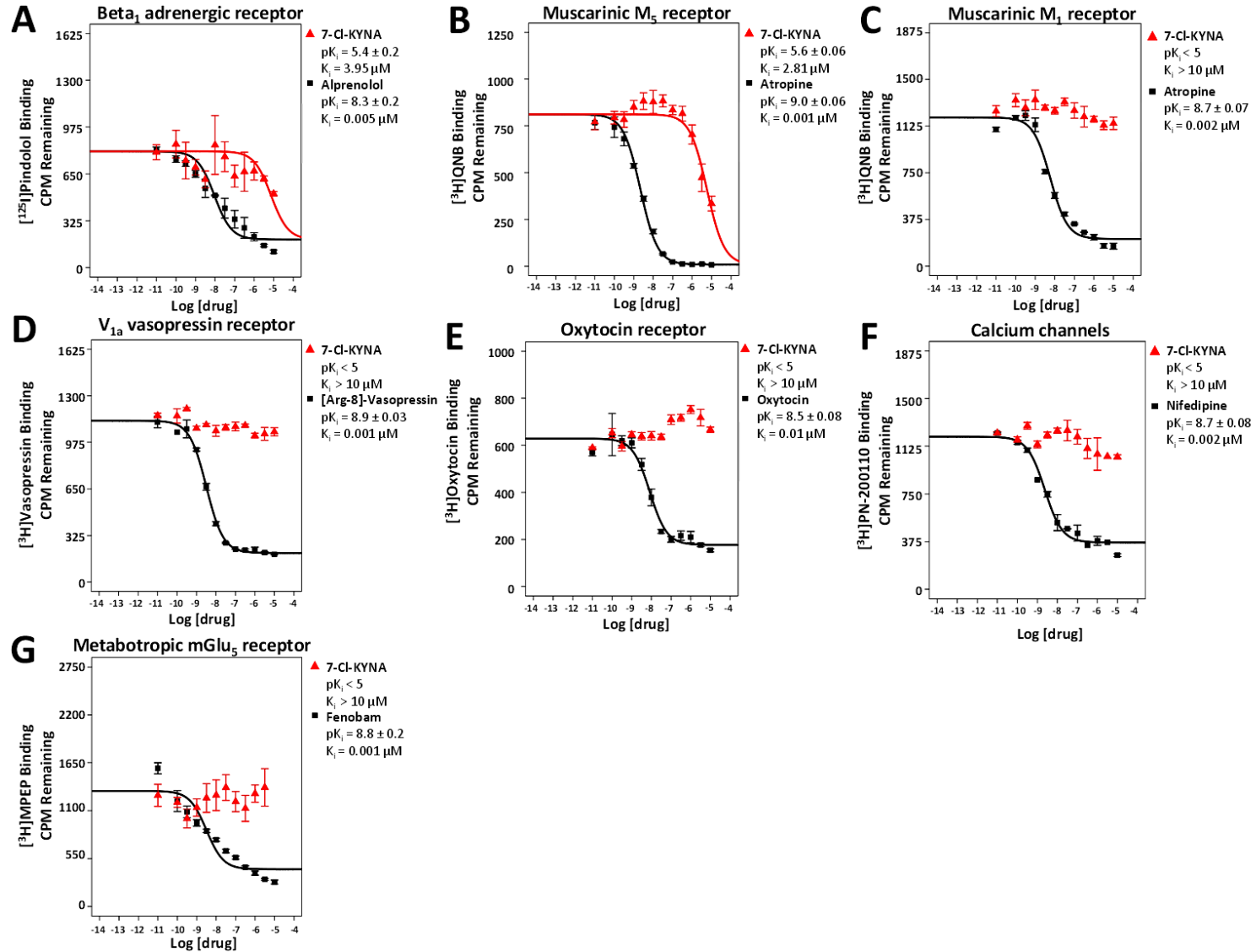


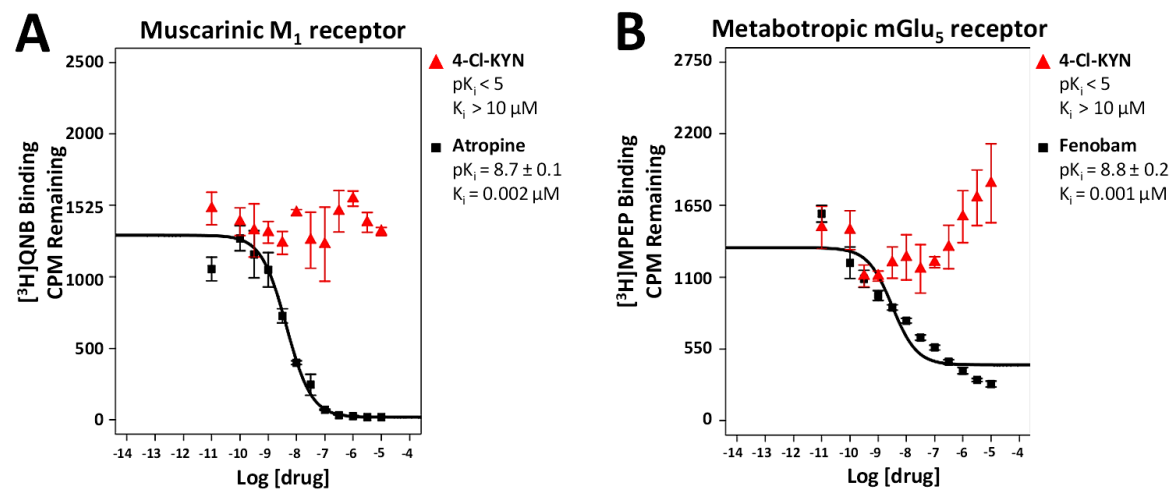
**The prodrug 4-chlorokynurenine causes ketamine-like antidepressant, but not side effects, by NMDA/glycine<sub>B</sub>-site inhibition**

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## SUPPLEMENTARY FIGURES/FIGURE LEGENDS



**Figure S1:  $K_i$  values for 7-Cl-KYNA against a panel of receptors.** Data represent  $K_i$  (μM) values obtained from non-linear regression of radioligand competition binding isotherms.  $K_i$  values are calculated from best fit IC<sub>50</sub> values using the Cheng-Prusoff equation. Data are the mean ± SEM (n=4/group in each experiment).



**Figure S2:  $K_i$  values for 4-Cl-KYN against a panel of receptors.** Data represent  $K_i$  ( $\mu\text{M}$ ) values obtained from non-linear regression of radioligand competition binding isotherms.  $K_i$  values are calculated from best fit  $\text{IC}_{50}$  values using the Cheng-Prusoff equation. Data are the mean  $\pm$  SEM (n=4/group in each experiment).

## SUPPLEMENTARY TABLES

Table S1: Potency at off-target G-protein coupled receptors, ion channels and transporters

Target	Receptor Family	7-chlorokynurenic acid	4-chlorokynurenine
		K <sub>i</sub> (μM) or % Inhibition	
5-HT <sub>1A</sub>	Serotonergic	0 %	11 %
5-HT <sub>1B</sub>		5 %	7 %
5-HT <sub>1D</sub>		6 %	0 %
