Supporting Information

Pyrrolidine analogs of lobelane: Relationship of affinity for the dihydrotetrabenazine binding site with function of the vesicular monoamine transporter 2 (VMAT2)

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General experimental procedures

THF and diethyl ether were distilled freshly from Na°/benzophenone ketyl under Ar prior to their utilization. All other solvents were used as received. Phenethylmagnesium bromide, benzylmagnesium chloride and Phenpropylmagnesium bromide were prepared freshly from Mg° turnings and the corresponding alkyl halides in THF or diethyl ether and the solutions were titrated for their organomagnesium content against menthol with 9,10-phenanthroline as the indicator. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer. Elemental analyses were conducted either by Atlantic Microlab, GA or by M-H-W Laboratories, AZ. Compounds evaluated for pharmacological properties were judged to be sufficiently pure (≥ 95%) when their percentage elemental compositions upon combustion were within ± 0.4% of the corresponding theoretical values. All of the compounds subjected to pharmacological evaluation were found to satisfy this requirement, as shown below:

Combustion analyses

Compound	Molecular Formula	Calculated			Found		
		C	H	N	C	H	N
6	$C_{20}H_{26}ClN$	76.05	8.30	4.43	76.40	8.22	4.40
7	$C_{21}H_{28}ClN \bullet 0.5\ H_2O$	74.42	8.62	4.13	74.08	8.80	3.83
8	$C_{20}H_{26}ClN$	76.05	8.30	4.43	75.96	8.30	4.18
9	$C_{21}H_{28}ClN \bullet H_2O$	72.49	8.69	4.03	72.15	9.00	3.79
10	$C_{20}H_{26}ClN \bullet H_2O$	71.94	8.45	4.19	71.86	4.21	4.05
11	$C_{21}H_{28}ClN$	76.45	8.55	4.25	76.42	8.40	4.48
14	$C_{28}\ H_{33}NO$	84.17	8.32	3.51	83.85	8.07	3.21
15	$C_{28}\ H_{33}NO \bullet 0.5\ H_2O$	82.31	8.39	3.43	81.97	8.17	3.40
18	$C_{28}\ H_{33}NO$	84.17	8.32	3.51	84.20	8.10	3.29
19	$C_{28}\ H_{33}NO$	84.17	8.32	3.51	84.41	8.02	3.16
22	$C_{18}H_{22}ClN$	75.11	7.70	4.87	74.72	8.01	4.96
23	$C_{19}H_{24}ClN \bullet H_2O$	71.34	8.19	4.38	70.95	8.49	4.18
25	$C_{22}H_{30}ClN$	76.83	8.79	4.07	76.81	8.72	4.00
26	$C_{23}H_{32}ClN \bullet H_2O$	73.47	9.11	3.73	73.12	9.48	3.51

Preparation of Compounds 21 and 24.

(2*S*)-2-[(2*R*,5*S*)-2,5-Di(2-benzyl)-tetrahydro-1*H*-1-pyrrolyl]-2-phenylethan-1-ol (21) A small crystal of iodine was added to a mixture of magnesium turnings (1.20 g) and diethyl ether (10 mL) without stirring. Benzyl bromide (0.2 mL) was added and the mixture was allowed to stand for 2 min. at room temperature. Vigorous stirring was then begun and the mixture changed color from brown to metallic gray, at which time, it was cooled to -15 ^{o}C in a salt-ice bath and diluted with diethyl ether (50 mL). Benzyl bromide (4 mL) was added over a period of 1 hour and the mixture was allowed to warm to room temperature and stirred for 30 min., after which, it was titrated for organomagnesium content (92% yield). In a separate flask, **12** (306 mg, 1 mmol) was suspended in THF (5 mL) and treated with $MgBr_2$ in THF (10 mmol, from Mg and dibromoethane). Benzylmagnesium chloride (6 mmol) were added via a syringe, and the resulting pale yellow suspension was left stirring for 4 h. HCl (1 mL) followed by NaOH (2*N*, 20 mL) was added and the mixture was extracted with 3 x 50mL portions of diethyl ether/hexanes (1:1). The organic layer was dried and evaporated under reduced pressure to yield a clear oil that was loaded onto a 10-g silica gel column and eluted with hexanes/EtOAc (15:1) to afford **21** (170 mg, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.20–7.06 (m, 15H, Ar), 4.14–3.95 (m, 2H), 3.60–3.50 (m, 1H), 3.33–3.10 (br, s, 1H), 3.02–2.88 (m, 2H), 2.85–2.33 (m, 2H), 2.07–0.98 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 143.7, 141.5, 141.2, 137.2, 129.1, 129.3, 128.7, 128.4, 128.5, 128.4, 128.2, 128.1,128.0, 127.9, 125.9, 64.8, 63.6, 61.9, 57.6, 38.1, 29.8, 29.2, 28.9. $[\alpha]_D^{20}$ +13.2° (*c* 1.00, CH_2Cl_2).

