Supplementary Data for JPET#174144

A novel family of negative and positive allosteric modulators of NMDA receptors

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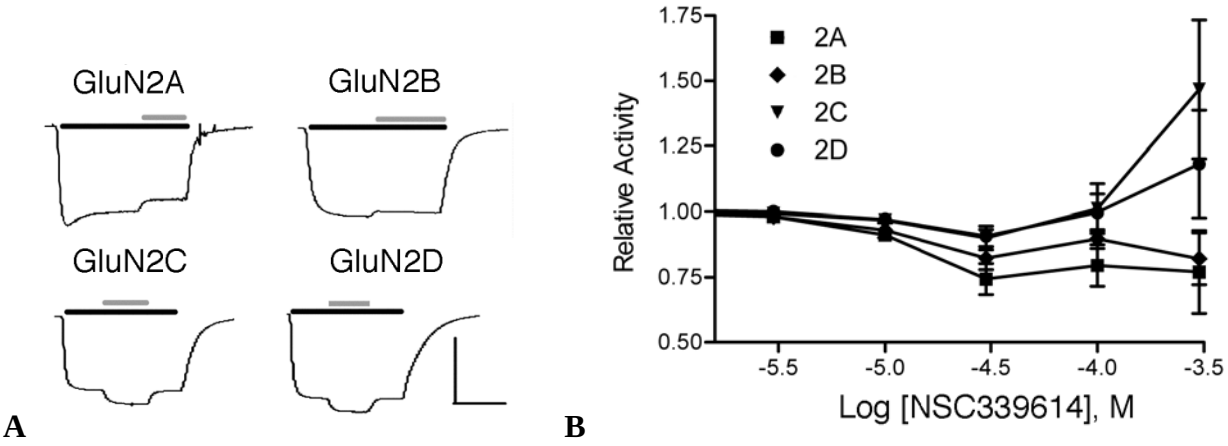
Figure 1. NSC339614 selectively potentiates GluN1/GluN2C and GluN1/GluN2D receptors.



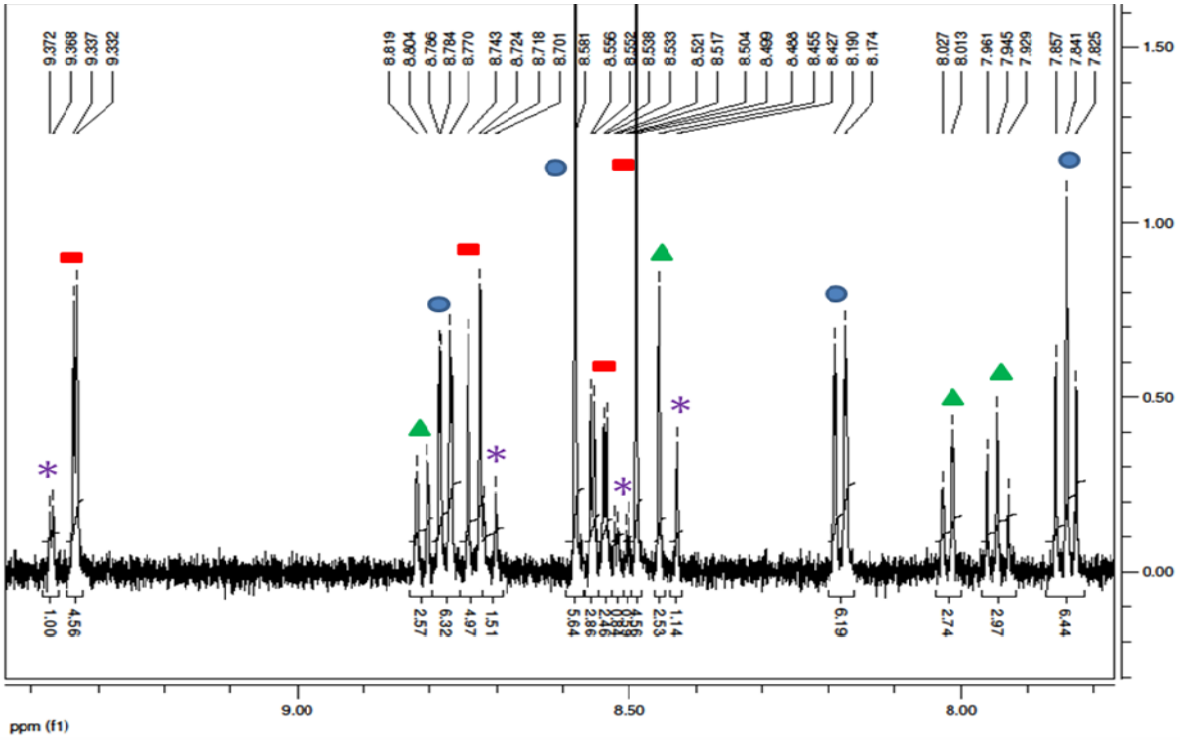
A. Agonist (10 μM L-glutamate plus 10 μM glycine; black bar) was added to Xenopus oocytes voltage-clamped at -60 mV expressing GluN1 + GluN2 (as indicated) to evoke a response. NSC339614 (100 μM) was then added as indicated by the gray bar. Scale bar values below. B. NSC339614 dose response for modulating agonist activation of GluN1/GluN2A (2A), GluN1/GluN2B (2B), GluN1/GluN2C (2C) and GluN1/GluN2D (2D) receptors. Values represent mean ± s.e.m. NSC339614 potentiated responses at both GluN1/GluN2C and GluN1/GluN2D receptors and had weak inhibitory activity at other NMDA receptors. The potentiating activity of NSC339614 did not saturate at 100 μM; greater potentiation was observed at the highest dose tested, 300 μM. However, the inhibitory activity

