Published in final edited form as:

Bioorg Med Chem Lett. 2015 July 1; 25(13): 2613–2616. doi:10.1016/j.bmcl.2015.04.105.

Quinolyl analogues of norlobelane: Novel potent inhibitors of [³H]dihydratetrabenazine binding and [³H]dopamine uptake at the vesicular monoamine transporter-2

Derong Ding^a, Justin R. Nickell^a, Linda P. Dwoskin^a, and Peter A. Crooks^{b,*}

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

^bDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

Abstract

We have previously shown that quinolyl moieties are attractive structural replacements for the phenyl groups in lobelane. These quinolyl analogues had improved water-solubility over lobelane and retained the potent vesicular monoamine transporter-2 (VMAT-2) inhibitory properties of the parent compound, with quinlobelane (**4**) exhibiting potent inhibition of uptake at VMAT-2 ($K_i = 51$ nM). However, the VMAT-2 inhibitory properties of quinolyl analogues of norlobelane, which is equipotent with lobeline as an inhibitor of [³H]dopamine (DA) uptake at VMAT-2, have not been reported. In the current communication, we describe the synthesis of some novel *des*-methyl quinolyl analogues of lobelane that exhibit greater affinity ($K_i = 178$ –647 nM) for the dihydratetrabenazine binding site located on VMAT-2 compared with lobelane ($K_i = 970$ nM), norlobelane ($K_i = 2310$ nM) and quinlobelane ($K_i = 2640$ nM). The most potent compounds, **14** and **15**, also exhibited inhibition of [³H]DA uptake at VMAT-2 ($K_i = 42$ nM) which was comparable to both lobelane ($K_i = 45$ nM) and norlobelane ($K_i = 43$ nM). Results reveal that binding affinity at VMAT-2 serves as an accurate predictor of inhibition of the function of VMAT-2 for the majority of these analogues. These novel analogues are under consideration for further development as treatments for methamphetamine abuse.

Graphical Abstract

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

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Keywords

Quinolyl analogues of norlobelane; vesicular [^3H]dopamine uptake; methamphetamine abuse; VMAT-2

Methamphetamine abuse and its complications are a global epidemic and pose a serious international public health concern due to methamphetamine's high liability for addiction together with a risk for neurotoxicity and long-lasting neurological impairment^{1,2}. Methamphetamine causes a number of medical problems, including myocardial infarction³, ischemic and hemorrhagic stroke⁴, and in some cases fatal hyperthermia⁵. Moreover, clinical reports suggest that methamphetamine abusers are predisposed to Parkinson's disease⁶. Currently, there is no clinically efficacious pharmacotherapy to treat methamphetamine addicts, necessitating the identification of novel therapeutics.

Our previous studies have demonstrated that lobeline (**1**, Fig. 1), a weakly basic lipophilic alkaloid isolated from *Lobelia inflata*, decreases the rewarding effects of methamphetamine in a rat behavioral model,^{7–10} and inhibits dopamine (DA) uptake into synaptic vesicles *via* interaction with the tetrabenazine binding site on the vesicular monoamine transporter-2 (VMAT-2).¹¹ However, lobeline has weak potency as an inhibitor of [^3H]DA uptake at the vesicular monoamine transporter-2 (VMAT-2) and is a relatively nonselective compound with poor drug-likeness properties. Subsequently, we identified lobelane (**2**, Fig. 1), a chemically defunctionalized analogue of lobeline, as a potent inhibitor of [^3H]DA uptake at VMAT-2 ($K_i = 45$ nM). Also, the *N*-demethylated analogue, norlobelane (**3**, Fig. 1) was found to be equipotent ($K_i = 43$ nM) with lobelane as an inhibitor of [^3H]DA uptake at VMAT-2, and both compounds exhibited 10 to 15-fold higher potency and selectivity for inhibition of [^3H]DA uptake into synaptic vesicles when compared to lobeline.^{12–15} Although more potent than lobeline, both lobelane and norlobelane exhibited less than optimal water-solubility. Consequently, in the search for more drug-like VMAT-2 inhibitors, we recently reported on a series of novel lobelane analogues in which the phenyl moieties were replaced with heterocyclic rings, such as indolyl, pyridyl, and quinolyl (e.g. compound **4**, Fig. 1)¹⁵. However, only the quinolyl analogues retained potent VMAT-2 inhibitory properties¹⁵, with quinlobelane (**4**) exhibiting improved water solubility over lobelane and norlobelane, and potent inhibition of [^3H]DA uptake at VMAT-2 ($K_i = 51$ nM).

