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Des-keto lobeline analogs with increased potency and selectivity at dopamine and serotonin transporters

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Abstract

A series of *des*-keto lobeline analogs has been synthesized and evaluated for their ability to inhibit the dopamine transporter (DAT) and serotonin transporter (SERT) function and for their affinity for the synaptic vesicle monoamine transporter (VMAT2), as well as for $\alpha4\beta2^*$ and $\alpha7^*$ neuronal nicotinic acetylcholine receptors (nAChRs). The enantiomers 8*R*-hydroxylobel-9-ene (**3a**) and 10*S*-hydroxylobel-7-ene (**3c**) exhibited high potency and selectivity at SERT and DAT, respectively.

Keywords

Lobeline; Dopamine transporter; Serotonin transporter; Vesicular monoamine transporter

Monoamine neurotransmitter transporters such as the dopamine transporter (DAT), the serotonin transporter (SERT), the norepinephrine transporter (NET), and the vesicular monoamine transporter (VMAT2) are considered valid targets for the development of therapeutic agents aimed at treating a variety of neurological and psychiatric diseases. For example, several antidepressant drugs, such as fluoxetine, bupropion, and reboxetine, act as SERT, DAT, and NET inhibitors, respectively. These antidepressants increase the extracellular concentration of the respective neurotransmitter by inhibiting transporter function.[1–7] Additionally, tetrabenazine, an inhibitor of VMAT2 function, is used to treat Huntington's Chorea.[8,9] Recently, DAT has also been considered as a primary target for the development of medications to treat cocaine abuse.[10–12]

(−)-Lobeline (the 2*R*,6*S*,10*S*-stereoisomer, **1**; Scheme 1), the major alkaloid of *Lobelia inflata*, has high affinity for several neuronal nicotinic acetylcholine receptor (nAChR) subtypes,[13–16] and interacts nonselectively with monoamine transporters (DAT, SERT, NET, and VMAT2).[15–18] Structural modification of lobeline revealed that the *des*-keto analog, 8*R*-hydroxylobel-9-ene (**3a**; Scheme 1), has high potency and selectivity for inhibition of [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) uptake over [^{3}H]dopamine ([^{3}H]DA) uptake, and also has increased selectivity for these transporters as a result of reduced affinity for nAChRs.[15] This intriguing result prompted us to carry out a more detailed investigation of the structure–activity relationships of various stereoisomeric forms of **3a** and the double bond reduced analog **4a**. The pharmacological profile of these compounds was expected to provide information on the importance of the C-8/C-10 stereochemistry on the interaction with DAT, SERT, and VMAT2. Thus, the present study investigated the synthesis and pharmacological activities of isomeric 8- and 10-hydroxy lobelenes, that is 8*R*-

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hydroxylobel-9-ene (**3a**), 8*S*-hydroxylobel-9-ene (**3b**), 10*S*-hydroxylobel-7-ene (**3c**), and 10*R*-hydroxylobel-7-ene (**3d**), as well as the isomeric 8- and 10-hydroxylobelanes, that is 8*R*-hydroxylobelane (**4a**), 8*S*-hydroxylobelane (**4b**), 10*S*-hydroxylobelane (**4c**), and 10*R*-hydroxylobelane (**4d**). These compounds were evaluated for their ability to inhibit [^{3}H]nicotine ([^{3}H]NIC) binding (probing $\alpha4\beta2^*$ nAChRs) and [^{3}H]methyllycaconitine ([^{3}H]MLA) binding (probing $\alpha7^*$ nAChRs) to rat brain membranes, to inhibit [^{3}H]5-HT and [^{3}H]DA uptake into rat hippocampal and striatal synaptosomes, respectively, and to inhibit [^{3}H]dihydrotetrabenazine ([^{3}H]DTBZ) binding to rat synaptic vesicle membranes.

The synthetic routes to compounds **3a**–**3d**[19] and **4a**–**4d**[19] are illustrated in Scheme 1. Compound **2** was prepared by dehydration of lobeline (**1**) with 85% H_3PO_4, to afford the *E*-isomer exclusively, according to a previously reported method.[20,21] Reduction of **2** gave a mixture of two isomers, **3a** and **3b**, in a ratio of 9:20 (determined by GC–MS). The pure form of **3a** was obtained by fractional recrystallization of this isomeric mixture. Compound **3b** was obtained by silica gel chromatography of the mother liquors from the crystallization of **3a**. Compound **3c**, which was prepared by Clemmensen reduction of lobeline, as previously reported,[20] was converted into compound **5** by Jones oxidation. Compound **3d** was obtained along with **3c**, from **5**, utilizing the same procedure as that employed in the synthesis of **3a** and **3b** from **2** (vide supra). Catalytic hydrogenation of the unsaturated compounds **3a**, **3b**, **3c**, and **3d** afforded the corresponding reduced compounds **4a**, **4b**, **4c**, and **4d**, respectively.

