Supporting Information

Conantokins Derived from the *Asprella* Clade Impart Con*Rl*-B, an NMDA Receptor Antagonist with a Unique Selectivity Profile for NR2B Subunits

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Table S1. Selected examples of antagonists for NMDARs containing NR2B subunit.

NR2B antagonist	**NR1/NR2B IC_{50} or K_i (μM)**	**Off Target Activity**	**References**
Ifenprodil	.03	Sigma-1, Sigma-2, Alpha-1 adrenergic receptor; GIRK channels, hERG channels; serotonin (5-hydroxytrypt amine; 5-HT) subtype 1A, 5-HT2 and 5-HT3 receptors, voltage-gated Ca^{2+} channels	Hashimoto & London 1993; Kobayashi et al., 2006; Monassier et al., 2007; Chenard et al., 1991; McCool & Lovinger, 1995; Church et al., 1994; Bath et al., 1996; Chenard & Menniti, 1999
Ro 25-6981	.009	Alpha-1 adrenergic receptor	Fischer et al., 1997; Pinard et al., 2001
CP 101,606	.014	Sigma-1 receptor; hERG channel	Hashimoto & London, 1993; Cougenhour & Barr, 2001; Kawai et al., 2007
Eliprodil	.128	Alpha-1 adrenergic receptors; Voltage-gated Na^{+} channels; Voltage-gated Ca^{2+} channels	Chenard & Menniti, 1999
N-(2-(3,4-Dichlorophenylamino)ethyl)-2-(4(methylsulfonamido)phenoxy)acetamide (Compound 52)	.054	Weakly targets hERG channel	Mosley et al., 2009
(*S*)-1-(4-Methanesulfonamidephenoxy)-3-(3,4-dichlorophenylethylamino)-2-propanol (Compound 29)	.050	hERG; Alpha-1 and alpha-2 adrenergic receptors; Serotonin, dopamine, and norepinephrine transporters	Tahirovic et al., 2008
Clobenpropit/ Iodophenpropit	1-1.4	Histamine 3 receptor	Hansen et al., 2010
7-methoxy-3-(4-phenylbutyl)-2,3,4,5,-tetrahydro-1H-3-benza-zepin-1-ol) (Compound 13)	.005	Some activity at sigma-1 and sigma-2 receptor at higher concentrations (K_i~180 nM, ~550 nM respectively)	Tewes et al., 2010; Beinat et al., 2010
4-Methylbenzyl 4-[(Pyrimidin-2-ylamino) methyl]piperidine-1-carboxylate (Compound 20j)	.003 (k_i)	IC50 for hERG channel >10μM; no other activity at any "off targets" tested (10 μM concentration) (disclaimer: data not shown.)	Liverton et al., 2007

Table S2. Purity, HPLC retention times and mass spectrometry results for conantokins studied in this work.

Peptide	Purity	RT[a] [min]	MS (calcul.) [M]	MS (found.) [M+1]
Con*Rl*-B	98%	24.38	2236.96	2237.94
Con*Rl*-B[O10P]	98%	23.00	2220.96	2221.95
Con*Rl*-B[O10A]	98%	32.60	2194.94	2195.92
Con*Rl*-B[K7X][b]	100%	29.64	2221.95	2222.90
Con*Rl*-B[desO10]	98%	20.87	2123.90	2124.88
Con*Rl*-B[L5Y]	99%	19.78[c]	2286.90	2287.50
Con*Rl*-B[desKAO;N8Q9]	100%	18.98[c]	2166.85	2167.40
Con*Rl*-C	100%	32.33	2224.91	2225.93
Con*Rl*-B[γ7K]	100%	26.15[c]	2192.02	2192.99
Con*Rl*-B[γ15K]	100%	25.60[c]	2192.02	2192.99

[a] RT determined on analytical C_{18} Vydac reversed-phase HPLC with a flow rate 1mL/min and linear gradient 20%-50% of solvent B (solvent A – 0.1% (v/v) TFA in water; solvent B – 0.1% TFA (v/v) TFA n 90% aqueous acetonitrile)

