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Exploring the effect of *N*-substitution in *nor*-lobelane on the interaction with VMAT2: discovery of a potential clinical candidate for treatment of methamphetamine abuse

Guangrong Zheng[a], **David B. Horton**[b], **Narsimha Reddy Penthala**[a], **Justin R. Nickell**[b], **John P. Culver**[b], **Agripina G. Deaciuc**[b], **Linda P. Dwoskin**[b], and **Peter A. Crooks**[*,a]

[a]Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

[b]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

Abstract

A series of *N*-substituted lobelane analogues was synthesized and evaluated for their [^{3}H]dihydrotetrabenazine binding affinity at the vesicular monoamine transporter and for their inhibition of vesicular [^{3}H]dopamine uptake. Compound 19a, which contains an *N*-1,2(*R*)-dihydroxypropyl group, had been identified as a potential clinical candidate for the treatment of methamphetamine abuse.

The rewarding and reinforcing effects of methamphetamine (METH) are believed to be through its interaction with the vesicular monoamine transporter-2 (VMAT2) and the dopamine (DA) transporter (DAT), resulting in elevated synaptic DA concentrations.[1] The involvement of VMAT2 in the mechanism of METH addiction is demonstrated also by the reduced drug-seeking behavior exhibited by heterozygous VMAT2 knockout mice compared to wild-type mice in condition place preference (CPP) experiments.[2] Thus, VMAT2 has been considered as a potential target for development of treatments for METH abuse.[3] Over the past decade, a research focus in our laboratories has been the discovery and development of novel therapeutic agents that target VMAT2.[4] The validation of VMAT2 as a therapeutic target for METH abuse was based on (−)-lobeline (**1**, Fig. 1), a natural alkaloid from *Lobelia inflata*. Lobeline inhibits METH-evoked DA release from rat striatal slices and attenuates METH-induced behavioral effects in rats.[5–7] The inhibitory effects of lobeline on METH are thought to result from its interaction with VMAT2. Lobeline binds to the tetrabenazine (TBZ) binding site on VMAT2 and inhibits DA uptake at VMAT2 at a concentration that does not interact with DAT and the serotonin transporter (SERT).[8] However, lobeline is also a nicotinic acetylcholine receptor (nAChR) ligand exhibiting high affinity at the α4β2* subtype.[8] Thus, the involvement of nAChRs as part of the mechanism by which lobeline inhibits the behavioral effects of METH cannot be ruled out. Structural modification of the lobeline molecule identified lobelane (**2**, Fig. 1) as a compound of interest. Compared to lobeline, lobelane has negligible activity at nAChRs, but has increased potency and selectivity in inhibiting VMAT2 function.[9,10] Importantly, lobelane also dose-dependently decreases METH self-administration (SA) in rats without altering food-maintained responding.[11] Collectively, these results suggest that VMAT2 is a valid target for METH abuse.

*Corresponding author. Tel.: +1-501-686-6495; fax: +1-501-686-6057; pacrooks@uams.edu.

To identify analogues with higher potency and selectivity in inhibiting VMAT2 function, extensive structure-activity relationship (SAR) studies have been carried out based on the lobelane scaffold, including structural modifications of the piperidine ring, the two phenyl rings, and the ethylene linkers bridging the piperidine ring and the phenyl rings.[4,9,12] These modifications have also included changing the chirality of the piperidinyl C-2 and C-6 stereo centers, altering the positions of the two phenethylsubstituents on the piperidine ring, replacing the piperidine ring with other aza-heterocyclic ring systems, adding various substituent groups on the phenyl rings, and varying the distance between the piperidine ring and the two phenyl rings.[4,9,12] A number of lead compounds have been identified based on these modifications; however, similar to lobelane, most of these analogues also exhibit low water solubility, which limits *in vivo* evaluation of their pharmacological properties. In addition, despite their high potency in the *in vitro* VMAT2 assays, *in vivo* preliminary screens indicate that these lead compounds have limited efficacy in inhibiting METH SA in rats (unpublished results). We reasoned that poor pharmacokinetic (PK) properties of these analogues might be responsible for the low *in vivo* efficacy.

