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Pyrrolidine analogs of GZ-793A: Synthesis and evaluation as inhibitors of the vesicular monoamine transporter-2 (VMAT2)

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Abstract

Central heterocyclic ring size reduction from piperidinyl to pyrrolidinyl in the vesicular monoamine transporter-2 (VMAT2) inhibitor **GZ-793A** and its analogs resulted in novel *N*-propane-1,2(*R*)-diol analogs **11a–i**. These compounds were evaluated for their affinity for the dihydrotetrabenazine (DTBZ) binding site on VMAT2 and for their ability to inhibit vesicular dopamine (DA) uptake. The 4-difluoromethoxyphenethyl analog **11f** was the most potent inhibitor of [^{3}H]-DTBZ binding (*Ki*=560 nM), with 15-fold greater affinity for this site than **GZ-793A** (*Ki*=8.29 μM). Analog **11f** also showed similar potency of inhibition of [^{3}H]-DA uptake into vesicles (*Ki*=45 nM) compared to that for **GZ-793A** (*Ki*=29 nM). Thus, **11f** represents a new water-soluble inhibitor of VMAT function. 2009 Elsevier Ltd. All rights reserved.

Keywords

Pyrrolidine analogs; GZ-793A; Dihydrotetrabenazine binding site; Vesicular monoamine transporter-2; *N*-propane-1,2(*R*)-diols

Methamphetamine (METH) abuse is a growing problem in the United States, especially in the rural West and Southwest. To date, there is no approved therapeutic agent for treating METH addiction. (−)-Lobeline (**1**, Fig. 1), the major alkaloid of *Lobelia inflata,* has been shown to decrease the rewarding effects of methamphetamine in a rat behavioral model,[1–4] and is believed to inhibit dopamine (DA) uptake into synaptic vesicles via an interaction with the tetrabenazine (TBZ) binding site on the vesicular monoamine transporter-2 (VMAT2).[5] METH is a substrate at the presynaptic dopamine transporter (DAT), and within the presynaptic terminal, METH interacts with VMAT2 located on synaptic vesicles, inhibiting DA uptake and promoting vesicular DA release, leading to increased cytosolic DA concentrations in the extracellular space.[6,7] Hence, VMAT2 has been identified as a valid target for the development of therapeutic agents that inhibit VMAT2 function, counteracting the effects of METH.

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The chemically defunctionalized lobeline analogue, lobelane (2*S*,6*R*-1-methyl-2,6-diphenethylpiperidine) (**2**, Fig. 1), and its analogues have recently been reported to have 10–15-fold more potency and selectivity for inhibition of [^{3}H]dopamine ([^{3}H]DA) uptake into synaptic vesicles when compared to lobeline.[8–11]

Additionally, an extensive study on the variation of methylene linker length between C-6 and C-2 of the piperidine ring and each of the two phenyl rings in the lobelane molecule confirmed that a minimum of two methylene units in the alkane linker moiety is essential for retention of binding affinity at the TBZ binding site on VMAT2.[12]

Recently, we have identified a novel structurally optimized *N*-propane-1,2-diol analogue of lobelane (GZ-793A; **3**, Fig. 1) which potently and selectively interacts with VMAT2 to inhibit DA uptake.[13,14] GZ-793A also blocked METH self-administration (SA) and reward in rats, and had no effect on responding for food.[15,16] The physicochemical properties of GZ-793A provide enhanced water-solubility and drug-likeness relative to the majority of previously reported lobelane analogs.

In a previous communication, we have reported on a series of pyrrolidino analogs of lobelane (**2**), and have shown that VMAT2 inhibition is increased when the piperidine ring of lobelane is replaced by a pyrrolidine ring.[10] The main aim of the current study was to determine [^{3}H]DTBZ binding affinity and inhibition of [^{3}H]DA uptake by VMAT2 by, of a series of novel *N*-1,2(*R*)-dihydroxylpropyl group (NDHP) substituted pyrrolidine analogs of GZ-793A.