was the same at 30 µM and 300 µM for GluN2A and GluN2B-containing receptors, suggesting that occupation at this site by NSC339614 does not fully inhibit receptor function – or that it is offset by a potentiating activity. In two of 15 cells tested, NSC339614 caused a weak inhibition of GluN1/GluN2C and GluN1/GluN2D receptor responses at 100 µM and 300 µM concentrations instead of potentiation. Thus, the ability of NSC339614 to potentiate GluN1/GluN2C and GluN1/GluN2D receptor responses may be state-dependent and not an intrinsic property of the receptor complex.

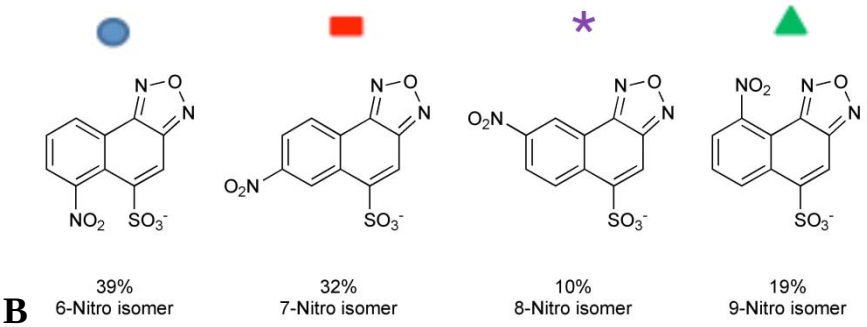
Scale bar values in A:

	GluN1/GluN2A	GluN1/GluN2B	GluN1/GluN2C	GluN1/GluN2D
nA	80	500	800	1600
sec	5	5	6	9

Figure 2. NSC339614 is a mixture of 4 isomers.



