrogenation. Using lithium tri-*sec*-butylborohydride (L-Selectride), **6** was then reduced stereoselectively to compound **7a**. Stereoselective reduction of **6** with amalgamated Zn provided compound **7b** (Scheme 1a). The structures of **7a** and **7b** were confirmed by X-ray crystallography.[15]

The above tropane analogues were evaluated at [^{3}H]nicotine ([^{3}H]NIC) binding sites (α4β2* nAChR) and [^{3}H]methyllycaconitine ([^{3}H]MLA) binding sites (α7* nAChR) on rat brain membranes, and at the [^{3}H]dihydrotetrabenazine ([^{3}H]DTBZ) binding site (VMAT2) on rat synaptic vesicle membranes (Table 1). None of these compounds showed any affinity for either α4β2* or α7* nAChRs. The trop-2-ene analogues **4a** and **4b** (K_i = 1.30 and 1.38 μM, respectively) exhibited similar affinity but higher selectivity at VMAT2 compared to lobelane (**2**) (K_i = 0.97 μM). Interestingly, compounds **5a** and **5b** (both with K_i >100 μM), saturated analogues of **4a** and **4b**, respectively, had no affinity at VMAT2. These results suggest that the double bond in compound **4a** or **4b** plays an important role in the recognition of the binding site of VMAT2. In this respect, the double bond affects both the configuration of the tropane ring and the orientations of two side chains at C-2 and C-4. In particular, the molecules of **4a** and **4b** are more extended than the corresponding reduced analogues, that is, **5a** and **5b**. Compound **4c** (K_i = 4.80 μM), in which an electron-donating methoxy group was introduced into the *para* position of each of the two phenyl rings, exhibited lower potency at VMAT2 compared to either lobelane (**2**), **4a**, or **4b**. Surprisingly, in contrast to **5a** and **5b**, when the double bond in **4c** was reduced to afford the tropane analogue **5c**, the affinity of **5c** (K_i = 3.88 μM) at VMAT2 was retained. It is possible that the electron density in the phenyl rings may influence binding at VMAT2. It should be noted, however, that compound **5c** is more extended than either **5a** or **5b**, because of the presence of the *para*-methoxy groups; thus, full extension of the molecule in these tropane analogues may be important for VMAT2 binding. As expected, compounds **6**, **7a**, and **7b**, analogues of compound **5a** in which oxygen-containing functional groups were present in the molecule, exhibited no affinity at α4β2* and α7* nAChRs, and at the VMAT2 binding site (Table 1).

Table 1. Inhibition constants (K_i) for lobelane analogues at the [^{3}H]NIC binding site (α4β2* nAChR) and the [^{3}H]MLA binding site (α7* nAChR) on rat brain membranes, and at the [^{3}H]DTBZ binding site (VMAT2) on rat synaptic vesicle membranes

Compound	K_i, μM, ±SEM[a]		
	[^{3}H]NIC binding	[^{3}H]MLA binding	[^{3}H]DTBZ binding
1	0.004 ± 0.000	6.26 ± 1.30	2.76 ± 0.64
2	14.9 ± 1.67	26.0 ± 6.57	0.97 ± 0.19
4a	>100	>100	1.30 ± 0.21
5a	>100	>100	>100
4b	>100	>100	1.38 ± 0.20
5b	>100	>100	>100
4c	>100	>100	4.80 ± 1.70
5c	>100	>100	3.88 ± 0.90
6	>100	>100	>100
7a	>100	>100	>100
7b	>100	>100	>100
9	>100	>100	3.95 ± 0.54
11	1.84 ± 0.32	15.24 ± 1.32	>100
12	6.57 ± 1.00	14.0 ± 0.63	>100

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

Compound **9**,[13] which incorporates an increased distance between the phenyl rings and the C-2, C-4 atoms of the tropane ring compared to **5a**, was synthesized from compound **8** via a similar procedure as that utilized for the synthesis of **5a** (Scheme 1b). Compared to **5a**, compound **9** (K_i = 3.95 μM, Table 1) showed increased affinity at VMAT2. It should be noted that, similar to compound **5c**, compound **9** is a more extended molecule than compound **5a**, which may be the reason for the observed increase in VMAT2 affinity.

In a previous study, we have demonstrated that both the C-2 and C-6 side chains of the piperidine ring of lobelane are essential for VMAT2 affinity and selectivity.[11] The high VMAT2 affinity and selectivity observed for **4a** in this series of compounds encouraged us to carry out a similar SAR investigation. Compounds **11** and **12**, which incorporate the double bond containing fragment and the single bond containing fragment present in compound **4a**, were synthesized from the mono-aldol adduct **10**[16] via a similar procedure as that utilized for the synthesis of compounds **4a** and **5a** (Scheme 1c). In contrast to the corresponding 2,4-disubstituted compounds, both **11** and **12** showed affinity at α4β2* and α7* nAChRs, but neither of them exhibited any affinity at VMAT2 (Table 1). This result is consistent with the findings from our previous studies on lobelane analogues[11] that the whole lobelane molecule is required for recognition by VMAT2.

In conclusion, a novel series of lobeline and lobelane analogues in which the piperidine ring has been replaced with a more rigid tropane ring has been synthesized. Some of these analogues exhibit good affinity and selectivity at VMAT2. This investigation indicates that there is potential for the design and synthesis of lobelane analogues with greater potency and selectivity in the development of novel VMAT2 ligands as treatments for methamphetamine abuse.

