Bioorganic & Medicinal Chemistry Letters 26 (2016) 2997–3000

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

1,4-Diphenalkylpiperidines: A new scaffold for the design of potent inhibitors of the vesicular monoamine transporter-2

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ARTICLE INFO

Article history:
Received 15 April 2016
Revised 6 May 2016
Accepted 9 May 2016
Available online 10 May 2016

Keywords:
Vesicular monoamine transporter-2
[^{3}H]DTBZ binding
[^{3}H]DA uptake inhibition at VMAT2
1,4 diphenethyl lobelane analogs
1-Phenethyl, 4-phenylmethylene derivatives of lobelane

ABSTRACT

A series of 1,4-diphenalkylpiperidine analogs were synthesized and evaluated for their affinity and inhibitory potency at the [^{3}H]dihydrotetrabenazine (DTBZ) binding site and [^{3}H]dopamine (DA) uptake site on the vesicular monoamine transporter-2 (VMAT2). Results revealed that translocation of the phenethyl side chains of lobelane from C2 and C6 to C1 and C4 around the central piperidine ring slightly reduces affinity and inhibitory potency at VMAT2 with respect to lobelane. However, methoxy and fluoro-substitution of either phenyl ring of these 1,4-diphenethyl analogs afforded VMAT2 inhibition comparable or higher (5-fold) affinity at the DTBZ binding and DA uptake sites relative to lobelane, whereas replacement of the 4-phenethyl moiety in these analogs with a 4-phenmethyl moiety markedly reduced affinity for the DTBZ binding and DA uptake sites by 3- and 5-fold, respectively. Among the twenty five 1,4-diphenethylpiperidine analogs evaluated, compounds containing a 4-(2-methoxyphenethyl) moiety exhibited the most potent inhibition of DTBZ binding and vesicular DA uptake. From this subgroup, analogs **8h**, **8j** and **8m** exhibited K_i values of 9.3 nM, 13 nM and 13 nM, respectively, for inhibition of [^{3}H]DA uptake by VMAT2, and represent some of the most potent inhibitors of VMAT2 function reported thus far.

Methamphetamine (METH) is a highly addictive psychostimulant and its abuse produces severe, deleterious health effects, including fatigue, dysphoric mood, anxiety, depression and symptoms of psychosis.[1–3] Currently, there are no FDA-approved treatments for METH abuse. The abuse liability of METH is, at least in part, due to its interaction with the brain dopamine (DA) reward system. METH reverses the normal function of the dopamine transporter (DAT) and the vesicular monoamine transporter-2 (VMAT2) greatly increasing DA concentrations in the cytosol of dopaminergic presynaptic terminals as well as in the extracellular compartment. METH also inhibits monoamine oxidase (MAO) activity, preventing the metabolism of cytosolic DA to 3,4-dihydroxyphenylacetic acid (DOPAC), consequently increasing the availability of cytosolic DA for METH-induced reverse transport by DAT and increasing DA concentrations in the extracellular compartment, resulting in subsequent stimulation of postsynaptic DA receptors.[4] Since METH inhibits DA uptake at VMAT2 and promotes DA release from vesicles to increase cytosolic DA, VMAT2 is considered an important molecular target in the search for a treatment for METH abuse.[5] Therefore, molecules that can modulate function and prevent the pharmacological effects of METH may be suitable candidates for the treatment of METH addiction.

In this respect, lobeline, the principal alkaloidal constituent of *Lobelia inflata*, has been shown to decrease amphetamine-induced and METH-induced hyperactivity in rats and mice, and to inhibit amphetamine-evoked and METH-evoked DA release from superfused rat brain slices.[6] Importantly, lobeline also decreases METH self-administration in rats without acting as a substitute reinforcer,[7,8] indicating a lack of abuse liability.

The mechanism by which lobeline reduces the reinforcing and rewarding effects of psychostimulants involves its ability to interact with VMAT2.[4,9] Lobeline inhibits VMAT2 with about 100-fold higher affinity compared to its affinity for DAT, indicating that the interaction with VMAT2 is essential for the observed decrease in the behavioral effects of METH.[4,9,10] Unlike METH, lobeline does not inhibit MAO activity, allowing DA within the cytosol to be metabolized to DOPAC.[11] Thus, lobeline diminishes the cytosolic DA pool available for METH-induced reverse transport by DAT.