In the present study, we focused on structural modifications that would increase the hydrophilicity of these lobelane analogues by introducing polar functional groups into the lobelane molecule. During our previous SAR studies, we observed that the *N*-methyl group of lobelane is not required for VMAT2 binding and inhibition, since nor-lobelane (**3**, Fig. 1) has equal potency to lobelane (Table 1).[10] In addition, replacement of the *N*-methyl group with an *N*-ethyl (compound **4**) or an *N*-*n*-propyl group (compound **5**)[12a] only slightly decreased the inhibitory effect of [^{3}H]DA uptake at VMAT2 when compared to lobelane (Table 1). These preliminary results indicate that modifications to the *N*-methyl group can be tolerated. Herein, we further investigate the structural modification of the *N*-methyl group of lobelane and its analogues.

Analogues **6–13**, **14a**, and **14b** were synthesized starting from the common intermediate, nor-lobelane (**3**), which was prepared as previously reported (Scheme 1 and 2).[12a]*N*-Alkylation of **3** with methyl bromoacetate or chloroacetonitrile afforded analogues **6** and **8**, respectively. Reduction of **6** and **8** with DIBAL-H furnished the corresponding amino alcohol analogue **7** and the diamino analogue **9**. **9** was converted into analogue **10** by *N*-methylation under the treatment of $NaBH_3CN/(CH_2O)_n$ and to guanidino analogue **11** by reacting with *N*,*N*′-di-(*tert*-butoxycarbonyl)thiourea, followed by treatment with CF_3COOH to remove the two BOC protective groups. Conversion of **3** to the corresponding hydrazino compound **12** was accomplished by a two-step procedure, i.e., *N*-nitrosation and a subsequent LAH reduction. Without further purification after work-up, the unstable compound **12** was converted into analogue **13** using the same *N*-methylation procedure as described for analogue **10** (Scheme 1). Analogues **14a** and **14b**, which contain an *N*-1,2(*R*)-dihydroxypropyl group and *N*-1,2(*S*)-dihydroxypropyl group, respectively, were prepared by reacting nor-lobelane with *S*-glycidol or *R*-glycidol, respectively (Scheme 2).

Phenyl ring modified nor-lobelane analogues **15–25** were synthesized as previously reported.[12a]**15a–25a** and **15b–22b** were synthesized by applying the same method for the synthesis of analogues **14a** and **14b**, from their corresponding precursors, **15–25** (Scheme 2, Table 2). Similarly, analogue **28a**, a homologue of **19a**, was prepared from the corresponding nor-compound **28**. **28** was synthesized by initial Kumada coupling[13] between 2,6-dibromopyridine (**26**) and 4-methoxybenzylmagnesium chloride to form compound **27**, followed by catalytic hydrogenation over Adams catalyst (PtO_2) (Scheme 3).

The ability of the above analogues[14] to interact with VMAT2 was assessed by determining their inhibition of [^{3}H]dihydrotetrabenazine ([^{3}H]DTBZ) binding to rat brain vesicle membranes, and their inhibition of [^{3}H]DA uptake into rat striatal synaptic vesicles.[10] The

results are summarized in Table 1 and 2. Initial structural exploration of *N*-substitution patterns based on lobelane led to analogues **6–13** (Table 1), **14a**, and **14b** (Table 2). Compound **13**, in which the *N*-methyl group is replaced by an *N,N*-dimethylhydrazino group, was the most potent analogue in this series, inhibiting [^{3}H]DA uptake at VMAT2, being equipotent to lobelane. Analogue **8**, in which the *N*-methyl group is replaced by an *N*-acetonitrile group, displayed 100-fold lower potency in inhibiting [^{3}H]DA uptake at VMAT2 compared to lobelane. The rest of the analogues in this series were 4–19 fold less potent compared to lobelane in the same assay. Interestingly, the most potent inhibitor of DA uptake at VMAT2, **13**, exhibited no affinity at the [^{3}H]DTBZ binding site, indicating that DA uptake at VMAT2 is not via interaction with the DTBZ recognition binding site on VMAT2. For this series of analogues, there is no correlation between affinity for the VMAT2 binding site and inhibition of DA uptake at VMAT2, which is in consistent with our previously reported studies on other lobelane analogues.[10,15]