From previous SAR studies, it was found that lobelane analogues containing either fluorophenethyl or methoxyphenethyl moieties were the most potent VMAT2 ligands.[14] These observations prompted us to focus on the synthesis and evaluation of *N*-1,2(*R*)-dihydroxylpropyl (NDHP) pyrrolidine analogues (**11a–i**) as inhibitors of VMAT2 function.

The synthesis of analogues **11a–i** was achieved via initial reaction of benzotriazole, (*S*)-phenylglycinol (formed from LAH reduction of (*S*)-phenylglycine), and succindialdehyde at room temperature to afford the key synthon, (3*S*,5*R*, 7*aR*)-5-(benzotriazol-1-yl)-3-phenyl[2,1-*b*]oxazolopyrrolidine (**6**),[17] which upon reaction with a variety of substituted phenethyl magnesium halides (**7a–i**) in tetrahydrofuran afforded a 2:1 mixture of the appropriate *cis*- and *trans*-isomers, **8** and **9**. These isomers were then separated by silica gel chromatography (8:2 hexane: ethyl acetate) to afford the appropriate 2*R*,5*S*- and 2*R*,5*R*-diastereomers **8a–i** and **9a–i**. Compounds **8a–i** were hydrogenolyzed by catalytic-transfer hydrogenation with palladium hydroxide-over-carbon, employing ammonium formate as the hydrogen source in refluxing methanol. These conditions afforded a quantitative conversion to the respective *N*-deprotected products **10a–i** within 30 min, as opposed to 12 h utilizing previously reported conditions (i.e., H_2/Pd-C).[10] The *cis-meso*-2,5-diarylethyl pyrrolidine free bases were each refluxed with *S*-glycidol in ethanol to afford a series of substituted *cis-meso*-2,5-diaryl ethylpyrrolidin-1-yl-propane-1,2(*R*)-diols (**11a–i**), which were then converted to their respective HCl salts using ethereal HCl. The products were fully characterized by ^{1}H and ^{13}C-NMR spectroscopy and mass spectrometry; representative examples are provided in the reference section.[18]

The hydrochloride salts of the pyrrolidino analogues (**11a–i**) were evaluated for their affinity for the DTBZ binding site on VMAT2 and for their ability to inhibit [^{3}H]DA uptake into synaptic vesicles (functional inhibition of VMAT2) (Table 1).

Nine GZ-793A analogues were evaluated. The binding affinity of these compounds at the DTBZ binding site on VMAT2 ranged from 560 nM to >100 μM. The most potent

compound was the 4-difluoromethoxy substituted 2,5-diphenethylpyrrolidin-1-yl-propane-1,2(*R*)-diol analogue, **11f** (*Ki*=560 nM) (Table 1,Fig. 2), which had a 15-fold greater affinity for this site than GZ-793A (**3**, *Ki*=8.29 μM). The binding affinity of the 2-, 3-, and 4-methoxy analogues **11b**–**11d** also exhibited slightly higher affinity than GZ-793A at the DTBZ binding site on VMAT2.

In the vesicular DA uptake assay, the 4-difluoromethoxy analogue, **11f** (K_i =45 nM) (Table 1, Fig. 3), and the 4-methoxy analogue, **11d** (K_i=49 nM) (Table 1, Fig. 4), both exhibited similar inhibition of [^{3}H]DA uptake when compared to GZ-793A (**3**, *Ki*=29 nM). Thus, if one considers the binding affinity and functional data for compounds **11d**, **11f**, and GZ-793A (**3**), ring size reduction of the piperidino moiety in GZ-793A appears to lead generally to an improvement in affinity for the DTBZ binding site, but does not alter inhibition of vesicular DA uptake.