The above lobeline analogs were evaluated as inhibitors of [^{3}H]NIC binding and [^{3}H]MLA binding to rat brain membranes, as inhibitors of [^{3}H]DA uptake into rat striatal synaptosomes to assess DAT function, as inhibitors of [^{3}H]5-HT uptake into rat hippocampal synaptosomes to assess SERT function, and as inhibitors of [^{3}H]DTBZ binding to rat synaptic vesicle membranes to assess interaction with VMAT2 (Table 1).[17] Analog-induced inhibition was compared with that induced by lobeline and the selective DAT, SERT, and VMAT2 transporter inhibitors GBR-12909, fluoxetine, and Ro 4–1284, respectively.[11,22,23] Lobeline potently inhibited [^{3}H]NIC binding with a K_i value of 4 nM, and had low affinity (K_i = 6.26 μM) for $\alpha7^*$ nAChRs.[24] *des*-Keto lobeline analogs exhibited diminished affinity at $\alpha4\beta2^*$ and $\alpha7^*$ nAChRs, except for compounds **3a** and **4a**, which had slightly higher potency than lobeline at $\alpha7^*$ nAChRs. These results indicate the importance of the keto group in lobeline for $\alpha4\beta2^*$ binding. Lobeline exhibited moderate selectivity for VMAT2 (K_i = 2.76 μM) over DAT (K_i = 28.2 μM) and SERT (K_i = 46.8 μM), and had relatively low affinity for the latter two transporters. However, most of the *des*-keto analogs exhibited higher potency as well as selectivity for DAT or SERT when compared to lobeline. None of these analogs exhibited high affinity and selectivity for VMAT2. All the *des*-keto analogs were generally equipotent with lobeline (within one order of magnitude of each other), and compound **3d** exhibited the highest affinity (K_i = 0.59 μM). These results are consistent with earlier results obtained with previously reported defunctionalized lobeline analogs.[15] In the current *des*-keto series, all analogs exhibited increased potency for inhibition of DAT and SERT compared to lobeline. Within this series, compound **3c** exhibited the highest affinity for DAT (K_i = 0.11 μM) and compound **3a**, the enantiomer of **3c**, exhibited the highest affinity for SERT (K_i = 0.044 μM).

Compound **3a** was 20-fold more selective in inhibiting SERT over DAT[15] and was 117-fold more selective for SERT over VMAT2. Compound **3b**, which has the antipodal chirality at the C8-hydroxyl group compared to compound **3a**, showed similar affinity for DAT and VMAT2 as **3a**, but was 2 orders of magnitude less potent than **3a** for SERT. Interestingly, compound **3c**, the enantiomer of **3a**, exhibited 220-fold greater selectivity in inhibiting DAT over SERT, which is the reverse of the selectivity observed with **3a**. However, this reversal

of selectivity did not occur in other pairs of enantiomers, that is, compounds **3b** and **3d**, both of which showed greater potency in inhibiting DAT over SERT (4- and 25-fold, respectively), and **3d** was more selective than **3b** at DAT. All four of these compounds inhibited DAT with K_i values all within one order of magnitude of each other. Thus, the binding site on SERT is more sensitive to stereochemical changes at the C-8/C-10 hydroxyl group than is the binding site on DAT.

Compounds **4a**–**4d** generally exhibited a similar potency and selectivity profile as their corresponding double bond unsaturated congeners, **3a**–**3d**. The four compounds **4a**–**4d** were slightly less potent than their corresponding precursors (**3a**–**3d**) in inhibiting DAT function. Moreover, compounds **4a**–**4d** exhibited similar potency as their corresponding precursors (**3a**–**3d**) in inhibiting SERT function. Thus, compounds **4a**–**4d** are less selective for DAT and SERT, compared to their corresponding double bond analogs. This indicates the double bond in these compounds is more important for the binding at DAT than at SERT.