Clinical evaluation of lobeline as a pharmacotherapy for METH abuse revealed that the alkaloid is safe in METH addicted individuals.[12] However, bitter taste and nausea were noted as relatively minor side-effects, likely the result of nicotinic acetylcholine

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http://dx.doi.org/10.1016/j.bmcl.2016.05.025
0960-894X/

receptor (nAChR) antagonism.[13,14] Another limitation was the short plasma half-life of lobeline, which would require multiple doses as a therapeutic, likely diminishing compliance. In an attempt to address these limitations, a significant drug discovery effort was initiated in which the lobeline molecule was structurally modified with the goal of improving potency and selectivity as an inhibitor of VMAT2, while eliminating affinity for nAChRs and other neurotransmitter transporters.[9] Lobelane (**1**), a chemically defunctionalized analog of lobeline, emerged as a lead analog that exhibited a greater potency inhibiting VMAT2 function compared to lobeline, while demonstrating little affinity for nAChRs.[13]

After carrying out comprehensive structure–activity relationship (SAR) studies[14] with lobelane analogs, it was determined that relocation of the 2,6-phenethyl moieties in the lobelane molecule (**1**) to the 1,4-positions on the central piperidine heterocycle to afford **2** ($R^1 = R^2 = H$) (Fig. 1), resulted in no loss of affinity for the [^{3}H]DBTZ binding site on VMAT2 when compared with lobelane. This structural change also maintained the low affinity for α4β2 and α7 nAChRs that was exhibited by lobelane. An added advantage was the achiral nature of **2**.

In the current study, this new scaffold was exploited by building a small library of aromatic substituted analogs of **2**, and these compounds exhibited inhibition of VMAT2 function with comparable or higher affinity relative to lobelane. Herein, we present the synthesis and evaluation of 1,4-diphenethylpiperidine and 1-phenethyl-4-benzylpiperidine analogs as inhibitors of vesicular DA uptake.

The general synthetic approach adopted for the preparation of the aromatic substituted 1,4-diphenethylpiperidine analogs is illustrated in Scheme 1. The synthesis utilizes 4-picoline (**4**) which is reacted with various substituted benzaldehydes (**3**) via Aldol condensation in acetic anhydride at reflux temperature to afford the corresponding (*E*)-4-styrylpyridine **5**. Compound **5** is then subjected to hydrogenation with Adams catalyst (PtO_2) in acetic acid under hydrogen gas (50 psi) at room temperature to afford the saturated piperidino intermediate (**6**). Intermediate **6** is alkylated with variously substituted phenethyl bromides (**7**) using K_2CO_3 in acetonitrile at reflux temperature to yield the corresponding 1,4-diphenethylpiperidine analogs **8a**–**8y**, which were further converted to hydrochloride salts with 2 M HCl in diethyl ether (Scheme 1).[15]

In addition, a small group of 1,4-diphenalkylpiperidine analogs were synthesized in which the 4-phenethyl moiety was replaced with a 4-benzyl moiety. The synthetic route for generating these compounds is shown in Scheme 2 and utilized 4-bromopyridine (**9**) as a starting point.

4-Bromopyridine was reacted with benzylmagnesium chloride using $NiCl_2$(dppp)/THF (Kumada coupling)[16] at ambient temperature to obtain the 4-benzylpyridine intermediate **10**. Compound **10** was then subjected to hydrogenation with Adams catalyst (PtO_2) in acetic acid under hydrogen gas (50 psi) at room temperature to obtain the saturated benzylpiperidino intermediate, **11**. Compound **11** was then reacted with variously substituted phenethyl bromides (**7**) in the presence of K_2CO_3 in acetonitrile at reflux temperature to yield the corresponding 1-phenethyl-4-benzylpiperidine derivatives **12a**–**12d**, which were further converted to hydrochloride salts with 2 M HCl in diethyl ether (Scheme 2).

All the synthesized compounds were evaluated for affinity for the [^{3}H]DTBZ binding site on VMAT2 and for affinity at the DA translocation site on VMAT2, using the [^{3}H]DA uptake assay in isolated synaptic vesicles (Table 1). In the 1,4-diphen-ethylpiperidine series of compounds (**8a**–**8y**, Table 1), the K_i values from the [^{3}H]DTBZ binding assay ranged from 0.15 to 2.8 μM. However, 80% of the analogs evaluated had K_i values <1 μM, notable exceptions being the 1-(3,4-dimethoxyphenethyl) analog **8c** (K_i = 2.8 μM), and the 1,4-bis(4-methoxyphenethyl) analog **8w** (K_i = 2.5 μM).