Compound **14a**, which bears an *N*-1,2(*R*)-dihydroxypropyl group, was chosen for further structural optimization based on preliminary data from *in vivo* screens (decrease in METH SA in rats). We incorporated the chiral *N*-1,2-dihydroxypropyl moiety into a series of phenyl ring substituted lobelane analogues, including **15–22** and their corresponding *N*-methylated analogues. Compared to compound **14a**, these analogues exhibited equal or improved binding affinity and potency for inhibiting [^{3}H]DA uptake at VMAT2, as shown in our previous study.[15] Accordingly, analogues **15a–22a**, which contain the *N*-1,2(*R*)-dihydroxypropyl group, and **15b–22b**, which contain the *N*-1,2(*S*)-dihydroxypropyl group, were synthesized and evaluated (Scheme 2 and Table 2). SAR on this series of analogues was discussed recently.[16] In general, analogues containing *N*-1,2-dihydroxypropyl moieties analogues exhibited lower potencies for inhibiting [^{3}H]DA uptake at VMAT2 compared to their corresponding *N*-methyl analogues. There are no apparent preferences in terms of [^{3}H]DTBZ binding affinities between *N*-1,2(*R*)-dihydroxypropyl containing analogues and their corresponding *N*-1,2(*S*)-dihydroxypropyl containing enantiomers. *N*-1,2(*R*)-Dihydroxypropyl containing analogues generally had a higher potency at the [^{3}H]DA uptake assay than their corresponding *S*-enantiomers, with the exception of an equipotent pair of analogues, **20a** and **20b**. Analogue **19a** (K_i = 29 nM) had the highest potency of the series inhibiting [^{3}H]DA uptake into synaptic vesicles, and was chosen for further neurochemical and behavioral studies.

Studies showed that **19a** had the best pharmacological profile in this series,[16] including over 50-fold selectivity for VMAT2 over DAT, SERT, and nAChRs; and decreasing METH-evoked DA release from vesicles and striatal slices without intrinsic activity. **19a** was also shown to have great selectivity over a series of off-target receptors/proteins (unpublished data).

Our recent studies have demonstrated that **19a** effectively decreases METH SA and CPP in rats without altering food-maintained responding. In addition, tolerance to these effects is not observed after repeated dosing of **19a**.[17] Importantly, **19a** is predicted to have low abuse liability, since it is not self-administered and does not produce CPP.[17] Compared to lobeline and lobelane, **19a** exhibited a significantly increased duration of action to inhibit METH SA within a behavioral session. In this respect, we reasoned that **19a** likely has a better PK profile than lobeline and lobelane due to the introduction of the hydrophilic *N*-1,2-dihydroxypropyl moiety. We are currently characterizing the PK profile of this molecule and results will be reported in due course.

We have continued the structural optimization studies based on **19a**. These studies include modifying the substituents on the two phenyl rings to improve metabolic stability (analogues **23a–25a**, Table 2) and modifying the two ethylene linkers bridging the phenyl rings and the

central piperidine ring (analogue **28a**). Further evaluation and modification of these compounds is ongoing currently.

Conclusions

In summary, a series of lobelane analogues, in which the *N*-methyl group of lobelane has been replaced by various hydrophilic moieties, has been synthesized and evaluated in the [^{3}H]DTBZ binding assay and the vesicular [^{3}H]DA uptake assay. Compound **19a**, which contains a *N*-1,2(*R*)-dihydroxypropyl group, has been identified as a potential clinical candidate for the treatment of METH abuse.

