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Synthesis and in vitro evaluation of water-soluble 1,4-diphenethylpiperazine analogs as novel inhibitors of the vesicular monoamine transporter-2

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Abstract

A small library of 1,4-diphenethylpiperazine analogs was synthesized and evaluated for inhibition of [³H]dihydrotrabenazine binding and [³H]dopamine uptake at the vesicular monoamine transporter-2 (VMAT2). Results from these studies identified three novel molecules, **6b**, **6e** and **9a** (K_i = 35 nM, 48 nM and 37 nM, respectively) that exhibit similar potency for inhibition of VMAT2 function compared with lobelane (K_i = 45 nM), and importantly, have enhanced water-solubility when compared to the previously reported 1,4-diphenethylpiperidine analogs. These 1,4-diphenethylpiperazine analogs constitute promising new leads in the discovery of potential pharmacotherapeutics for treatment of methamphetamine use disorders.

Graphical abstract

Keywords

Lobelane; Piperazine analogs; VMAT2; Phenethyl bromides; Dopamine uptake

Methamphetamine (METH) use disorders continue to present a formidable challenge to the international healthcare community. METH use disorders result in severe and persistent

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physiological and psychological untoward effects. The mechanism of action underlying the effects of METH involves both the plasmalemmal dopamine transporter (DAT) and the vesicular monoamine transporter-2 (VMAT2) expressed by dopamine (DA) containing presynaptic terminals. METH gains entry to presynaptic terminals via diffusion across the plasmalemmal lipid bilayer and by acting as a substrate for DAT¹⁻³. Once within the cytosolic compartment of the dopaminergic presynaptic terminals, METH disrupts the proton gradient across the vesicular membrane due to its weak basicity³. The proton gradient provides the driving force for VMAT2-mediated sequestration of DA from the cytosol into the vesicle. Thus, METH acts as an inhibitor of VMAT2 function⁴. Consequently, METH releases DA from the vesicles and inhibits DA uptake into the vesicles, increasing DA concentrations in the cytosol. METH also inhibits mitochondrial monoamine oxidase (MAO) activity⁵, which is the principal mechanism responsible for DA metabolism to dihydroxyphenylacetic acid.

As a substrate for DAT, METH reverses the transport of DA from the cytosol to the extracellular compartment⁶. The subsequent increase in extracellular DA concentration leads to activation of both presynaptic and postsynaptic DA receptors.

Lobeline (Fig 1, 1), a naturally occurring alkaloidal constituent of *Lobelia inflata*, attenuates the reinforcing properties of METH in a rat self-administration model. Pretreatment with lobeline decreases responding for intravenous METH which is not surmounted by increasing unit doses of METH^{7,8}. Importantly, tolerance does not develop to the lobeline-induced decrease in METH self-administration⁹. Also, lobeline does not serve as a substitute reinforcer⁹, indicating a lack of abuse liability. Neurochemical studies reveal that lobeline inhibits METH-evoked DA release from rat striatal slices⁴. Concentrations of lobeline that inhibit METH-evoked DA release from striatal slices are similar to concentrations of lobeline that inhibit VMAT2 function, whereas 100-fold greater concentrations are required to inhibit DAT function¹⁰. Thus, VMAT2 has been proposed as the primary biological target for lobeline with respect to inhibition of the pharmacological effects of METH. In contrast to METH, lobeline does not inhibit MAO activity, allowing for cytosolic DA to be metabolized, resulting in a reduction in the DA concentration available for reverse transport by DAT.¹⁰

Enthusiasm for the clinical development of lobeline as a pharmacotherapeutic to treat METH use disorders was diminished because lobeline has a relatively short plasma half-life, and an undesirable pharmacokinetic profile. Also, nausea was a common adverse effect due to lobeline's extremely bitter taste, which would likely limit compliance when administered via the oral route. However, lobeline was found to be safe when administered to study participants who had a METH use disorder¹¹. Another limitation was that lobeline exhibits high affinity for $\alpha 4\beta 2$ and $\alpha 7$ nicotinic acetylcholine receptor (nAChR) subtypes, such that the alkaloid is not selective for the VMAT2 target¹²⁻¹⁷.

Continuing efforts were aimed at modifying the chemical structure of lobeline to afford compounds with enhanced selectivity for VMAT2. This led to the discovery of lobelane (Fig. 1, 2), a chemically defunctionalized analog of lobeline, which exhibited high affinity for VMAT2 and low affinity for nAChRs, thus revealing a greater selectivity for VMAT2

relative to lobeline¹⁸. Similar to lobeline, lobelane decreased METH self-administration in rats; however, tolerance developed to this effect,¹⁹ precluding its development as a therapeutic.