In this respect, the VMAT-2 inhibitory properties of quinolyl analogues of norlobelane (i.e. *N*-*des*-methyl analogues) have not been previously studied. In this communication, we report on several *des*-methyl analogues of quinlobelane and determine affinity for the dihydrotetrabenazine (DTBZ) binding site on VMAT-2 and inhibition of DA uptake at VMAT-2. Thus, novel 3-quinolyl, 4-quinolyl, and 6-quinolyl analogues of *cis*-norlobelane were synthesized and evaluated for their inhibition of [^3H]DTBZ binding and [^3H]DA uptake at VMAT-2. We also prepared an *N*-2,3-dihydroxypropyl derivative of *cis*-2,6-di-[(2-ethylquinolyl)]-piperidine as a structural analogue of the preclinical candidate GZ-793A¹⁶ and evaluated this compound for VMAT-2 inhibitory potency.

The synthesis of the above compounds from the common intermediate *cis*-*N*-carbobenzyloxy-2,6-piperidine dicarboxaldehyde (**6**) is illustrated in Scheme 1, and was

based upon our recently reported procedure for the synthesis of *cis*-2,6-di-(2-quinolyl)piperidines¹⁷. Starting material **6** was prepared from pyridine-2,6-dicarboxylic acid via a 5-step procedure¹⁷ involving initial esterification and exhaustive reduction to dimethyl *cis*-2,6-piperidine dicarboxylate, followed by *N*-carbobenzyloxylolation, LiBH₄ reduction to the 2,6-methylenediol derivative, and finally, Swern oxidation to afford **6**. Reaction of **6** with the appropriate phosphonium salt (**7–9**) afforded the corresponding Wittig product (**10–12**), which was then *N*-deprotected in refluxing 6N HCl and reduced with 10% Pd/C to afford the corresponding *des*-methyl quinlobelane analogues **13–15**. The preparation of compound **16**¹⁸, a structural analogue of the potent VMAT2 inhibitor, GZ-793A (**17**), which has been extensively studied as a preclinical candidate for treatment of methamphetamine abuse^{19–24}, is also shown in Scheme 1. Compound **16** was prepared via a one-step reaction of **13**¹⁷ with (*S*)-(-)-glycidol in EtOH in 66% yield. Quinolyl analogues (**13–16**) were converted to their respective HCl salts using ethereal HCl and evaluated as inhibitors of [³H]DTBZ binding and [³H]DA uptake at VMAT-2 using preparations of isolated rat brain synaptic vesicles. Inhibition constants (*K_i*) were obtained and are provided in Table 1.

Results from the [³H]DTBZ binding assay show that introduction of the quinolyl heterocyclic ring groups to the structure of norlobelane (*K_i* = 2310 nM) markedly improved affinity for the high-affinity binding site located on VMAT-2 (Table 1). In comparison to quinlobelane (*K_i* = 2640 nM), compounds **15** (*K_i* = 647 nM) and **16** (*K_i* = 627 nM) exhibited 4-fold greater affinity. Compounds **13** (*K_i* = 293 nM) and **14** (*K_i* = 178 nM) exhibited affinities for the binding site which were 9- and 15-fold, respectively, greater than that of quinlobelane. Thus, the methyl group present on the central nitrogen atom of quinlobelane compromises affinity for the [³H]DTBZ binding site.

In the vesicular DA uptake assay, the 2-quinolyl analogue, **13** (*K_i* = 57 nM), the 4-quinolyl analogue, **14** (*K_i* = 42 nM), and the 6-quinolyl analogue, **15** (*K_i* = 42 nM) (Table 1), all exhibited similar inhibition of VMAT-2 function when compared to lobelane (*K_i* = 45 nM), norlobelane (*K_i* = 43 nM) and quinlobelane (*K_i* = 51 nM), indicating that neither the quinolyl moiety nor the *N*-methyl group are critical structural moieties for inhibition of VMAT-2 function. The comparable inhibitory potencies of these isomeric quinolyl analogues on [³H]DA uptake at VMAT-2 are informative for further structural optimization studies. Interestingly, compounds **13**, **14** and **15** were about 6-fold more potent inhibiting [³H]DA uptake at VMAT-2 compared to the *N*-2,3-dihydroxypropyl derivative **16**.