In summary, a series of *des*-keto lobeline analogs has been synthesized, in which the oxygen of the keto group of lobeline has been eliminated. Pharmacological evaluation shows that all the analogs have diminished affinity at $\alpha4\beta2^*$ nAChRs and most of them also have diminished affinity at $\alpha7^*$ nAChRs. In addition, all the analogs are equipotent with lobeline at VMAT2. Moreover, some of these analogs have high potency and selectivity at either DAT or SERT. The current study indicates that the stereochemistry at C-8/C-10 in these molecules is important for inhibition of SERT, but not for inhibition of DAT. In contrast, the double bond in these analogs is more important for inhibition of DAT than for inhibition of SERT function. Further structural modification based on this series of analogs may reveal important information about the DAT and SERT pharmacophores.

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19. Compound **3a**: $[\alpha]_D^{25}$ 41.5° (*c* 1.0, $CHCl_3$); mp 104–105 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42–1.72 (m, 6H), 1.87 (m, 1H), 2.04 (m, 1H), 2.32 (s, 3H), 2.93 (m, 1H), 3.28 (m, 1H), 4.98 (dd, *J* = 10.5, 3.3 Hz, 1H), 6.23 (dd, *J* = 16.2, 6.3 Hz, 1H), 6.45 (d, *J* = 16.2 Hz, 1H), 7.20–7.42 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.6, 26.7, 33.0, 41.5, 63.4, 65.5, 74.6, 125.6, 126.3, 127.2, 127.5, 128.4, 128.7, 130.4, 133.1, 137.2, 145.4 ppm; MS *m/z* 321 (M^+); Anal. Calcd for $C_{22}H_{27}NO{\cdot}HCl{\cdot}1.0H_2O$: C, 70.29; H, 8.04; N, 3.73. Found: C, 70.20; H,8.28; N, 4.01.Compound **3b**: $[\alpha]_D^{25}$ –131.0° (*c* 1.0, $CHCl_3$); mp 117– 118 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.35–1.64 (m, 3H), 1.66–1.80 (m, 2H), 1.87 (m, 1H), 2.02 (m, 1H), 2.45 (m, 1H), 2.47 (s, 3H), 2.65 (m, 1H), 5.20 (dd, *J* = 11.1, 3.3 Hz, 1H), 6.13 (dd, *J* = 16.2, 8.4 Hz, 1H), 6.47 (d, *J* = 16.2 Hz, 1H), 7.20–7.42 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.0, 30.0, 33.7, 40.2, 41.6, 63.3, 68.6, 72.1, 125.6, 126.3, 127.0, 127.5, 128.3, 128.7, 130.7, 134.1, 137.0, 145.4 ppm; MS *m/z* 321 (M^+); Anal. Calcd for $C_{22}H_{27}NO{\cdot}HCl{\cdot}1/3H_2O$: C, 72.61; H, 7.94; N, 3.85. Found: C, 72.43; H, 8.02; N, 3.97.Compound **3c**: $[\alpha]_D^{25}$ –44.8° (*c* 1.0, $CHCl_3$); mp 107–108 °C; ^1H, ^{13}C NMR and MS are same as those of compound **3a**; Anal. Calcd for $C_{22}H_{27}NO{\cdot}HCl{\cdot}0.5H_2O$: C, 72.01; H, 7.97; N, 3.82. Found: C, 71.96; H, 8.12; N, 3.77.Compound **3d**: $[\alpha]_D^{25}$ 128.8° (*c* 1.0, $CHCl_3$); mp 118–119 °C; ^1H, ^{13}C NMR and MS are same as those of compound **3b**; Anal. Calcd for $C_{22}H_{27}NO{\cdot}HCl{\cdot}0.1H_2O$: C, 73.46; H, 7.90; N, 3.89. Found: C, 73.47; H, 7.78; N, 4.24.Compound **4a**: $[\alpha]_D^{25}$ 75.5 °(*c* 1.0, $CHCl_3$); mp 83–84 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.14 (m, 1H), 1.25 (m, 1H), 1.45–1.70 (m, 5H), 1.76–2.04 (m, 3H), 2.30 (s, 3H), 2.57– 2.79 (m, 2H), 2.87 (m, 1H), 3.20 (m, 1H), 5.01 (dd,*J* = 10.8, 3.0 Hz, 1H), 7.15–7.43 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7, 23.9, 25.4, 25.7, 32.6, 36.0, 40.2, 62.0, 65.0, 76.4, 125.6, 125.9, 127.1, 128.4, 128.5, 128.6, 142.3, 145.2 ppm; MS *m/z* 323 (M^+); Anal. Calcd for $C_{22}H_{29}NO{\cdot}HCl{\cdot}2/3H_2O$: C, 71.04; H, 8.49; N, 3.77. Found: C, 71.00; H, 8.62; N, 4.09.Compound **4b**: $[\alpha]_D^{25}$ –75.4° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.26–1.44 (m, 2H), 1.44–1.57 (m, 2H), 1.67–2.00 (m, 6H), 2.36 (m, 1H), 2.39 (s, 3H), 2.60– 2.72 (m, 3H), 5.14 (t, *J* = 6.0 Hz, 1H), 7.15–7.40 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.7, 26.7, 31.5, 33.7, 36.1, 39.1, 61.8, 62.8, 72.5, 125.7, 125.9, 126.8, 128.3, 128.5, 142.4, 145.7 ppm; MS *m/z* 323 (M^+); Anal. Calcd for $C_{22}H_{29}NO{\cdot}HCl{\cdot}2/3H_2O$: C, 71.04; H, 8.49; N, 3.77. Found: C, 70.84; H, 8.27; N, 4.06.Compound **4c**: $[\alpha]_D^{25}$ –77.9° (*c* 1.0, $CHCl_3$); mp 83–84 °C; ^1H, ^{13}C NMR and MS are same as those of compound **4a**; Anal. Calcd for $C_{22}H_{29}NO{\cdot}HCl{\cdot}1.0H_2O$: C, 69.91; H, 8.53; N, 3.71. Found: C, 69.63; H, 8.36; N, 3.88.Compound **4d**: $[\alpha]_D^{25}$ 74.4° (*c* 1.0, $CHCl_3$); ^1H, ^{13}C NMR and MS are same as those of compound **4b**; Anal. Calcd for $C_{22}H_{29}NO{\cdot}HCl{\cdot}1/3H_2O$: C, 72.21; H, 8.45; N, 3.83. Found: C, 72.05; H, 8.71; N, 4.06.

