Identification of AICP as a GluN2C-selective NMDA receptor superagonist at the GluN1 glycine site

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MOLECULAR PHARMACOLOGY

SUPPLEMENTAL DATA

Supplemental Table 1. Influence of glutamate concentrations on AICP concentration-response data at rat NMDA receptor subtypes.

	GluN1/2A					GluN1/2B				
	EC_{50} [µM]	$pEC_{50} \pm$ SEM	Rel. I_{max} (%)	n_H	N	EC_{50} [µM]	$pEC_{50} \pm$ SEM	Rel. I_{max} (%)	n_H	N
AICP (+ 30 µM Glu)	**0.048**	7.33 ± 0.03	**83 ± 2**	1.2	12	**0.014**	7.88 ± 0.06	**7 ± 0**	1.1	6
AICP (+ 100 µM Glu)	**0.041**	7.39 ± 0.02	**88 ± 2**	1.2	16	**0.013**	7.90 ± 0.03	**8 ± 0**	1.1	9
AICP (+ 300 µM Glu)	**0.043**	7.37 ± 0.01	**95 ± 1[†]**	1.2	8	**0.016**	7.80 ± 0.05	**9 ± 1[†]**	1.1	5

	GluN1/2C					GluN1/2D				
	EC_{50} [µM]	$pEC_{50} \pm$ SEM	Rel. I_{max} (%)	n_H	N	EC_{50} [µM]	$pEC_{50} \pm$ SEM	Rel. I_{max} (%)	n_H	N
AICP (+ 30 µM Glu)	**0.0004**	9.37 ± 0.02	**310 ± 6**	1.0	6	**0.032**	7.50 ± 0.05	**22 ± 1**	1.6	6
AICP (+ 100 µM Glu)	**0.0010**	9.04 ± 0.04[†]	**340 ± 12**	1.1	14	**0.029**	7.54 ± 0.04	**28 ± 2[†]**	1.4	5
AICP (+ 300 µM Glu)	**0.0010**	9.03 ± 0.03[†]	**338 ± 16**	0.9	3	**0.033**	7.48 ± 0.03	**25 ± 1**	1.5	6

Concentration-response data for AICP at recombinant rat NMDA receptor subtypes measured using two-electrode voltage-clamp electrophysiology in the presence of 30, 100, or 300 µM glutamate (Glu). Relative (Rel.) I_{max} is the fitted maximal response relative to the maximal response to glycine, n_H is the Hill coefficient, and N is the number of oocytes. [†] significantly different from AICP data at the same receptor in 30 µM glutamate ($p < 0.05$, one-way ANOVA with Tukey's post-test).

Supplemental Table 2. Concentration-response data for AICP and DCS at wild type and mutant rat GluN1/2A and GluN1/2C receptors.

Receptor	Compound	EC_{50} [µM]	$pEC_{50} \pm SEM$	n_H	N	Fold increase in EC_{50} compared to wild type
GluN1/2A	AICP	0.036	7.49 ± 0.05	1.3	29	-
	DCS	18	4.75 ± 0.01	1.6	13	-
GluN1(F484A)/2A	AICP	13	4.98 ± 0.14	1.5	5	360
	DCS	> 10 mM	-	-	3	-
GluN1(R523A)/2A	AICP	4.9	5.38 ± 0.12	1.7	5	140
	DCS	880	3.06 ± 0.04	1.7	4	49
GluN1(T518L)/2A	AICP	16	4.82 ± 0.05	1.6	8	440
	DCS	N.R.	N.R.	N.R.	4	-
GluN1(F484A/T518L)/2A	AICP	N.R.	N.R.	N.R.	6	-
	DCS	N.R.	N.R.	N.R.	4	-
GluN1/2C	AICP	0.0010	9.04 ± 0.03	1.0	17	-
	DCS	2.8	5.55 ± 0.01	1.5	18	-
GluN1(F484A)/2C	AICP	9.7	5.01 ± 0.01	1.6	6	9700
	DCS	> 10 mM	-	-	4	-
GluN1(R523A)/2C	AICP	3.6	5.44 ± 0.01	1.6	6	3600
	DCS	250	3.61 ± 0.01	1.6	5	89
GluN1(T518L)/2C	AICP	1.2	5.93 ± 0.01	1.8	8	1200
	DCS	> 10 mM	-	-	4	-
GluN1(F484A/T518L)/2C	AICP	N.R.	N.R.	N.R.	6	-
	DCS	N.R.	N.R.	N.R.	4	-

Concentration-response data for AICP and DCS at recombinant wild type or mutated rat GluN1/2A and GluN1/2C receptors measured using two-electrode voltage-clamp electrophysiology in the presence of 100-300 µM glutamate. Relative (Rel.) I_{max} is the fitted maximal response, n_H is the Hill coefficient, and N is the number of oocytes. N.R. indicates < 10% response to 30-100 µM AICP, 30 mM DCS, or 30 mM glycine, and - indicates not determined.