Synaptic vesicles were prepared as described previously.[19] Briefly, fresh whole brain (excluding cerebellum) was homogenized in 28 mL of ice-cold 0.32 M sucrose using a glass homogenizer (7 up and down strokes of a Teflon pestle, clearance 0.003 in). Homogenates were centrifuged at 1000*g* for 12 min at 4°C. Resulting supernatants (S1) were centrifuged at 22,000*g* for 10 min. The resulting pellets (P2), containing the synaptosomes, were resuspended in 18 mL of ice-cold Milli-Q water for 5 min with 7 up and down strokes of the Teflon pestle homogenizer. Osmolarity was restored by immediate addition of 2 mL of 25 mM HEPES and 100 mM K_2-tartrate buffer (pH 7.5). Samples were centrifuged at 20,000*g* for 20 min. $MgSO_4$ (final concentration, 1 mM) was added to the resulting supernatants (S3). Final centrifugations were performed at 100,000g for 45 min. Pellets (P4) were resuspended immediately in ice-cold buffer providing ~15 μg of protein/100 μL.

[^{3}H]DTBZ binding to synaptic vesicle membranes was performed according to previously described procedures.[20] Briefly, 100 μL of vesicles suspension was incubated in assay buffer (in 25 mM HEPES, 100 mM K_2-tartrate, 5 mM $MgSO_4$, 0.1 mM EDTA, and 0.05 mM EGTA, pH 7.5, 25°C) in the presence of 5 nM [^{3}H]DTBZ and 1 nM-1 mM lobelane analogs (final concentrations) for 30 min at room temperature. Nonspecific binding was determined in the presence of 20 μM TBZ. Assays were performed in duplicate using Unifilter 96-well GF/B filter plates (presoaked in 0.5% polyethylenimine) and terminated by harvesting using a FilterMate harvester. After washing five times with 350 μL of the ice-cold wash buffer (in 25 mM HEPES, 100 mM K_2-tartrate, 5 mM $MgSO_4$, and 10 mM NaCl, pH 7.5), filter plates were dried, bottoms were sealed, and each well was filled with 40 μL of Packard's MicroScint 20 cocktail. Bound [^{3}H]DTBZ was measured using a Packard Top Count NXT scintillation counter and a Packard Windows NT-based operating system.

Inhibition of [^{3}H]DA uptake was conducted as previously described,[22] utilizing a striatal synaptic vesicle preparation. Briefly, rat striata were homogenized with 10 up and down strokes of a Teflon pestle homogenizer (clearance ~ 0.003″) in 14 ml of 0.32 M sucrose solution. Homogenate was centrifuged (2,000 *g* for 10 min at 4°C), and the resulting supernatant was centrifuged again (10,000 *g* for 30 min at 4°C). The pellet was 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 immediate restoration of osmolarity by adding 900 μl of 0.25M HEPES buffer and 900 μL 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 hr at 4°C), followed by the addition of 100 μL of 10 mM MgSO4, 100 μL of 0.25 M HEPES and 100 μL of 1.0 M potassium tartrate solution prior to the final centrifugation (100,000 *g* for 45 min at 4°C). The final pellet was resuspended in 2.4 mL of assay buffer (25 mM HEPES, 100 mM potassium tartrate, 50 μM EGTA, 100 μM EDTA, 1.7 mM ascorbic acid, 2 mM ATP-Mg^{2+}, pH 7.4). Aliquots of the vesicular suspension (100

μL) were added to tubes containing assay buffer, various concentrations of inhibitor (0.1 nM - 10 mM) and 0.1 μM [^{3}H]DA in a final volume of 500 μL. Nonspecific uptake was determined in the presence of Ro4-1284 (10 μM). Reactions were terminated by filtration, and radioactivity retained by the filters was determined by liquid scintillation spectroscopy (Liquid scintillation analyzer; PerkinElmer Life and Analytical Sciences, Boston, MA).

In summary, reduction in ring size of the central heterocyclic piperidine ring of GZ-793A has led to the discovery of the 4-methoxy and 4-difluoromethoxy *cis-meso*-2,5-disubstitued pyrrolidine analogues **11d** and **11f**. These two analogues showed similar inhibitory potency of [^{3}H]DA uptake into vesicles (*Ki* = 49 and 45 nM, respectively) when compared to that for GZ-793A (*Ki* =29 nM), and were the most potent analogues in the series in this assay. Analogue **11f** was also the most potent inhibitor of [^{3}H]DTBZ binding in the series (*Ki* =560 nM), and had a 15-fold greater affinity for this site than GZ-793A (*Ki* =8.29 μM). Thus, **11d** and **11f** represent new water-soluble analogues of GZ-793A that inhibit VMAT2 function.