Of note, all of the compounds had a higher affinity (3 to 15-fold) for inhibition of [³H]DA uptake than for inhibition of [³H]DTBZ binding. However, affinity for the [³H]DTBZ binding site served as an accurate predictor of rank-order of inhibition of [³H]DA uptake at VMAT-2 for compounds **13**, **14** and **16**. One exception was compound **15**, which displayed the greatest potency in the functional assay, but exhibited the lowest affinity for the [³H]DTBZ binding site. These results suggest that compound **15** may be interacting with a different site on the VMAT-2 protein than the other analogues.

Analogue-induced inhibition of [³H]DTBZ binding was determined as previously described¹³. Rat whole brain (excluding cerebellum) was homogenized in 20 ml of ice-cold 0.32 M sucrose solution with 7 up-and-down strokes of a Teflon pestle homogenizer

(clearance $\approx 0.003''$). Homogenates were centrifuged at 1,000 g for 12 min at 4 °C, and the resulting supernatants were again centrifuged at 22,000 g for 10 min at 4 °C. Resulting pellets were incubated in 18 ml of ice-cold water for 5 min, and 2 ml of HEPES (25 mM) and potassium tartrate (100 mM) solution were subsequently added. Samples were centrifuged (20,000 g for 20 min at 4 °C), and 20 μ l of MgSO_4 (1 mM) solution was added to the supernatants. Solutions were centrifuged (100,000 g for 45 min at 4 °C) and pellets resuspended in ice-cold binding assay buffer (25 mM HEPES, 100 mM potassium tartrate, 5 mM MgSO_4 , 0.1 mM EDTA, and 0.05 mM EGTA, pH 7.5). Assays were performed in duplicate using 96-well plates. Aliquots of vesicular suspension (15 μ g protein in 100 μ l) were added to wells containing 5 nM [^3H]DTBZ, 50 μ l of analogue (1 nM–1 mM), and 50 μ l of buffer. Nonspecific binding was determined in the presence of Ro4-1284 (20 μ M). Reactions were terminated by filtration (Packard Filtermate harvester; PerkinElmer Life and Analytical Sciences) onto Unifilter-96 GF/B filter plates (presoaked in 0.5% PEI). Filters were washed 5 times with 350 μ l of ice-cold buffer (25 mM HEPES, 100 mM potassium tartrate, 5 mM MgSO_4 , and 10 mM NaCl, pH 7.5). Filter plates were dried and bottom-sealed, and each well was filled with 40 μ l of scintillation cocktail (MicroScint 20; PerkinElmer Life and Analytical Sciences). Radioactivity on the filters was determined by liquid scintillation spectrometry (TopCount NXT; PerkinElmer Life and Analytical Sciences, Boston, MA).

Inhibition of [^3H]DA uptake at VMAT-2 was conducted as previously described²⁵ using isolated rat synaptic vesicle preparations. Briefly, rat striata were homogenized with 10 strokes of a Teflon pestle homogenizer (clearance $\approx 0.003''$) in 14 ml of 0.32 M sucrose solution. Homogenates were centrifuged (2000 g for 10 min at 4 °C), and the resulting supernatants were centrifuged again (10,000 g for 30 min at 4 °C). Pellets were resuspended in 2 ml of 0.32 M sucrose solution and subjected to osmotic shock by adding 7 ml of ice-cold water to the preparation, followed by the immediately adding 900 μ L of 0.25 M HEPES buffer and 900 μ L of 1.0 M potassium tartrate solution. Samples were centrifuged (20,000 g for 20 min at 4 °C), and the resulting supernatant was centrifuged again (55,000 g for 1 h at 4 °C), followed by the addition of 100 μ L of 10 mM MgSO_4 , 100 μ L of 0.25 M HEPES and 100 μ L of 1.0 M potassium tartrate solution prior to the final centrifugation (100,000 g for 45 min at 4 °C). Final pellets were resuspended in 2.4 ml of uptake assay buffer (25 mM HEPES, 100 mM potassium tartrate, 50 μ M EGTA, 100 μ M EDTA, 1.7 mM ascorbic acid, 2 mM ATP- Mg^{2+} , pH 7.4). Aliquots of the vesicular suspension (100 μ L) were added to tubes containing assay buffer, various concentrations of inhibitor (0.1 nM–10 mM) and 0.1 μ M [^3H]DA in a final volume of 500 μ L. Nonspecific uptake was determined in the presence of Ro4-1284 (10 μ M). Reactions were terminated by filtration, and radioactivity retained by the filters was determined by liquid scintillation spectrometry (PerkinElmer Life and Analytical Sciences, Boston, MA).