Acknowledgments

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Notes and references

1. Sulzer D, Sonders MS, Poulsen NW, Galli A. Prog. Neurobiol. 2005; 75:406. [PubMed: 15955613]
2. Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR. *Proc. Natl. Acad. Sci.* USA. 1997; 94:9938. [PubMed: 9275230]
3. (a) Zheng G, Dwoskin LP, Crooks PA. AAPS J. 2006; 8:E682. [PubMed: 17233532] (b) Dwoskin LP, Crooks PA. Biochem. Pharmacol. 2002; 63:89. [PubMed: 11841781]
4. Crooks PA, Zheng G, Vartak AP, Culver JP, Zheng F, Horton DB, Dwoskin LP. Curr. Top. Med. Chem. 2011; 11:1103. [PubMed: 21050177]
5. Miller DK, Crooks PA, Teng L, Witkin JM, Munzar P, Goldberg SR, Acri JB, Dwoskin LP. J. Pharmacol. Exp. Ther. 2001; 296:1023. [PubMed: 11181937]
6. Harrod SB, Dwoskin LP, Crooks PA, Klebaur JE, Bardo MT. J. Pharmacol. Exp. Ther. 2001; 298:172. [PubMed: 11408539]
7. Harrod SB, Dwoskin LP, Green TA, Gehrke BJ, Bardo MT. Psychopharmacology (Berl). 2003; 165:397. [PubMed: 12459929]
8. (a) Teng L, Crooks PA, Sonsalla PK, Dwoskin LP. J. Pharmacol. Exp. Ther. 1997; 280:1432. [PubMed: 9067333] (b) Teng L, Crooks PA, Sonsalla PK, Dwoskin LP. J. Neurochem. 1998; 71:258. [PubMed: 9648873]
9. Zheng G, Dwoskin LP, Deaciuc AG, Norrholm SD, Crooks PA. J. Med. Chem. 2005; 48:5551. [PubMed: 16107155]
10. Nickell JR, Krishnamurthy S, Norrholm S, Deaciuc G, Siripurapu KB, Zheng G, Crooks PA, Dwoskin LP. J. Pharmacol. Exp. Ther. 2010; 332:612. [PubMed: 19855096]
11. Neugebauer NM, Harrod SB, Stairs DJ, Crooks PA, Dwoskin LP, Bardo MT. Eur. J. Pharmacol. 2007; 571:33. [PubMed: 17612524]
12. (a) Zheng G, Dwoskin LP, Deaciuc AG, Zhu J, Jones MD, Crooks PA. Bioorg. Med. Chem. 2005; 13:3899. [PubMed: 15911306] (b) Zheng G, Dwoskin LP, Deaciuc AG, Crooks PA. Bioorg. Med. Chem. Lett. 2005; 15:4463. [PubMed: 16112864] (c) Zheng G, Dwoskin LP, Deaciuc AG, Crooks PA. Bioorg. Med. Chem. Lett. 2008; 18:6509. [PubMed: 18976906] (d) Vartak AP, Nickell JR, Chaqkutip J, Dwoskin LP, Crooks PA. J. Med. Chem. 2009; 52:7878. [PubMed: 19691331]
13. Kumada M. Pure Appl. Chem. 1980; 52:669.
14. Selected spectra data: compound 6, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12–7.32 (m, 10H); 3.65 (s, 3H), 3.35 (s, 2H), 2.77 (m, 2H), 2.68 (t, J = 8.1 Hz, 4H), 1.77–1.90 (m, 3H), 1.50–1.65 (m, 4H), 1.20–1.40 (m, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 142.6, 128.5, 128.4, 125.8, 67.8, 51.8, 46.3, 36.5, 32.6, 27.4, 24.9 ppm; EI-MS m/z 365 (M^+); compound 7, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14–7.34 (m, 10H); 3.50 (br s, 2H), 2.50–2.78 (m, 8H), 1.95–2.05 (m, 2H), 1.52–1.65 (m, 4H), 1.32–1.45 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9, 128.53, 128.50, 126.0, 62.6, 60.5, 46.9, 36.0, 33.5, 26.2, 23.1 ppm; EI-MS m/z 336 $(M-1)^+$; compound 8, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15–7.33 (m, 10H); 3.67 (s, 2H), 2.68 (t, J = 8.4 Hz, 4H), 2.52 (m, 2H), 1.62–1.92 (m, 8H), 1.30–1.52 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.1, 128.5, 128.3, 126.0,