Recently, we have reported on a new series of compounds based on a 1,4-diphenethylpiperidine scaffold (Fig. 1, 3), from which were identified numerous potent inhibitors of DA uptake at VMAT2²⁰. Analogs in this series exhibit K_i values in the range of 9.3-13 nM in the [³H]DA uptake assay, which represent some of the most potent VMAT inhibitors heretofore discovered. In the present study, the 1,4-diphenethylpiperidine scaffold (**3**) was modified by replacing the central piperidine moiety with a 1,4-piperazine moiety to enhance water-solubility, and the effects of 1,4-diphenethylpiperazine analogs on VMAT2 binding and function determined. We also investigated the water solubility of these novel 1,4-diphenethylpiperazine analogs. Water solubility is an important factor in drug design²¹, since oral ingestion is the most common method of drug delivery and poorly water soluble orally delivered drugs are not absorbed as efficiently as water soluble drugs at the site of absorption in the gastrointestinal tract, leading to low bioavailability.

Two subsets of symmetrical and nonsymmetrical 1,4-diphenethylpiperazine analogs were synthesized (Scheme 1). The 1,4-symmetrical diphenethylpiperazine analogs were prepared from piperazine (**4**) and various phenethyl bromides (**5**). Piperazine (**4**) (1 equiv) was reacted with an appropriate phenethyl bromide (**5**) (2 eq) in the presence of K₂CO₃/acetonitrile at reflux temperature for 6 h to afford the corresponding symmetrical 1,4-piperazine analogs **6a-6f**, which were then converted to their hydrochloride salt forms by treatment with 2M HCl in diethyl ether (Scheme 1).

For the synthesis of nonsymmetrical 1,4-diphenethylpiperazine analogs **9a-9e** (Scheme 1), piperazine (**4**) (5 eq) was reacted with phenethyl bromide (**7**) in toluene at reflux temperature for 8 h to afford 1-phenethylpiperazine (**8**). In this reaction, excess piperazine was utilized to ensure selective mono-*N*-alkylation. 1-Phenethylpiperazine (**8**) was then reacted with an appropriate phenethyl bromide (**5**) in the presence of K₂CO₃/acetonitrile at reflux temperature for 6 h to afford compounds **9a-9e**, which were then converted to their hydrochloride salt forms by treatment with 2M HCl in diethyl ether (Scheme 1). All final products were purified by silica gel column chromatography utilizing 30-40% EtOAc in hexane as eluting solvent to afford products with high purity (yield 75-80%). The ¹H NMR, ¹³C NMR and mass spectral data for the above compounds can be found in the Supporting Information.

The above 1,4-diphenethylpiperazine analogs were evaluated for inhibition of [³H]dihydrotetrabenazine (DTBZ) binding to and [³H]DA uptake by VMAT2 utilizing previously described methods²⁰ (see Supporting Information). In the [³H]DTBZ binding assay, the K_i values for the eleven 1,4-diphenethylpiperazine analogs ranged from 0.37-6.63 μ M (Table 1). Compounds **6e**, **6c**, **6b**, **9a** and **9b** exhibited K_i values similar to that for lobelane ($K_i = 0.97 \mu$ M)¹⁸ in this assay. As examples, concentration-response curves for compounds **6b** and **9a** are illustrated in Fig. 2. Analogs incorporating a 4-substituent into the phenyl ring of the *N*-4-phenethyl moiety, e.g. analogs **6d** and **9c** (*N*-4-(4-

methoxyphenethyl), and analogs **6f** and **9e** (*N*-4-(4-fluorophenethyl)), generally had lower affinity for the DTBZ binding site on VMAT2 in comparison to lobelane.

In the [³H]DA uptake assay, *K_i* values for the 1,4-diphenylpiperazine analogs ranged from 35-410 nM (Table 1). Compounds **6b**, **6c**, **6e**, **6f** and **9a** exhibited *K_i* values not different from lobelane (45 nM)²². As examples, concentration-response curves for compounds **6b** and **9a** are illustrated in Fig. 3. In contrast, compounds containing an *N*-4-phenethyl or *N*-4-(4-methoxyphenethyl) moiety exhibited lower affinity in the [³H]DA uptake assay; compounds **6a**, **6d** and **9c** had a 2- to 9-fold lower affinity for VMAT2 compared to lobelane.

Thus, simultaneous translocation of the 2,6-diphenethyl linker units of lobelane to the 1,4-position around the central heterocyclic ring, and conversion of the piperidine ring in lobelane to a more polar piperazine ring, afforded a series of aromatic substituted analogs which retain affinity at the DTBZ and DA recognition sites on VMAT2 when compared to lobelane. When compared to the structurally related 1,4-diphenethylpiperidine analogs²⁰, these 1,4-diphenethyl-piperazine analogs appear to be slightly less potent as VMAT2 inhibitors in both the [³H]DTBZ binding and [³H]DA uptake assays.