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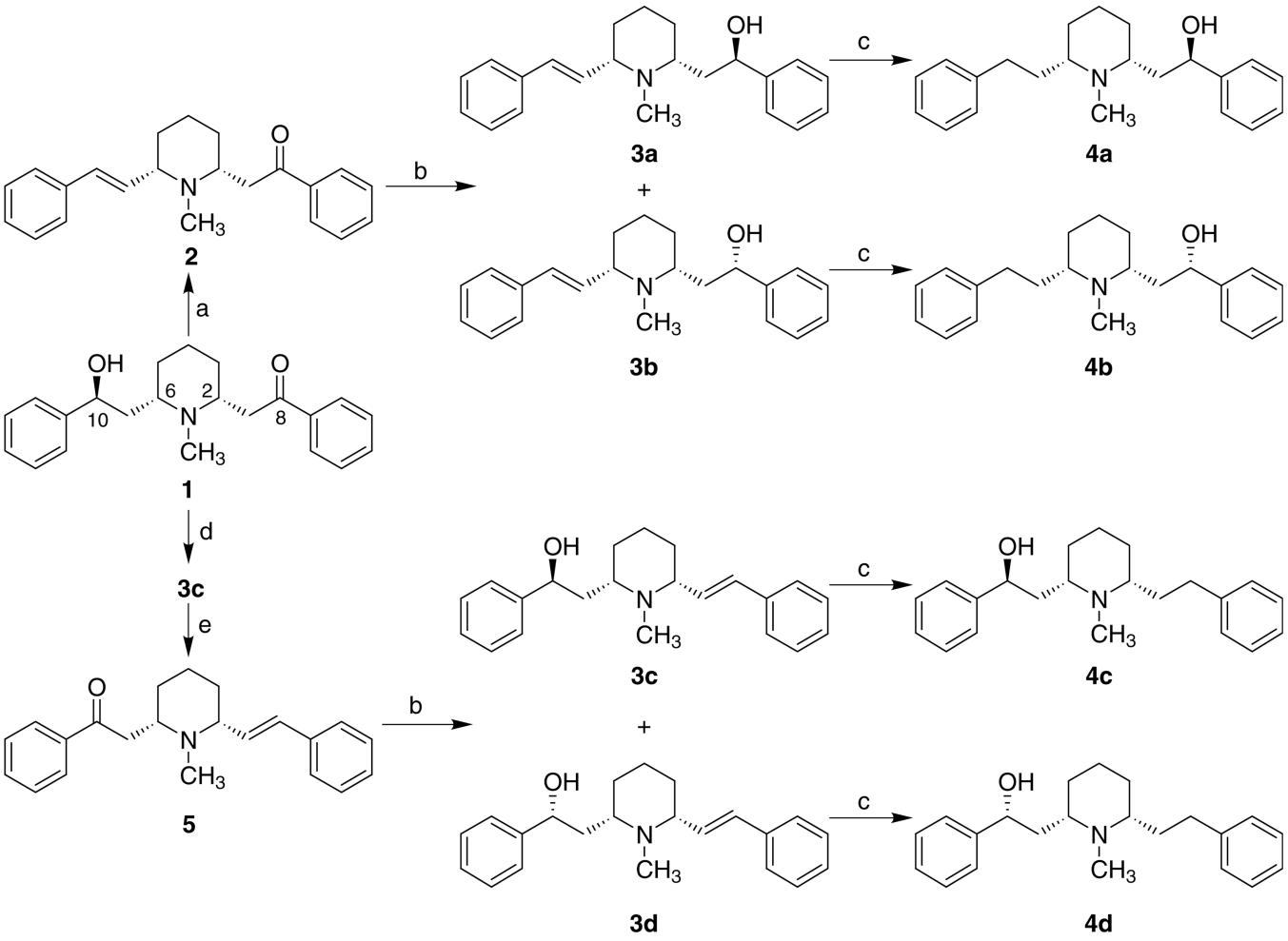