115.5, 59.9, 36.3, 35.7, 31.0, 30.2, 24.3 ppm; EI-MS m/z 332 (M^+); compound 9, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05–7.30 (m, 10H); 2.40–2.75 (m, 10H), 1.78–1.95 (m, 2H), 1.30–1.65 (m, 8H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.5, 128.4, 128.3, 125.7, 62.1, 49.0, 43.1, 36.7, 33.3, 26.6, 24.0 ppm; EI-MS m/z 335 $(M-1)^+$; compound 10, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15–7.32 (m, 10H); 2.55–2.75 (m, 8H), 2.37 (t, J = 8.1 Hz, 2H), 2.22 (s, 6H), 1.85–2.00 (m, 2H), 1.78 (m, 1H), 1.50–1.72 (m, 4H), 1.33–1.45 (m, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.4, 128.5, 128.4, 125.8, 62.5, 60.0, 46.1, 43.6, 36.2, 33.1, 27.0, 24.4 ppm; EI-MS m/z 364 (M^+); compound 13, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15–7.35 (m, 10H); 2.66–2.80 (m, 2H), 2.54 (s, 6H), 2.40–2.60 (m, 4H), 1.95–2.10 (m, 2H), 1.78–1.90 (m, 2H), 1.58–1.75 (m, 4H), 1.23–1.38 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.3, 128.5, 128.4, 125.6, 61.1, 40.9, 36.7, 32.3, 31.9, 24.1 ppm; EI-MS m/z 336 (M^+); compound 19a, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (dd, J = 8.4, 2.1 Hz, 4H), 6.82 (d, J = 8.4 Hz, 4H), 3.78 (s, 6H), 1.22–1.63 (m, 7H), 3.70 (dd, J = 11.4, 3.3 Hz, 1H), 3.45–3.54 (m, 1H), 3.37 (dd, J = 11.4, 3.9 Hz, 1H), 1.72–1.92 (m, 3H), 2.42–2.74 (m, 8H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 134.2, 134.1, 129.4, 114.0, 68.2, 64.9, 63.1, 61.6, 55.5, 46.5, 36.5, 36.2, 32.8, 32.7, 26.6, 25.9, 23.1 ppm; LC-ESI-MS m/z 428 $(M+1)^+$. All compounds are estimated >95% pure based on GC-MS or LC-MS data

15. Nickell JR, Zheng G, Deaciuc G, Crooks PA, Dwoskin LP. J. Pharmacol. Exp. Ther. 2011; 336:724. [PubMed: 20876747]
16. Horton DB, Siripurapu KB, Zheng G, Crooks PA, Dwoskin LP. J. Pharmacol. Exp. Ther. 2011; 339:286. [PubMed: 21778282]
17. Beckmann JS, Denehy ED, Zheng G, Crooks PA, Dwoskin LP, Bardo MT. Psychopharmacology (Berl). 2012; 220:395. [PubMed: 21938414]

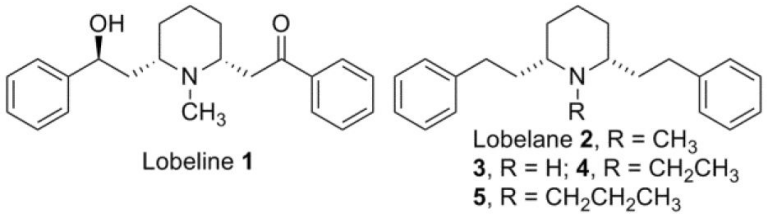
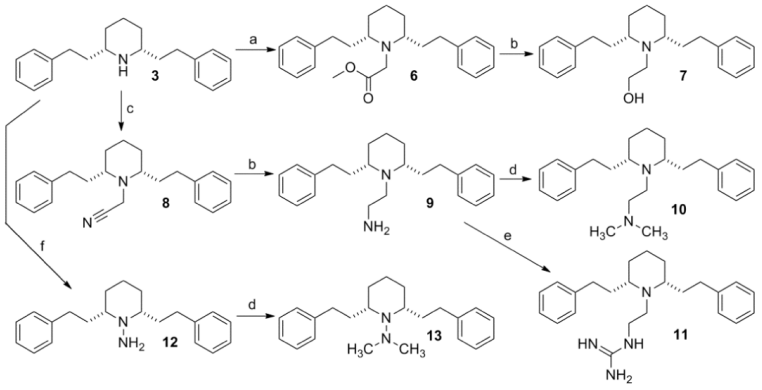
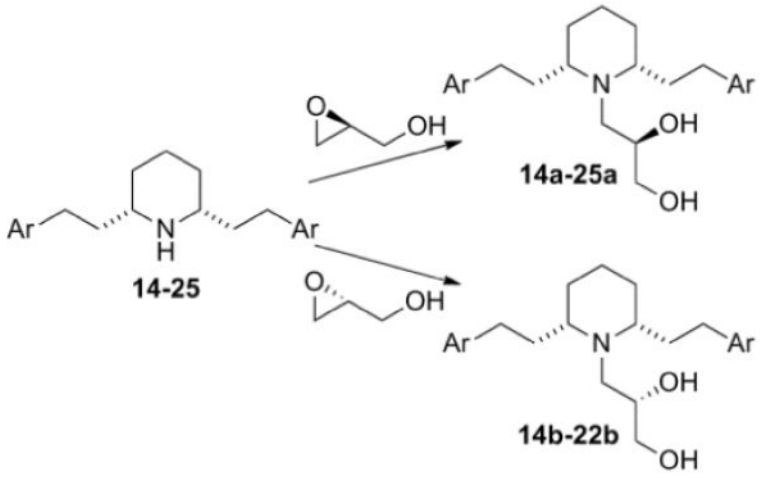