5-HT <sub>1F</sub>		0 %	0 %
5-HT <sub>2A</sub>		0 %	0 %
5-HT <sub>2B</sub>		0 %	0 %
5-HT <sub>2C</sub>		4 %	0 %
5-HT <sub>3</sub>		5 %	12 %
5-HT <sub>5A</sub>		0 %	0 %
5-HT <sub>6</sub>		1 %	4 %
5-HT <sub>7</sub>		13 %	0 %
Serotonin transporter		32 %	5 %
Alpha <sub>1A</sub>		Adrenergic	2 %
Alpha <sub>1B</sub>	38 %		0 %
Alpha <sub>1D</sub>	0 %		1 %
Alpha <sub>2A</sub>	0 %		0 %
Alpha <sub>2B</sub>	6 %		0 %
Alpha <sub>2C</sub>	0 %		20 %
Beta <sub>1</sub>	<b>3.95 μM</b>		0 %
Beta <sub>2</sub>	0 %		0 %
Beta <sub>3</sub>	0 %		0 %
AMPA	Glutamatergic	ND	9 %
mGlu <sub>5</sub> R		<b>&gt;10 μM</b>	<b>&gt;10 μM</b>
Kainate		ND	0 %
D1	Dopaminergic	0 %	11 %
D2		0 %	0 %
D3		0 %	4 %
D4		8 %	2 %
D5		3 %	0 %
Dopamine transporter	9 %	0 %	
δ	Opioid	1 %	0 %
μ		10 %	0 %
κ		0 %	0 %
GABA <sub>A</sub>	GABAergic	1 %	5 %
H <sub>1</sub>	Histaminergic	6 %	0 %
H <sub>2</sub>		0 %	0 %
H <sub>3</sub>		0 %	0 %
H <sub>4</sub>		0 %	3 %
M <sub>1</sub>	Muscarinic Acetylcholine	<b>&gt;10 μM</b>	<b>&gt;10 μM</b>
M <sub>2</sub>		8 %	5 %
M <sub>3</sub>		0 %	1 %
M <sub>4</sub>		10 %	0 %
M <sub>5</sub>		<b>2.81 μM</b>	7 %
Norepinephrine	Norepinephrinergic	0 %	0 %
PBR	Peripheral	2 %	0 %
σ <sub>1</sub>	Dimethyl-tryptamine	0 %	0 %
σ <sub>2</sub>	Unknown	0 %	12 %
Calcium Channels		<b>&gt;10 μM</b>	0 %
Oxytocin	Oxytocinergic	<b>&gt;10 μM</b>	0 %
V <sub>1A</sub>	Vasopressinergic	<b>&gt;10 μM</b>	48 %