Acknowledgments

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References and notes

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18. Spectral data of some potent compounds; Compound **11a**: (free base), ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12–7.40 (m, 10H), 3.90–3.95 (m, 1H), 3.80-3.46 (m, 3H), 3.40-2.95 (m, 4H), 2.72-2.40 (m, 4H), 2.38-1.95 (m, 6H), 1.90-1.82 (m, 2H) *ppm*; ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.0, 128.8, 128.5, 126.6, 72.1, 69.7, 67.6, 56.1, 32.7, 32.3, 27.8 *ppm*; MS (EI) *m/z* 353 (M^+). Compound **11c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.38 (brs, 1H), 7.16 (t, 2H, *J*=7.5 Hz), 6.74-6.70 (m, 6H), 4.15 (m, 1H), 3.75 (s, 6H), 3.58-3.35 (m, 2H), 3.13-2.78 (m, 4H), 2.77-2.74 (m, 2H), 2.50-2.38 (m, 6H), 2.16-1.99 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.9, 141.2, 129.9, 120.8, 114.3, 112.0, 70.5, 69.32, 67.6, 64.3, 55.5, 32.7, 31.5, 27.6; MS (EI) m/z; 413 (M^+). Compound **11d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.86 (brs, 1H), 7.05-6.95 (m, 4H), 6.95-6.82 (m, 4H), 4.05 (brs, 1H), 3.80-3.42 (m, 12H), 3.28-2.95 (m, 2H), 2.80-1.79 (m, 10H) *ppm*; ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.2, 131.7, 129.5, 114.2, 70.3, 69.0, 67.7, 64.4, 55.6, 32.1, 31.9, 27.8 *ppm*; MS (EI) m/z; 413 (M^+). Compound **11f**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.85 (brs, 1H), 7.35-7.15 (m, 4H), 6.95–7.10 (m, 4H), 6.47 (t, 2H, *J* = 72.0 Hz), 4.19 (brs, 1H), 3.60-3.32 (m, 6H), 3.15-2.85 (m, 4H), 2.75-1.70 (m, 8H) *ppm*; ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.7, 137.2, 129.7, 119.7, 115.9, 67.9, 65.9, 64.0, 48.6, 32.9, 31.3, 27.5 *ppm*; MS (EI) m/z; 485 (M^+). Compound **11g**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.42 (brs, 1H), 6.70-6.59 (m, 6H), 5.89 (s, 4H), 5.32 (brs, 1H), 4.20 (m, 1H), 3.73-3.50 (m, 2H), 2.33–3.98 (m, 3H), 2.82-2.62 (m, 2H), 2.60-2.28 (m, 6H), 2.16-2.00 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.9, 146.2, 133.3, 121.3, 108.6, 101.1, 70.6, 69.3, 67.7, 64.3, 57.5, 32.4, 31.8, 27.6; MS (EI) m/z 441 (M^+). Compound **11i**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.92 (brs, 1H), 6.91-6.64 (m, 6H), 4.30-4.21 (m, 1H), 3.65-3.55 (m, 2H), 3.25-2.95 (m, 4H), 2.92-2.83 (m, 4H), 2.62-2.25 (m, 4H), 2.25-1.90 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.2, 141.3, 112.3, 102.1, 69.3, 67.7, 65.3, 49.5, 32.4, 31.8, 27.6; MS (EI) m/z; 425(M^+).

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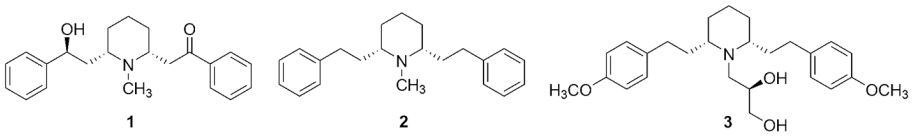


Fig 1.
Molecular structures of lobeline (**1**), lobelane (**2**), and GZ-793A (**3**).