Fig. 1.
Structures of lobeline **1**, lobelane **2**, and lobelane analogues.

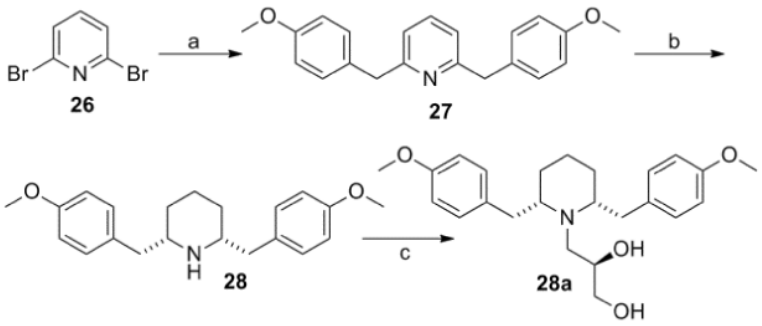


Scheme 1.

Reagents and conditions: a) $BrCH_2COOMe$, K_2CO_3, CH_3CN, reflux, 72 h, 77%; (b) DIBAL-H, toluene, 0 °C, 1 h, 83% for **7**, 79% for **9**; (c) $ClCH_2CN$, K_2CO_3, NaI, CH_3CN, reflux, 96 h, 66%; (d) $NaCNBH_3$, $(CH_2O)_n$, HOAc, MeOH, rt, 4 h, 60% for **10**, 35% for **13**; (e) i. N,N′-di-(tertbutoxycarbonyl)thiourea, DMF, rt, 18 h, 58%; ii. CF_3COOH, CH_2Cl_2, rt, 30 min, 74%; (f) i. $NaNO_2$, HOAc, MeOH/H_2O, 0 °C-reflux, 20 h, 82%; ii. LAH, THF, reflux, 1 h, 33%.



Scheme 2.
Synthesis of analogues containing *N*-1,2-dihydroxypropyl group.



Scheme 3.

Reagents and conditions: (a) $MeOPhCH_2MgCl$, $NiCl_2$(dppp), THF, rt, 18 h, 97%; (b) H_2, PtO_2, HOAc, 50 psi, rt, 36 h, 84%; (c) (*S*)-glycidol, EtOH, reflux, 48 h, 76%.

Table 1

Analogue inhibition of [^{3}H]DTBZ binding to rat synaptic vesicle membranes and inhibition of [^{3}H]DA uptake into rat striatal synaptic vesicles.