Supplemental Table 3. Concentration-response data for AICP at human NMDA receptor subtypes in the presence of glycine.

Receptor	Glycine [μM]	EC_{50} [μM] / IC_{50} [μM]	$pEC_{50} \pm SEM$ / $pIC_{50} \pm SEM$	Rel. I_{max} (%)	Rel. I_{min} (%)	n_H	N
GluN1/2A	0.6	**0.027**	7.56 ± 0.07	**99** $\pm$ 2	**29** $\pm$ 3	1.2	5
	100	**ND**	ND	**102** $\pm 2^{\#}$	**95** $\pm$ $3^{\#}$	ND	10
GluN1/2B	0.6	**0.013**	7.88 ± 0.18	**59** $\pm$ 5	**14** $\pm$ 2	-0.9	6
	100	**5.1**	$5.29 \pm 0.11^{\dagger}$	**93** $\pm$ 2	**20** $\pm$ 6	-1.1	5-6
GluN1/2C	0.6	**0.013**	7.88 ± 0.07	**337** $\pm$ 8	**60** ± 12	1.2	5-6
	100	**1.4**	$5.85 \pm 0.10^{\dagger}$	**321** ± 20	**105** $\pm$ 4	1.1	4-6
GluN1/2D	0.6	**1.1**	5.97 ± 0.07	**92** $\pm$ 2	**45** $\pm$ 3	-2.5	5-8
	100	**42**	$4.38 \pm 0.55^{\dagger}$	**104** $\pm$ 2	**48** ± 37	-1.3	5-8

Concentration-response data for AICP at recombinant human NMDA receptor subtypes measured using two-electrode voltage-clamp electrophysiology in the presence of 100 μM glutamate and 100 μM or 0.6 μM glycine. Relative (Rel.) I_{max} and Rel. I_{min} are the fitted maximal and minimal responses relative to the maximal response to glycine, n_H is the Hill coefficient, and N is the number of oocytes. Negative n_H indicates inhibition by AICP, ND indicates not determined, and # indicates that Rel. I_{max} and Rel. I_{min} are determined as the average responses to the lowest and highest concentrations of AICP, respectively, relative to the maximal response to glycine. † significantly different from pEC_{50} or pIC_{50} at the same receptor in 0.6 μM glycine (p < 0.05, one-way ANOVA with Tukey's post-test).

Supplemental Table 4. Concentration-response data for AICP and DCS at mutant and chimeric GluN1/2A, GluN1/2B, and GluN1/2C receptors.

Receptor	Compound	EC_{50} [µM]	$pEC_{50} \pm SEM$	Rel. I_{max} (%)	n_H	N
GluN1/2A	AICP	0.036	7.49 ± 0.05	**92** $\pm$ 2	1.3	29
	DCS	18	4.75 ± 0.01	**91** $\pm$ 1	1.6	13
GluN1/2B	AICP	0.014	7.86 ± 0.03	**9** $\pm$ 0	1.1	14
	DCS	8.3	5.08 ± 0.01	**61** $\pm$ 0	1.4	12
GluN1/2C	AICP	0.0010	9.04 ± 0.03	**339** $\pm$ 10	1.0	17
	DCS	2.8	5.55 ± 0.01	**197** $\pm$ 2	1.5	18
GluN1/2B E790Q	AICP	0.0050	8.31 ± 0.03	**9** $\pm$ 1	1.7	11
	DCS	11	4.97 ± 0.01	**81** $\pm$ 0	1.4	9
GluN1/2C Q800E	AICP	0.012	7.93 ± 0.02	**607** $\pm$ 22	1.3	9
	DCS	4.4	5.35 ± 0.01	**143** $\pm$ 3	1.5	8
GluN1/2A-(2C ATD)	AICP	0.025	7.60 ± 0.02	**97** $\pm$ 5	1.2	6
	DCS	33	4.48 ± 0.03	**75** $\pm$ 2	1.5	6
GluN1/2C-(2A ATD)	AICP	0.0013	8.89 ± 0.04	**98** $\pm$ 7	0.8	6
	DCS	5.6	5.26 ± 0.03	**104** $\pm$ 0	1.4	7

Concentration-response data for AICP and DCS at recombinant rat NMDA receptors measured using two-electrode voltage-clamp electrophysiology in the presence of 100-300 µM glutamate. Relative (Rel.) I_{max} is the fitted maximal response relative to the maximal response to glycine, n_H is the Hill coefficient, and N is the number of oocytes.