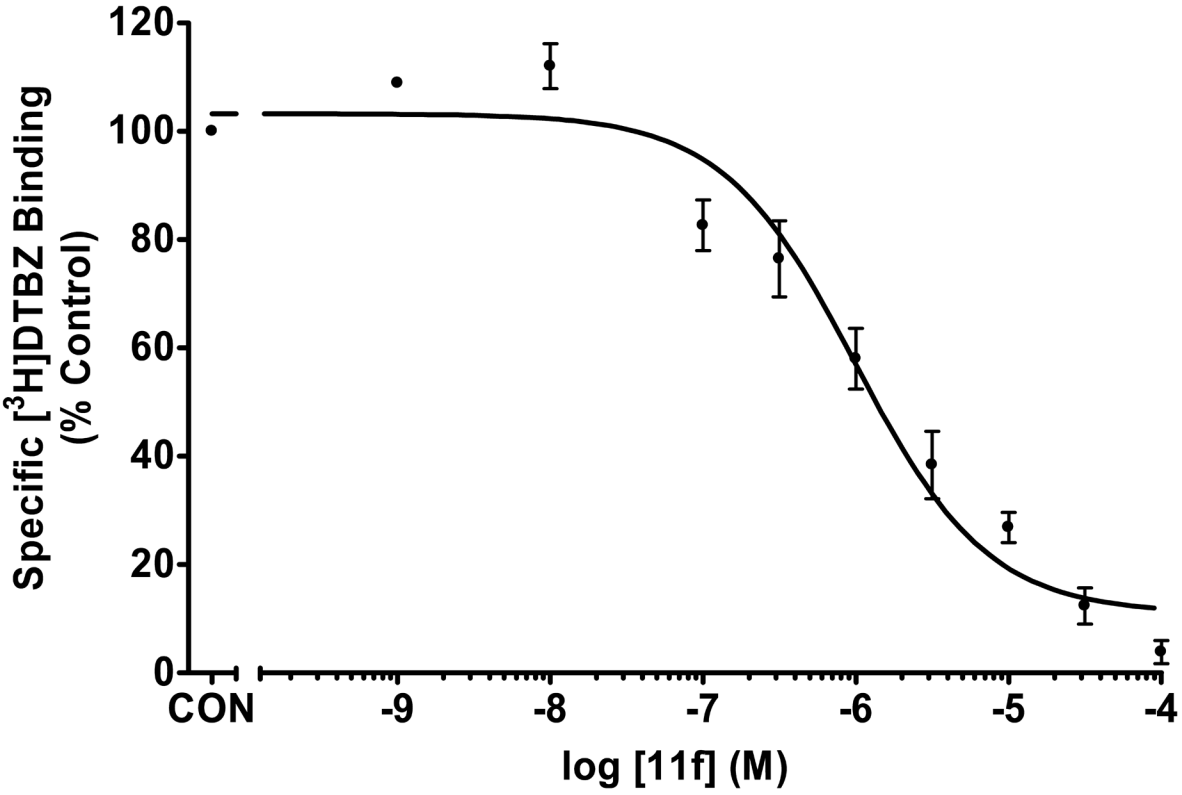


Fig. 2.
Affinity of Analogue **11f** at the TBZ binding site on VMAT2 in the [^{3}H]DTBZ binding assay.