[b] X denotes L-norleucine

[c] RT determined on analytical C_{18} Vydac reversed-phase HPLC with a flow rate 1mL/min and linear gradient 15%-45% of solvent B

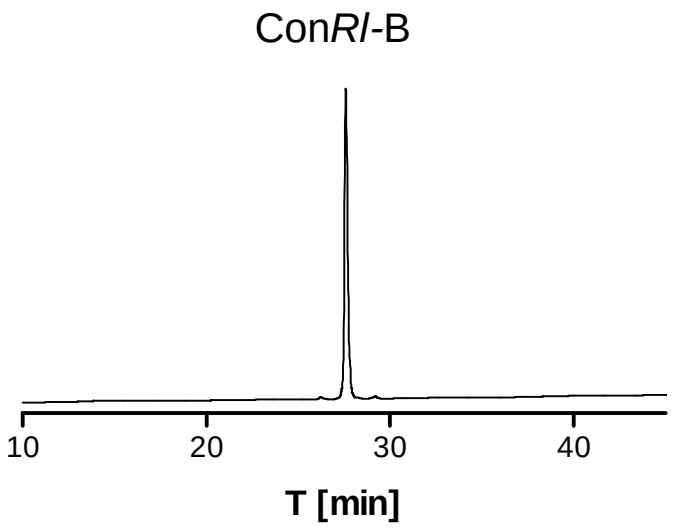

Figure S1. Example of an HPLC trace of synthetic Con*Rl*-B recorded on analytical analytical C_{18} Vydac reversed-phase HPLC with a flow rate 1mL/min and linear gradient 20%-50% of solvent B (solvent A – 0.1% (v/v) TFA in water; solvent B – 0.1% TFA (v/v) TFA in 90% aqueous acetonitrile).

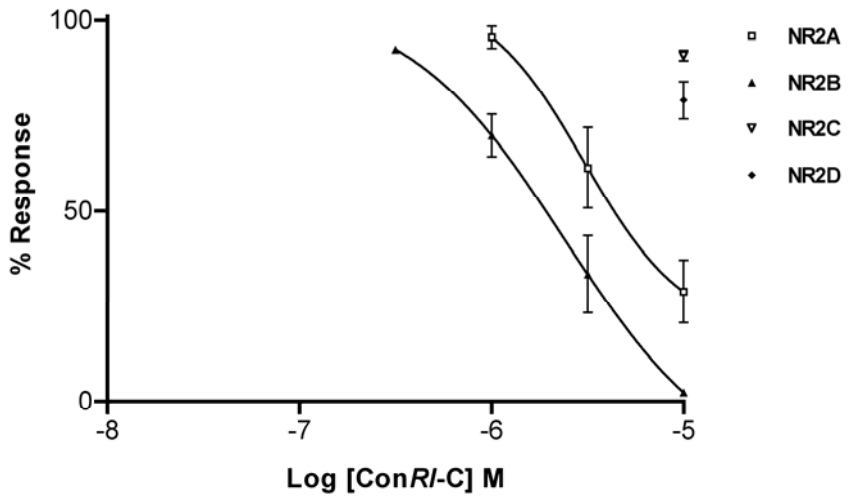

Figure S2. Concentration-response curve of Con*RI*-C on the four different NR2 subunits of NMDA receptor separately co-expressed with NR1-2b in *Xenopus* oocytes (n=3 tests for each data point; error bars represent standard error of the mean (SEM). Curve fitting was performed as described in experimental procedures.