(2*S*)-2-[(2*R*,5*S*)-2,5-Di(3-phenylpropyl)-tetrahydro-1*H*-1-pyrrolyl]-2-phenylethan-1-ol (24) To 306 mg (1 mmol) of **1** suspended in 10 mL of THF was added $MgBr_2$ (10 mmol, from Mg and dibromoethane). A solution of phenpropylmagnesium bromide in THF (6 equiv.) was added over a period of 10 minutes at 0 ^{o}C. The solution was allowed to warm to ambient temperature overnight, after which 10 mL of 2*N* NaOH were added and the biphasic mixture was extracted with diethyl ether (3 × 100 mL). The

etheral layers were combined, diluted with 50 mL of CH_2Cl_2, dried with Na_2SO_4, and evaporated under reduced pressure to afford a yellow oil. The oil was dissolved in 20 mL of dry diethyl ether and HCl gas was bubbled through the solution with stirring for 10 minutes. The supernatant was decanted and the gummy residue was triturated with 5 x 50-mL portions of diethyl ether. A solution of NaOH (1*N*, 20 mL) was then added and the suspension extracted with diethyl ether (2 × 100 mL). The organic layers were washed with brine, dried and evaporated, and the resulting oily residue was applied to a silica gel column (10 g). The column was eluted with 40:1 (hexanes/diethyl ether) followed by 15:1 (hexanes/diethyl ether) to afford **24** (205 mg, 48%) as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.60–6.98 (m, 15H, Ar), 3.99–3.43 (m, 3H), 3.43 (overlap)–3.18 (br, s, 1H), 3.24–2.86 (m, 2H), 2.76–2.35 (m, 4H), 2.03–1.38 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 143.7, 139.2, 137.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.5, 127.9, 127.4, 127.1, 127.0, 125.9, 121.4, 64.8, 63.6, 61.9, 57.6, 38.1, 36.4, 29.8, 29.7, 29.2, 29.0 28.9, 28.86 $[\alpha]_D^{20}$ +33.2° (*c* 0.4, CH_2Cl_2).

Preparation of rat brain synaptic vesicles. Fresh whole brain (excluding cerebellum and brain stem) was homogenized in 20 vol of ice-cold 0.32 M sucrose using a glass homogenizer (7 strokes of a Teflon pestle, clearance = 0.003 in). Homogenates were centrifuged at 1000 g for 12 min at 4 ºC. Resulting supernatants (S1) were centrifuged at 22,000 g for 10 min. Resulting pellets (P2), containing the synaptosomes, were resuspended in 18 mL ice-cold Milli-Q water for 5 min with 7 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 100,000 g for 45 min. Pellets (P4) were resuspended immediately in ice-cold buffer (see below) providing ~15 μg protein/100 μL.

[^{3}H]DTBZ binding assay. 100 μL of vesicles suspension was incubated in assay buffer (in mM: 25 HEPES, 100 K_2-tartrate, 5 $MgSO_4$, 0.1 EDTA and 0.05 EGTA, pH 7.5, 25 ºC) in the presence of 5 nM [^{3}H]DTBZ and 1 nM –1 mM lobelane analogues (final concentrations) for 30 min at room temperature. Nonspecific binding was determined in the presence of 10 μM Ro4-12084. Assays were performed in duplicate using the Unifilter-96 96-well GF/B filter plates (presoaked in 0.5% polyethylenimine) plates and terminated by harvesting using the FilterMate harvester. After washing 5 times with 350 μL of the ice-cold wash buffer (in mM: 25 HEPES, 100 K_2-tartrate, 5 $MgSO_4$ and 10 NaCl, pH 7.5), filter plates were dried, bottoms-sealed and each well filled with 40 μL Packard's MicroScint 20 cocktail. Bound [^{3}H]DTBZ was measured using a Packard TopCount NXT scintillation counter and a Packard Windows NT-based operating system.

[^{3}H]-DA uptake inhibition assay. Inhibition of [^{3}H]-DA uptake was conducted in a preparation of isolated synaptic vesicles.[1] Briefly, rat striata were homogenized with 10 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).

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(1) Teng, L.; Crooks, P.A.; Sonsalla, P.K.; Dwoskin, L.P. Lobeline and nicotine evoked [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]dopamine: differential inhibition of synaptosomal and vesicular [^{3}H]dopamine uptake. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 1432–1444.