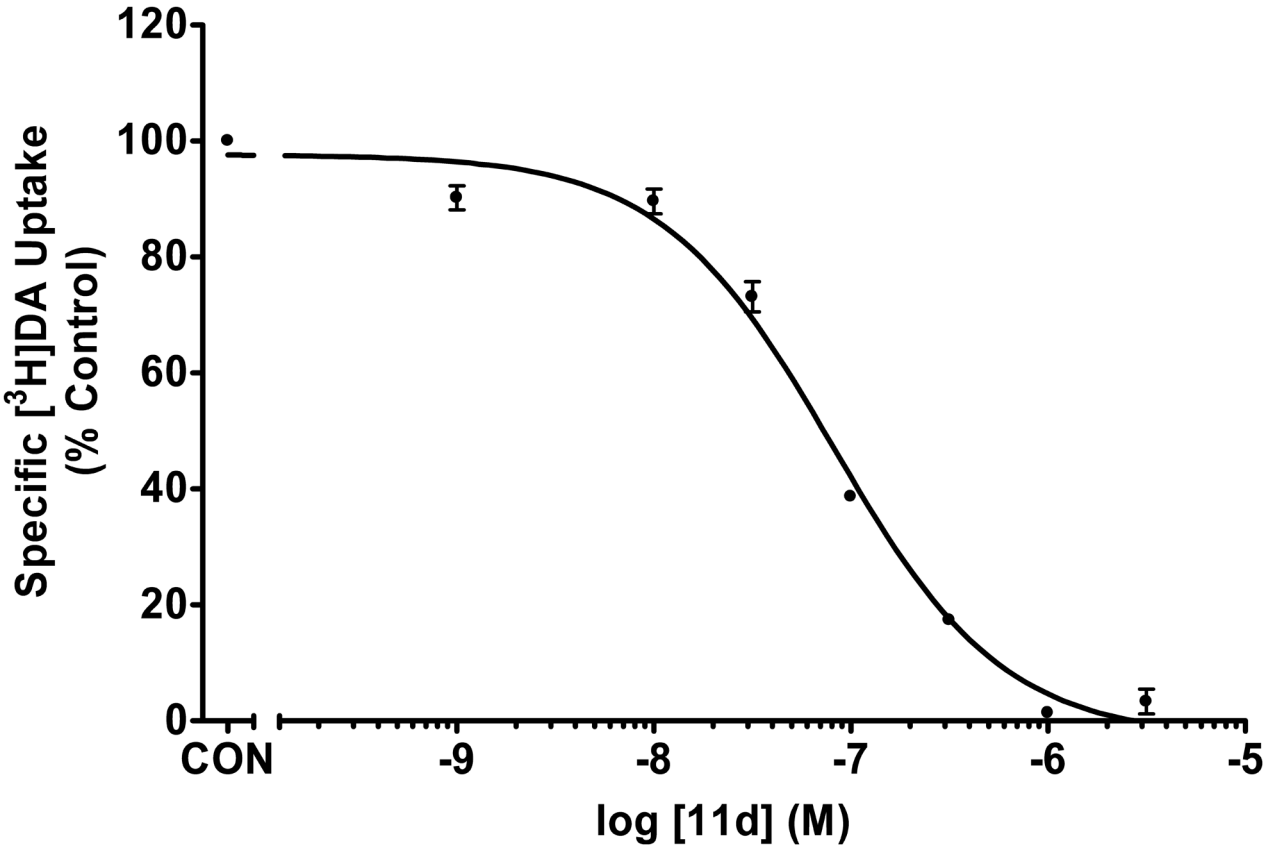


Fig. 3.
Inhibition of [^{3}H]DA uptake into rat synaptic vesicles by analogue **11d**