In conclusion, we report herein a novel series of quinolyl analogues of norlobelane that exhibit potent inhibition of VMAT-2 binding and function. The isomeric quinolines **14** and **15** show similar potency inhibiting vesicular [^3H]DA uptake. In addition, all analogues exhibited greater affinity for the DTBZ binding site located on VMAT-2 than lobelane, norlobelane and quinlobelane. With the exception of compound **15**, affinity binding data (K_i

for [³H]-DTBZ binding) served as an accurate predictor of rank order of inhibition of transporter function in this study. With respect to structure-activity relationships, the *N*-methyl group appears not to be a structural requirement necessary for interaction with VMAT-2. Surprisingly, the *N*-2,3-dihydroxypropyl analogue **16**, a structural analogue of the preclinical candidate, GZ-793A¹⁹, had 4-fold, and 8-fold lower inhibitory potency, respectively, than either **13** or GZ-793A ($K_i = 29$ nM)¹⁹ in the vesicular [³H]DA uptake assay.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Ms. Agripina G. Deaciuc and funding support from NIH grant U01 DA13519.

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18. Spectroscopic data for compounds 14–16. Compound 16: colorless oil; ¹H NMR (300 MHz, CDCl₃): δ 8.08–7.99 (m, 4H), 7.79–7.62 (m, 4H), 7.51–7.45 (m, 2H), 7.32–7.26 (m, 2H), 3.77–3.50 (m, 3H), 3.10–2.99 (m, 4H), 2.75–2.56 (m, 6H), 2.18–1.95 (m, 3H), 1.85–1.67 (m, 3H), 1.59–1.42 (m, 4H) ppm, HRMS (EI) Calcd for C₃₀H₃₅N₃O₂: 469.2729. Found: 469.2732; Compound 14: viscous oil, ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.76 (d, *J* = 4.0 Hz, 2H), 8.13 (d, *J* = 8.0 Hz, 2H),

8.00 (d, $J = 8.0$ Hz, 2H), 7.75-7.73 (m, 2H), 7.64-7.62 (m, 2H), 7.39 (d, $J = 4.5$ Hz, 2H), 3.18-3.14 (m, 8H), 2.10-1.98 (m, 2H), 1.91-1.78 (m, 3H), 1.58-1.31 (m, 3H). HRMS (EI) Calcd for $C_{27}H_{29}N_3$: 395.2361. Found: 395.2364. Compound 15: viscous oil, 1H NMR (300 MHz, $CDCl_3$): δ 8.79- 8.77 (m, 2H), 7.96-7.88 (m, 5H), 7.37-7.23 (m, 5H), 2.79 (br, 3H), 2.62-2.55 (m, 7H), 2.08-1.87 (m, 7H) *ppm*. HRMS (EI) Calcd for $C_{27}H_{29}N_3$: 395.2361. Found: 395.2359.

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Figure 1.
Structures of lobeline (1), lobelane (2), norlobelane (3) and quinlobelane (4).

Scheme 1.
Synthetic routes to compounds **13–16**.

Table 1

Inhibition constants (K_i) for analogue-induced inhibition of [^3H]DTBZ binding and [^3H]DA uptake at VMAT-2.

Compound ^a	Structure	VMAT-2	
		[^3H]DTBZ Binding K_i (nM); mean $\pm$ SEM ^b	[^3H]DA uptake K_i (nM); mean $\pm$ SEM ^b
Methamphetamine		80100 $\pm$ 19500 ^c	2460 $\pm$ 8.3 ^c
Lobeline		970 $\pm$ 190 ^c	45 $\pm$ 8 ^c
Norlobeline		2310 $\pm$ 190 ^d	43 $\pm$ 2 ^d
Quinlobeline		2640 $\pm$ 1410 ^d	51 $\pm$ 5 ^d
13		293 $\pm$ 35.7	57 $\pm$ 5
14		178 $\pm$ 14.4	42 $\pm$ 1
15		647 $\pm$ 86.5	42 $\pm$ 9
16		627 $\pm$ 127	240 $\pm$ 4

^a All compounds were evaluated as their water-soluble HCl salts.

^b Each K_i value represents mean $\pm$ SEM from 3–4 animals, with each experiment performed in duplicate.

^c Data from reference 13.

^dData from reference 15.

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