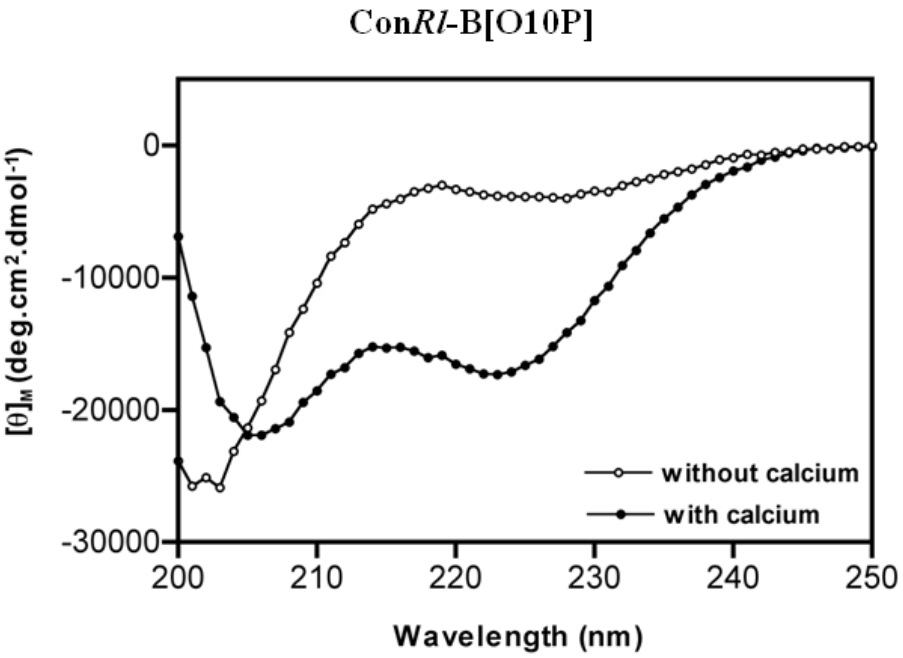

Figure S3. Circular dichroism spectroscopy of Con*Rl*-B[O10P]. Spectra were recorded with (or) without 2mM CaCl2 containing 10 mM HEPES buffer at pH 7.0 and shown is an average spectra obtained from five independent scans (n=5). Estimated percentage of helicity of peptide in the absence of calcium is 11% and in the presence of calcium is 51%.

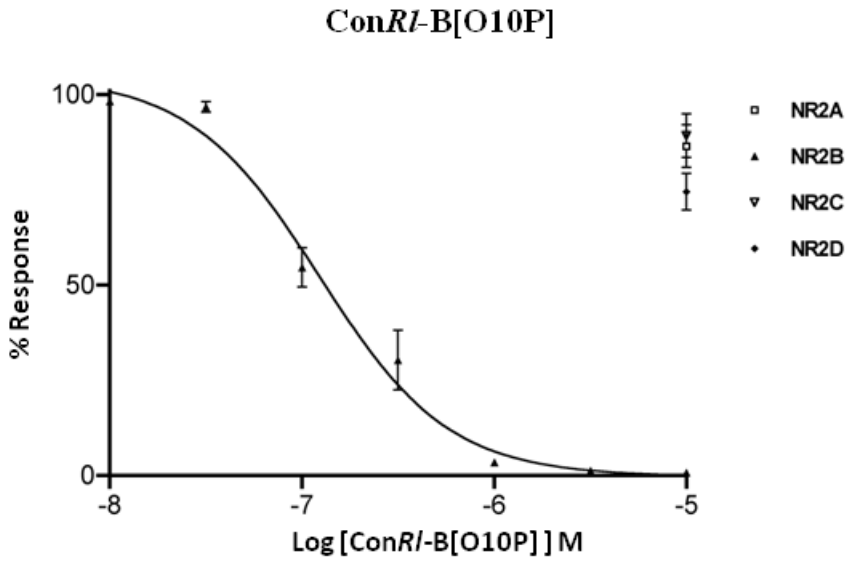

Figure S4. Concentration-response curve of Con*Rl*-B[O10P] on the four different NR2 subunits of NMDA receptor separately co-expressed with NR1-2b in *Xenopus* oocytes (n=3 tests for each data point; error bars represent standard error of the mean (SEM). Curve fitting was performed as described in experimental procedures. The approximate IC_{50} value of Con*Rl*-B[O10P] for NR2B subunit of NMDA receptor is 0.12μM, similar to that of Con*Rl*-B having 0.1μM.

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