All experiments were binding assays performed by PDSP. %<sub>4</sub> values are the mean of 4 replicates and were derived from single concentration (10μM). Negative values are represented as 0% here. K<sub>i</sub> values (μM; in bold) were determined from full concentration curves only for receptors displaying >50% inhibition.

Table S2: Assay conditions for radioligand binding assays

Receptor	Radioligand (Assay Concentration)	Reference compound	Binding Buffer	pH
5-HT <sub>1A</sub>	[ <sup>3</sup> H]8-OH-DPAT (0.5 nM)	Methysergide	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>1B</sub>	[ <sup>3</sup> H]GR127543 (0.3 nM)	Ergotamine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>1D</sub>	[ <sup>3</sup> H]GR127543 (0.3 nM)	Ergotamine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>1E</sub>	[ <sup>3</sup> H]5-HT (3 nM)	5-HT	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>2A</sub>	[ <sup>3</sup> H]Ketanserin (0.5 nM)	Chlorpromazine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>2B</sub>	[ <sup>3</sup> H]LSD (1 nM)	Methysergide	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>2C</sub>	[ <sup>3</sup> H]Mesulergine (0.5 nM)	Chlorpromazine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>3</sub>	[ <sup>3</sup> H]LY278584 (0.3 nM)	LY278584	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>5A</sub>	[ <sup>3</sup> H]LSD (1 nM)	Ergotamine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>6</sub>	[ <sup>3</sup> H]LSD (1 nM)	Chlorpromazine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
5-HT <sub>7</sub>	[ <sup>3</sup> H]LSD (1 nM)	Chlorpromazine	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
Serotonin transporter	[ <sup>3</sup> H]Citalopram (0.5 nM)	Amitriptyline	50 mM Tris HCl, 150 mM NaCl, 5 mM KCl	7.4
Alpha <sub>1A</sub>	[ <sup>3</sup> H]Prazosin (0.5 nM)	Prazosin	20 mM Tris HCl, 145 mM NaCl	7.4
Alpha <sub>1B</sub>	[ <sup>3</sup> H]Prazosin (1.5 nM)	Prazosin	20 mM Tris HCl, 145 mM NaCl	7.4
Alpha <sub>1D</sub>	[ <sup>3</sup> H]Prazosin (0.5 nM)	Prazosin	20 mM Tris HCl, 145 mM NaCl	7.4
Alpha <sub>2A</sub>	[ <sup>3</sup> H]Clonidine (1 nM)	Oxymetazoline	50 mM Tris HCl, 5 mM MgCl <sub>2</sub>	7.7
Alpha <sub>2B</sub>	[ <sup>3</sup> H]Clonidine (1 nM)	Prazosin	50 mM Tris HCl, 5 mM MgCl <sub>2</sub>	7.7
Alpha <sub>2C</sub>	[ <sup>3</sup> H]Clonidine (1 nM)	Prazosin	50 mM Tris HCl, 5 mM MgCl <sub>2</sub>	7.7
Beta <sub>1</sub>	[ <sup>125</sup> I]Iodopindolol (0.1 nM)	Atenolol	50 mM Tris HCl, 3 mM MnCl <sub>2</sub>	7.7
Beta <sub>2</sub>	[ <sup>125</sup> I]Iodopindolol (0.1 nM)	ICI118551	50 mM Tris HCl, 3 mM MnCl <sub>2</sub>	7.7