In the 1,4-diphenethylpiperazine series, the *K_i* values for the five most potent compounds (**6b**, **6c**, **6e**, **9a** and **9b**) in the [³H]DTBZ binding assay ranged from 0.37-1.30 μM, whereas *K_i* values for the five most potent compounds (**6b**, **6c**, **6e**, **6f** and **9a**) in the [³H]DA uptake assay ranged from 35-60 nM. Interestingly, by comparison, in the 1,4-diphenethylpiperidine series the ranges of *K_i* values for the five most potent compounds in the [³H]DTBZ binding and [³H]DA uptake assays were 0.19-0.38 μM and 9.3-46 nM, respectively. Thus, the piperazine analogs generally have a reduced affinity at both binding and uptake sites on VMAT2 relative to the related piperidine analogs.

While these VMAT2 uptake data are promising, the possibility exists that these compounds may interact with additional targets. In order to address this concern, the three piperazino compounds which exhibited the greatest inhibitory potency for VMAT2 (**6b**, **6e**, and **9a**) were evaluated further for their ability to inhibit the dopamine (DAT) and serotonin (SERT) transporters²⁴, in addition to the human-ether-a-go-go-related gene (hERG) channel²⁵ (Table 2). Compound **6b** exhibited a remarkable 411-fold greater selectivity for VMAT2 versus DAT, while having 45- and 29-fold greater affinity for VMAT2 in comparison to SERT and the hERG channel, respectively. Compound **6e** demonstrated a similar profile, exhibiting 131-, 70- and 23-fold greater selectivity for VMAT2 versus DAT, SERT and hERG, respectively. This investigation revealed compound **9a** to have the optimal profile, bearing VMAT2 selectivity ratios of 346-, 70-, and 79-fold with respect to DAT, SERT and hERG. Prior reports illustrate that the preponderance of drug discovery candidates which progress to the development phase exhibit hERG binding IC₅₀ values within the relatively restricted range of 1-10 μM²⁶. As such, it is noteworthy that compounds **6b**, **6e** and **9a** all exhibited affinities for the hERG channel which were >1 μM. Collectively, these data indicate that compounds within this series are associated with low risk for abuse liability, and are not expected to induce cardiotoxicity.

Table 3 provides comparative data on the predicted water solubility and ClogP values of the 1,4-diphenethylpiperazine analogs **6a-6f** and **9a-9e** and their corresponding 1,4-diphenethylpiperidine analogs **10-20**. All water solubility values were calculated utilizing the ACD/ADME algorithm suite 5.0²⁷. The data clearly indicate that in every case, substituting an *N*-atom for the piperidine 4-CH moiety in the 1,4-diphenethylpyridine analogs **10-20** results in a predicted improvement, in some cases as much as 20- to 30-fold, in water solubility. Within the 1,4-diphenethylpiperazine series, two groups of compounds are identified. Compounds containing only one or no aromatic substituents (**9a-9e**, and **6a**) exhibit water solubility values 12-30 times greater than their 1,4-diphenethylpiperidine counterparts. The 1,4-phenethyl-piperazine analogs containing one aromatic substituent on both phenyl rings (**6b-6f**) exhibit water solubility values only 4-8 times greater than their 1,4-diphenethylpiperidine counterparts. Experimental water solubility values for the hydrochloride salts of the three most potent compounds (**6b**, **6e** and **9a**) were also determined utilizing the thermodynamic solubility method (Table 3)²⁸, and afforded values of 33.3 mg/mL, 25.0 mg/mL and 13.5 mg/mL, respectively.

The ClogP value is a measure of the hydrophilicity of a drug. High ClogP values indicate low hydrophilicities and poor absorption or permeation properties. Compounds that are poorly absorbed usually have ClogP values > 5.0. From Table 3 it can be seen that the 1,4-diphenylethylpiperazine analogs have ClogP values in the range 3.12-4.48, whereas the 1,4-diphenylethylpiperidine analogs ClogP values were in the range 5.52-6.88. The three most promising compounds **6b**, **6e** and **9a** have ClogP values ranging from 3.12 to 4.48.

In summary, replacement of the piperidine ring in the 1,4-diphenethylpiperidine analog series with a piperazine ring leads to improvement in water solubility and a small decrease in affinity for VMAT2 relative to their 1,4-piperidino counterparts. Nevertheless, the structural modifications evaluated herein have led to the identification of three molecules, **6b**, **6e** and **9a**, that retain high affinity (K_i = 35 nM, 48 nM and 37 nM, respectively) for VMAT2 in the DA uptake assay relative to lobelane (K_i = 45 nM), and have the advantage of improved water solubility (13.5-33.3 mg/mL). As such, these 1,4-diphenethylpiperazine analogs constitute promising leads in the development of a pharmacotherapy for METH use disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structures of lobeline (1), lobelane (2) and the 1,4-phenethylpiperidine scaffold (3).