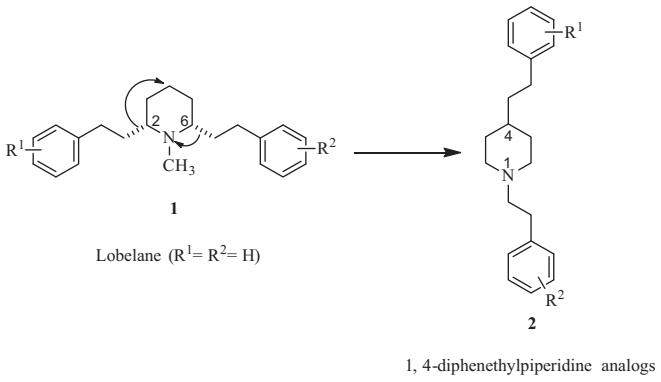
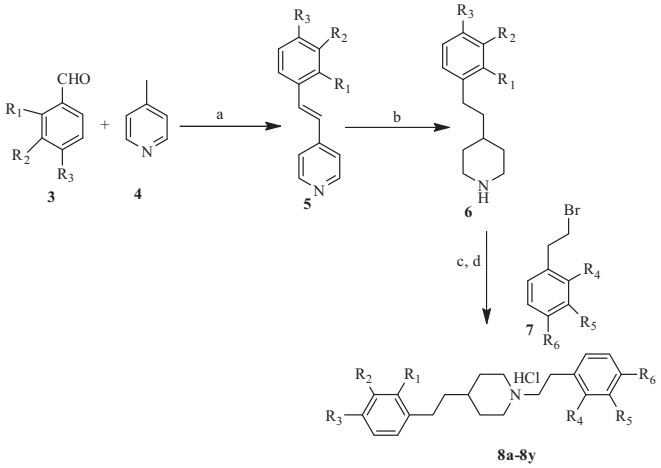
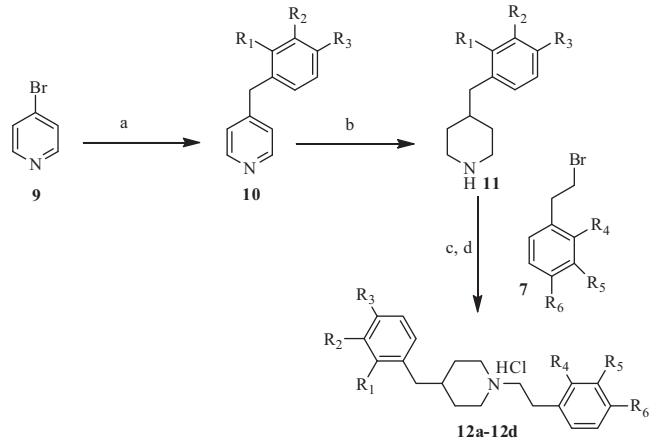


Figure 1. Relocation of the 2,6-phenethyl moieties of lobelane (**1**, $R^1 = R^2 = H$) to the 1,4-positions on the central piperidine heterocycle to afford 1,4-diphenethylpiperidine (**2**, $R^1 = R^2 = H$).



Scheme 1. Synthesis of 1,4-diphenethylpiperidine analogs. Reagents and conditions: (a) Ac_2O, reflux, 24 h, 48–60%; (b) 10% (w/v) PtO_2/H_2, AcOH, 50 psi, rt, 12 h, 75–80%; (c) K_2CO_3/acetonitrile, 80 °C, 8 h, 75–80%; (d) 2 M HCl in ether.



Scheme 2. Synthesis of 1-phenethyl-4-benzylpiperidine analogs. Reagents and conditions: (a) benzylmagnesium chloride, $NiCl_2$ (dppp), THF, rt 15 h, 85–90%; (b) H_2, PtO_2, AcOH, 50 psi, rt, 12 h, 75–80%; (c) K_2CO_3/acetonitrile, 80 °C, 8 h, 75–80%; (d) 2 M HCl in ether.

The two most potent compounds in this series were analogs **8j** and **8h** (Fig. 2), which exhibited similar affinity in the [^{3}H]DTBZ binding assay (K_i = 0.15 μM, and 0.19 μM, respectively), revealing a 5 to 6-fold higher affinity than lobelane (K_i = 0.97 μM)[14] for the