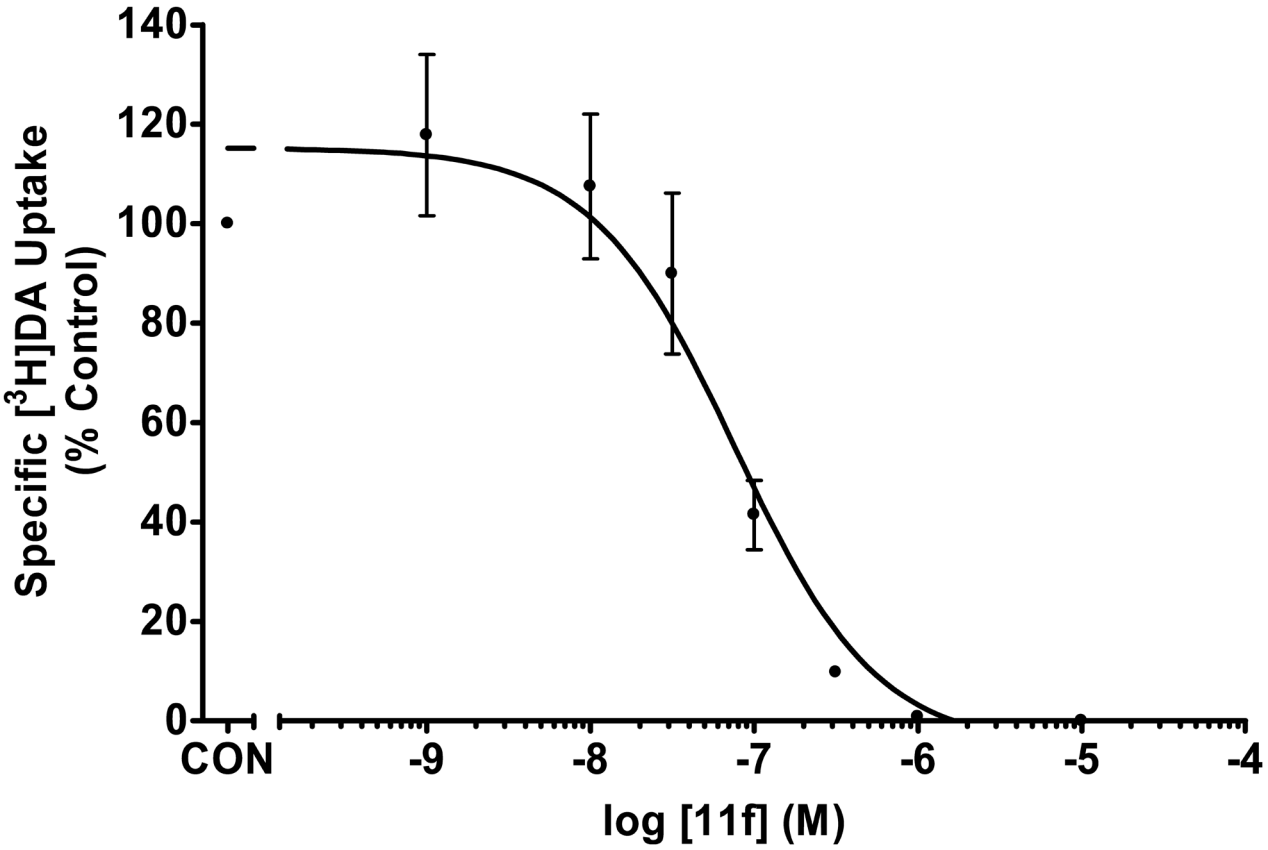
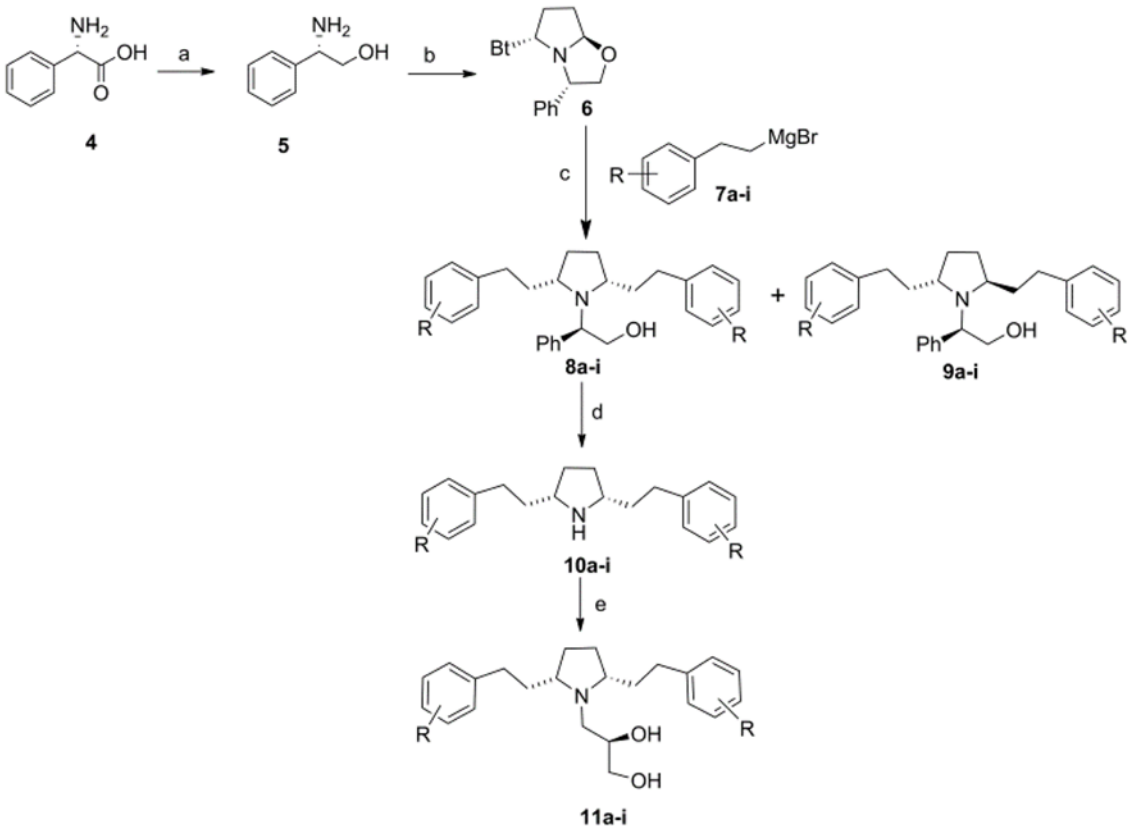