Fig. 2. 1,4-Diphenethylpiperazines inhibit [³H]DTBZ binding to VMAT2 in rat brain vesicle preparations. Concentration-response curves for inhibition of [³H]DTBZ binding for compounds **6b** and **9a**. Control (CON) represents [³H]DTBZ binding in the absence of compound. Data are mean (± S.E.M.) specific [³H]DTBZ binding expressed as a percentage of control (1430 ± 74.7 fmol/mg, n = 3 rats/compound).

Fig. 3. 1,4-Diphenethylpiperazines inhibit [³H]DA uptake at VMAT2 in rat striatal vesicle preparations. Concentration-response curves for inhibition of [³H]DA uptake for compounds **6b** and **9a**, which exhibited the highest affinity for VMAT2. Control (CON) represents [³H]DA uptake in the absence of compound. Data are mean ($\pm$ S.E.M.) specific [³H]DA uptake expressed as a percentage of control (12.1 ± 1.97 pmol/min/mg), $n = 4$ rats/compound.

Scheme 1.

Synthesis of 1,4-disubstituted symmetrical and nonsymmetrical 1,4-diphenethylpiperazine analogs. *Reagents and conditions:* (a) toluene/reflux, 8-12 h; (b) 2M HCl in diethyl ether; (c) toluene/reflux, 8 h; (d) K₂CO₃/acetonitrile, 80 °C, 6 h.

Table 1
1,4-Diphenethylpiperazine analogs inhibit [³H]DA uptake by VMAT.

Compound	R ₁	R ₂	R ₃	[³ H]DTBZ Binding K _i (μM)	[³ H]DA Uptake K _i (nM)
Lobeline (1)				2.76 ± 0.64 ^f	470 ± 45 ^f
Lobeline (2)				0.97 ± 0.19 ^f	45 ± 8 ²
6a	H	H	H	3.05 ± 0.64	110 ± 12
6b	OCH ₃	H	H	1.17 ± 0.13	35 ± 1.2
6c	H	OCH ₃	H	1.05 ± 0.087	60 ± 4
6d	H	H	OCH ₃	6.63 ± 0.84	410 ± 5
6e	Cl	H	H	0.37 ± 0.038	48 ± 3
6f	H	H	F	3.71 ± 0.48	58 ± 12
9a	OCH ₃	H	H	1.07 ± 0.25	37 ± 0.5
9b	H	OCH ₃	H	1.30 ± 0.33	98 ± 16
9c	H	H	OCH ₃	4.63 ± 1.31	100 ± 11
9d	Cl	H	H	1.59 ± 0.26	63 ± 6.9
9e	H	H	F	3.86 ± 1.01	88 ± 6.9

²Data taken from reference 22 (Ding et al., 2015)

^fData taken from reference 23 (Vartak et al., 2009)

Table 2

Inhibition of [³H]DA uptake by DAT, [³H]5-HT uptake by SERT and [³H]dofetilide binding to hERG by lobelane and compounds **6b**, **6e**, and **9a**.

Compound	DAT [³ H]DA Uptake Ki ± SEM (μM)	SERT [³ H]5-HT Uptake Ki ± SEM (μM)	hERG [³ H]Dofetilide Binding Ki ± SEM (μM)
Lobelane (2)	1.57 ± 0.38	3.60 ± 0.35	0.238 ± 0.0389
6b	14.4 ± 0.456	1.58 ± 0.415	1.02 ± 0.180
6e	6.31 ± 1.01	3.34 ± 1.53	1.08 ± 0.175
9a	12.8 ± 4.46	2.58 ± 0.415	2.94 ± 0.302

Table 3
Water solubility and ClogP values for 1,4-diphenethylpiperidine and 1,4-diphenethylpiperazine derivatives

1,4-Diphenethylpiperidines ¹ 10-20	Solubility (pH = 7.4) mg/mL	ClogP values	1,4-Diphenethylpiperazines 6a-6f and 9a-9e	Solubility (pH = 7.4) mg/mL	ClogP values
0.27	5.69		8.08	3.29
0.12	5.60		3.16 (33.3)²	3.21
0.11	5.60		3.16	3.21
0.16	5.60		3.71	3.21
0.11	6.28		1.33	3.89
0.37	5.74		5.94	3.34
0.30	5.52		1.61 (25.0)²	3.12
0.26	5.52		1.54	3.12
0.38	5.52		1.94	3.12
0.01	6.88		0.08 (13.5)²	4.48
0.58	5.79		2.39	3.39

¹ Structures taken from reference²⁰ (Nickell et al., 2016)

Experimentally determined water solubility of the hydrochloride salt

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