Acknowledgments

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13. Compound **4a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.11–7.32 (m, 10H), 5.08 (br s, 1H), 3.22 (ABq, 2H), 2.83–3.03 (m, 3H), 2.47–2.63 (m, 2H), 2.24 (s, 3H), 1.76–2.02 (m, 3H), 1.63 (m, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.8, 140.2, 139.2, 129.1, 129.0, 128.4, 128.3, 126.2, 126.0, 121.8, 62.4, 62.0, 41.8, 40.5, 37.8, 36.9, 32.1, 22.8 ppm; MS (EI) *m/z* 303 (M^+); Anal. Calcd for $C_{22}H_{25}N{\cdot}HCl{\cdot}0.2H_2O$: C, 76.92; H, 7.75; N, 4.08. Found: C, 76.78; H, 7.73; N, 3.97. Compound **4b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05–7.16 (m, 4H), 6.92–7.01 (m, 4H), 5.02 (br s, 1H), 3.19 (ABq, 2H), 2.92–3.01 (m, 2H), 2.83 (m, 1H), 2.45–2.60 (m, 2H), 2.24 (s, 3H), 1.73–2.03 (m, 3H), 1.63 (m, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.1, 163.0, 159.9, 159.8, 141.2, 135.8, 135.7, 134.8, 134.7, 130.50, 130.44, 130.40, 130.3, 121.6, 115.4, 115.3, 115.2, 115.1, 62.5, 62.1, 40.9, 37.3, 37.0, 32.1, 22.7 ppm; MS (EI) *m/z* 339 (M^+); Anal. Calcd for $C_{22}H_{23}F_2N{\cdot}HCl{\cdot}1/3H_2O$: C, 69.19; H, 6.51; N, 3.67. Found: C, 69.17; H, 6.72; N, 3.56. Compound **4c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.00–7.14 (m, 4H), 6.78–6.86 (m, 4H), 5.05 (br s, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.16 (ABq, 2H), 2.91–3.01 (m, 2H), 2.83 (m, 1H), 2.42–2.56 (m, 2H), 2.24 (s, 3H), 1.73–2.03 (m, 3H), 1.62 (m, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.0, 157.8, 141.2, 132.3, 131.3, 130.0, 121.5, 113.9, 113.8, 62.4, 62.0, 55.4, 40.9, 37.0, 36.9, 32.1, 22.7 ppm; MS (EI) *m/z* 363 (M^+); Anal. Calcd for $C_{24}H_{29}NO_2{\cdot}HCl$: C, 72.07; H, 7.56; N, 3.50. Found: C, 71.70; H, 7.46; N, 3.32. Compound **5a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10–7.33 (m, 10H), 3.32 (br s, 2H), 2.88 (m, 2H), 2.55 (s, 3H), 2.36–2.63 (m, 4H), 2.01 (br s, 4H), 1.69 (dt, J = 14.1, 4.5 Hz, 1H), 0.92 (dd, J = 25.8, 12.0 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9, 129.0, 128.8, 126.7, 66.0, 39.1, 38.9, 29.6, 20.7 ppm; MS (EI) *m/z* 305 (M^+); Anal. Calcd for $C_{22}H_{27}N{\cdot}HCl$: C, 77.28; H, 8.25; N, 4.10. Found: C, 76.97; H, 8.54; N, 3.99. Compound **5b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.89–7.10 (m, 8H), 2.78 (br d, J = 3.6 Hz, 2H), 2.36 (d, J = 7.8 Hz, 4H), 2.18 (s, 3H), 1.98 (m, 2H), 1.83 (m, 2H), 1.59 (m, 2H), 1.39 (dt, J = 13.5, 4.5 Hz, 1H), 0.69 (dd, J = 25.2, 12.0 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.9, 159.6, 136.01, 135.97, 130.4, 130.3, 115.2, 114.9, 64.7, 42.7, 41.3, 39.4,30.7, 21.4 ppm; MS (EI) *m/z* 341 (M^+); $C_{22}H_{25}F_2N{\cdot}HCl{\cdot}0.2H_2O$: C, 69.26; H, 6.97; N, 3.67. Found: C, 69.33; H, 6.90; N, 3.66. Compound **5c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.02 (d, J = 8.7 Hz, 4H), 6.80 (d, J = 8.7 Hz, 4H), 3.78 (s, 6H), 2.88 (br s, 2H), 2.30–2.42 (m, 4H), 2.24 (s, 3H), 2.13 (m, 2H), 1.84 (m, 2H), 1.65 (m, 2H), 1.46 (dt, J = 13.5, 4.5 Hz, 1H), 0.72 (dd, J = 25.2, 12.0 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 132.1, 129.9, 113.8, 65.0, 55.4, 42.1, 41.0, 39.2, 30.7, 21.3 ppm; MS (EI) *m/z* 365 (M^+); Anal. Calcd for $C_{24}H_{31}NO_2{\cdot}HCl$: C, 71.71; H, 8.02; N, 3.48. Found: C, 71.33; H, 7.80; N, 3.42. Compound **9**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12–7.32 (m, 10H), 2.89 (br d, J = 3.3 Hz, 2H), 2.57 (t, J = 7.8 Hz, 4H), 2.28 (s, 3H), 1.37–1.82 (m, 11H), 1.02–1.26 (m, 4H), 0.49 (dd, J = 25.2, 12.0 Hz, 1H) ppm; ^{13}CNMR (75 MHz, $CDCl_3$): δ 142.7, 128.5, 128.3, 125.7, 65.6, 41.3, 40.4, 36.4, 33.5, 31.0, 29.2, 21.5 ppm; MS (EI) *m/z* 361 (M^+); Anal. Calcd for $C_{26}H_{35}N{\cdot}HCl$: C, 78.46; H, 9.12; N, 3.52. Found: C, 78.13; H, 8.80; N, 3.37.
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