A

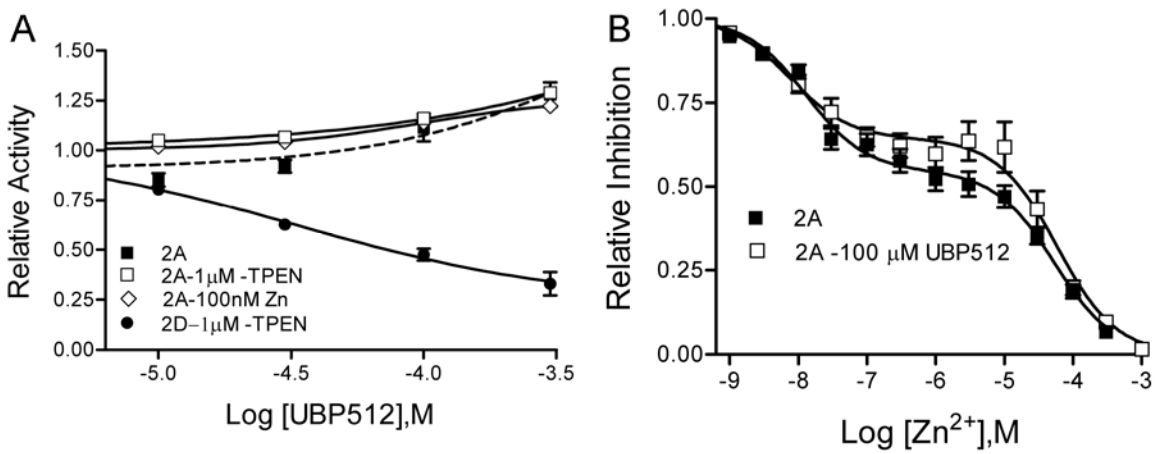


B

A. A ^{1}H NMR spectrum of NSC339614 shows that it is a mixture of four nitro-substituted positional isomers: 6-nitro isomer (peaks with blue circles), 7-nitro isomer (peaks with red squares), 8-nitro isomer (peaks with purple asterisks) and 9-nitro isomer (peaks with green triangles). B. The ratio of the peaks at 8.43, 8.46, 8.49 and 8.58 ppm suggests a 39:32:10:19 ratio for the 6-nitro, 7-nitro, 8-nitro and 9-nitro isomers, respectively. It is not known which isomer(s) is the pharmacologically active form; NSC339614 is defined as the 7-nitro isomer.

Figure 3 UBP512 does not potentiate NMDA receptor responses by chelating Zn^{++}

Zn^{++} is a high affinity negative modulator of GluN1/GluN2A receptors that binds to the N-terminal regulatory domain (NTD) of GluN2A(1, 2). The selective potentiation of GluN2A-containing receptors by UBP512 could potentially be due to the reversal of Zn^{++}-inhibition by Zn^{++} chelation. However, UBP512 potentiation was not affected by Zn^{++} chelation, nor by the addition of 100 nM Zn^{++} (Supplementary Fig. 1). Conversely, UBP512 addition did not alter the EC_{50}s for either the high affinity or the low affinity components of buffered(1) Zn^{++} inhibition at GluN1/GluN2A receptors.



(A) UBP512 activity was determined at GluN1/GluN2A (2A) receptors in the presence or absence (dotted line) of the zinc chelator TPEN (N,N,N',N'-tetrakis-(2-pyridylmethyl)ethylenediamine) or $ZnCl_2$. TPEN did not alter UBP512 activity at GluN1/GluN2D (2D) receptors. (B) $ZnCl_2$ inhibition of GluN1/GluN2A (2A) responses was unaltered by the addition of UBP512.

Table 1. X-axis and Y-axis scales for Figure 1.

		GluN1/GluN2A	GluN1/GluN2D
UBP512	nA	110	380
	sec	9	26
UBP551	nA	80	280
	sec	18	13
UBP608	nA	220	120
	sec	4	12
UBP618	nA	420	420
	sec	7	10
UBP710	nA	170	750
	sec	10	15
UBP646	nA	520	137
	sec	23	60

Table 2. Compounds do not display agonist or partial agonist activity nor alter the holding current. Compounds were tested for excitatory activity in the presence or absence of L-glutamate or glycine. Values represent % activation ± s.e.m., n = 4. G + E = 10 μM glycine + 10 μM L-glutamate, TC = test compound (100 μM), G = 10 μM glycine, E = 10 μM L-glutamate.

		UBP512	**UBP710**	**UBP618**	**UBP551**	**NSC 339614**	**UBP608**	**UBP646**
GluN1/ GluN2A	**G + E**	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	TC	-2.2 ± 1.2	2.7 ± 3.3	0.3 ± 2.4	-1.0 ± 2.9	-0.02 ± 0.3	0.2 ± 0.2	1.0 ± 0.1
	TC + G	4.1 ± 2.1	7.6 ± 4.8	3.5 ± 4.2	7.2 ± 3.3	0.3 ± 0.1	0.1 ± 0.2	0.7 ± 0.3
	TC + E	1.8 ± 1.2	0.03 ± 1.2	5.0 ± 2.7	-1.0 ± 4.1	0.4 ± 0.9	3.2 ± 3.1	1.8 ± 0.6
GluN1/ GluN2D	**G + E**	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	TC	-1.4 ± 1.9	0.6 ± 1.6	-0.3 ± 0.2	0.1 ± 0.5	0.1 ± 0.8	1.4 ± 2.3	0.6 ± 0.2
	TC + G	3.7 ± 0.7	3.2 ± 2.1	3.0 ± 3.0	0.4 ± 1.3	2.1 ± 0.8	3.3 ± 1.7	0.9 ± 0.3
	TC + E	1.2 ± 0.5	-0.7 ± 0.7	0.7 ± 0.4	1.6 ± 0.5	1.8 ± 0.3	-1.2 ± 0.7	0.9 ± 0.3

1. Paoletti P, Ascher P, & Neyton J (1997) High-affinity zinc inhibition of NMDA NR1-NR2A receptors *J Neurosci* 17(15):5711-5725.
2. Paoletti P, *et al.* (2000) Molecular organization of a zinc binding n-terminal modulatory domain in a NMDA receptor subunit. *Neuron* 28(3):911-925.