Scheme 1.

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

Table 1

Inhibition of [^{3}H]NIC binding (probing α4β2** nAChRs) and the [^{3}H]MLA binding (probing α7** nAChRs) on rat brain membranes, [^{3}H]DTBZ binding (probing VMAT2) on rat synaptic vesicle membranes, [^{3}H]DA uptake into rat striatal synaptosomes, and [^{3}H]5-HT uptake into rat hippocampal synaptosomes by lobeline and its *des*-keto analogs

	K_i, μM, ±SEM[a]					K_i ratio[b]
	[^{3}H]NIC (α4β2*)	[^{3}H]MLA (α7*)	[^{3}H]DA (DAT)	[^{3}H]5-HT (SERT)	[^{3}H]DTBZ (VMAT2)	DAT/SERT/VMAT2
Fluoxetine	—	—	—	0.041[c]	—	—
GBR-12909	—	—	0.018[c]	—	—	—
Ro 4–1284	—	—	—	—	0.028 ± 0.03	—
Lobeline	0.004 ± 0.000	6.26 ± 1.30	28.2 ± 6.73	46.8 ± 3.7	2.76 ± 0.64	10.2/17.0/1
3a	4.19 ± 0.80	1.70 ± 0.32	0.86[c]	0.044[c]	5.16 ± 0.30	19.5/1/117.3
3b	>100	>100	0.96 ± 0.11	3.75 ± 0.75	6.06 ± 0.45	1/3.9/6.3
3c	9.75 ± 0.91	>100	0.11 ± 0.003	19.0 ± 3.9	6.44 ± 0.54	1/173/58.5
3d	>100	>100	0.29 ± 0.02	7.50 ± 1.80	0.59 ± 0.15	1/26/2.0
4a	2.36 ± 0.18	1.21 ± 0.09	1.88 ± 0.12	0.15 ± 0.02	1.98 ± 0.31	13/1/13.2
4b	33.6 ± 8.54	> 100	1.26 ± 0.17	2.22 ± 0.36	3.01 ± 0.44	1/1.8/2.4
4c	1.77 ± 0.61	39.3 ± 12.9	1.39 ± 0.08	4.27 ± 1.00	3.09 ± 0.41	1/3.1/2.2
4d	>100	>100	0.57 ± 0.04	7.30 ± 0.50	6.60 ± 2.96	1/12.8/11.6

GBR-12909 (a specific DAT inhibitor), fluoxetine (a specific SERT inhibitor), and Ro 4–1284 (a specific VMAT2 inhibitor) were used as standards for comparison.

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

[b] For ratios between three monoamine transporters (DAT, SERT, and VMAT2), the highest affinity value was taken as 1.

[c] Data as reported in Ref. 15.