Fig. 4.
Inhibition of [^{3}H]DA uptake into rat synaptic vesicles by analogue **11f**

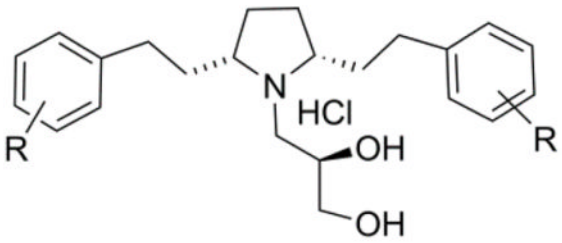


Scheme 1.

Reagents and conditions: a) $LiAlH_4$, THF, reflux, 24h; b) 2,5 dimethoxy tetrahydrofuran, benzotriazole, 10% aq HCl, rt, 12h: c) Substituted phenethyl magnesium bromides (**7a–i**), THF, rt, 12h; d) $Pd(OH)_2$, NH_4HCO_2, MeOH, reflux, 30 min; e) (*S*)-glycidol, EtOH, reflux, 24h.

Table 1

Inhibition constants (K_i) for GZ-793A (**3**) analogs at the [^{3}H]DTBZ binding site and [^{3}H]-DA uptake site on VMAT2



Comp	R	[^{3}H]DTBZ Binding[a]	VMAT2 [^{3}H]DA uptake[c]
		Ki±SEM (μM)[b]	*Ki*±SEM (nM)
3	-	8.29 ± 2.79	29 ± 8
11a	H	5.91 ± 1.57	279 ± 35
11b	2-OCH_3	2.35 ± 0.44	270 ± 6
11c	3-OCH_3	2.28 ± 0.44	160 ± 10
11d	4-OCH_3	3.94 ± 1.12	49 ± 9
11e	3-F	6.76 ± 1.68	170 ± 15
11f	4-$OCHF_2$	0.56 ± 0.23	45± 7
11g	3,4-methylenedioxy	8.47 ± 5.19	269 ± 28
11h	3,4-dimethoxy	> 100	3780 ± 230
11i	3,5-difluoro	4.06 ± 0.81	200 ± 1

[a] All binding experiments have n=3,

[b] Each K_i value represents mean ±SEM.

[c] All uptake experiments represent n=4