Compd.	[^{3}H]DTBZ K_i ± SEM (μM)[a]	VMAT2 [^{3}H]DA Uptake K_i ± SEM (μM)[a]
1	2.76 ± 0.64[b]	0.47 ± 0.045[b]
2	0.97 ± 0.00[b]	0.045 ± 0.002[b]
3	2.31 ± 0.21[b]	0.044 ± 0.008[b]
4	3.41 ± 0.67[c]	0.060 ± 0.005
5	1.87 ± 0.25[c]	0.087 ± 0.04
6	4.4 ± 0.76	0.34 ± 0.13
7	1.43 ± 0.136	0.17 ± 0.01
8	22.57 ± 0.23	4.49 ± 0.35
9	5.59 ± 0.94	0.51 ± 0.089
10	9.59 ± 1.47	0.18 ± 0.024
11	3.49 ± 0.63	0.68 ± 0.18
13	> 100	0.054 ± 0.042

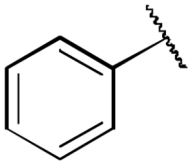
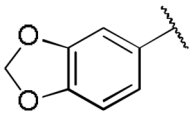
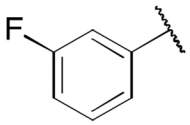
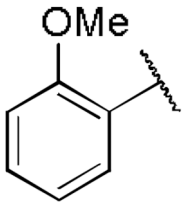
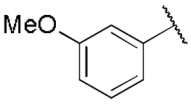
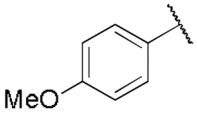
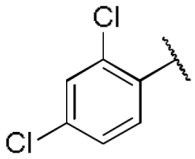
[a] Each K_i value represents data from at least three independent experiments, evaluated in duplicate;

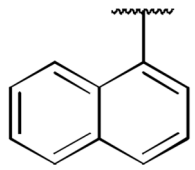
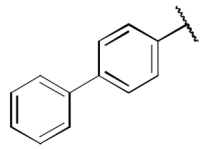
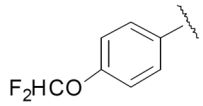
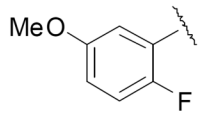
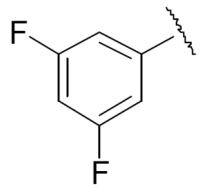
[b] data taken from reference 10;

[c] data taken from reference 12b.

Table 2

Analogue inhibition of [^{3}H]DTBZ binding to rat synaptic vesicle membranes and inhibition of [^{3}H]DA uptake into rat striatal synaptic vesicles.

Compd.	Ar	[^{3}H]DTBZ K_i ± SEM (μM)[a]	VMAT2 [^{3}H]DA Uptake K_i ± SEM (μM)[a]
14a		0.56 ± 0.08	0.19 ± 0.05
14b		1.28 ± 0.13	0.86 ± 0.12
15a		1.30 ± 0.05	0.16 ± 0.04
15b		5.61 ± 0.62	0.76 ± 0.04
16a		1.00 ± 0.16	0.19 ± 0.06
16b		1.08 ± 0.38	1.03 ± 0.16
17a		1.04 ± 0.73	0.49 ± 0.06
17b		1.87 ± 0.69	0.79 ± 0.08
18a		0.46 ± 0.22	0.14 ± 0.02
18b		2.73 ± 0.68	0.52 ± 0.04
19a		8.29 ± 2.79	0.029 ± 0.008
19b		7.74 ± 2.34	0.18 ± 0.04
20a		10.4 ± 0.65	0.14 ± 0.04
20b		13.9 ± 0.38	0.09 ± 0.04

Compd.	Ar	[^{3}H]DTBZ K_i ± SEM (μM)[a]	VMAT2 [^{3}H]DA Uptake K_i ± SEM (μM)[a]
21a		0.31 ± 0.07	0.03 ± 0.002
21b		0.13 ± 0.07	0.08 ± 0.01
22a		> 100	0.79 ± 0.23
22b		90.2 ± 9.70	2.25 ± 1.30
23		1.75 ± 0.36	0.023 ± 0.006
23a		0.53 ± 0.053	0.060 ± 0.006
24		0.93 ± 0.22	0.039 ± 0.002
24a		0.21 ± 0.02	0.16 ± 0.035
25		5.24 ± 1.81	0.043 ± 0.003
25a		0.62 ± 0.10	0.104 ± 0.004
28		13.1 ± 7.4	0.048 ± 0.005
28a	-	91 ± 40	0.57 ± 0.05

[a]Each K_i value represents data from at least three independent experiments, evaluated in duplicate; The data of **14a–22a** and **14b–22b** were taken from reference 16.