Beta <sub>3</sub>	[ <sup>125</sup> I]Iodopindolol (0.1 nM)	ICI118551	50 mM Tris HCl, 3 mM MnCl <sub>2</sub>	7.7
AMPA	[ <sup>3</sup> H]AMPA (1 nM)	Glutamic Acid	50 mM Tris HCl, 2.5 mM CaCl <sub>2</sub>	7.4
mGlu <sub>5</sub> R	[ <sup>3</sup> H]MPEP (3.8 nM)	Fenobam	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
Kainate	[ <sup>3</sup> H]Kainic Acid (10.7 nM)	Glutamic Acid	50 mM Tris HCl, 2.5 mM CaCl <sub>2</sub>	7.4
D <sub>1</sub>	[ <sup>3</sup> H]SCH233930 (0.2 nM)	SKF38393	50 mM HEPES, 50 mM NaCl, 5 mM MgCl <sub>2</sub> , 0.5 mM EDTA	7.4
D <sub>2</sub>	[ <sup>3</sup> H]N-methylspiperone (0.2 nM)	Haloperidol	50 mM HEPES, 50 mM NaCl, 5 mM MgCl <sub>2</sub> , 0.5 mM EDTA	7.4
D <sub>3</sub>	[ <sup>3</sup> H]N-methylspiperone (0.2 nM)	Chlorpromazine	50 mM HEPES, 50 mM NaCl, 5 mM MgCl <sub>2</sub> , 0.5 mM EDTA	7.4
D <sub>4</sub>	[ <sup>3</sup> H]N-methylspiperone (0.3 nM)	Chlorpromazine	50 mM HEPES, 50 mM NaCl, 5 mM MgCl <sub>2</sub> , 0.5 mM EDTA	7.4
D <sub>5</sub>	[ <sup>3</sup> H]SCH233930 (0.2 nM)	SKF38393	50 mM HEPES, 50 mM NaCl, 5 mM MgCl <sub>2</sub> , 0.5 mM EDTA	7.4
Dopamine transporter	[ <sup>3</sup> H]WIN35428 (0.5 nM)	GBR12909	50 mM Tris HCl, 150 mM NaCl, 5 mM KCl	7.4
δ	[ <sup>3</sup> H]DADLE (0.3 nM)	Naltrindole	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
μ	[ <sup>3</sup> H]U69593 (0.3 nM)	Salvinorin A	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
κ	[ <sup>3</sup> H]DAMGO (0.3 nM)	DAMGO	50 mM Tris HCl, 10 mM MgCl <sub>2</sub> , 0.1 mM EDTA	7.4
GABA <sub>A</sub>	[ <sup>3</sup> H]Muscimol (1 nM)	GABA	50 mM Tris Acetate	7.4
H <sub>1</sub>	[ <sup>3</sup> H]Pyrilamine (0.9 nM)	Chlorpheniramine	50 mM Tris HCl, 0.5 mM EDTA	7.4
H <sub>2</sub>	[ <sup>3</sup> H]Tiotidine (3 nM)	Cimetidine	50 mM Tris HCl, 0.5 mM EDTA	7.4
H <sub>3</sub>	[ <sup>3</sup> H]alpha-methylhistamine (0.4 nM)	Histamine	50 mM Tris HCl, 0.5 mM EDTA	7.4
H <sub>4</sub>	[ <sup>3</sup> H]Histamine (5 nM)	Clozapine	50 mM Tris HCl, 0.5 mM EDTA	7.4
M <sub>1</sub>	[ <sup>3</sup> H]QNB (0.5 nM)	Atropine	50 mM Tris HCl	7.7
M <sub>2</sub>	[ <sup>3</sup> H]QNB (0.5 nM)	Atropine	50 mM Tris HCl	7.7

M <sub>3</sub>	[ <sup>3</sup> H]QNB (0.5 nM)	Atropine	50 mM Tris HCl	7.7
M <sub>4</sub>	[ <sup>3</sup> H]QNB (0.5 nM)	Atropine	50 mM Tris HCl	7.7
M <sub>5</sub>	[ <sup>3</sup> H]QNB (0.5 nM)	Atropine	50 mM Tris HCl	7.7
Norepinerphine transporter	[ <sup>3</sup> H]Nisoxetine (0.5 nM)	Desipramine	50 mM Tris HCl, 150 mM NaCl, 5 mM KCl	7.4
PBR	[ <sup>3</sup> H]PK11195 (1 nM)	PK11195	50 mM Tris HCl	7.4
σ <sub>1</sub>	[ <sup>3</sup> H]Pentazocine (3 nM)	Haloperidol	50 mM Tris HCl	8.0
σ <sub>2</sub>	[ <sup>3</sup> H]DTG (3 nM)	Haloperidol	50 mM Tris HCl	8.0
Calcium Channels	[ <sup>3</sup> H]Nitrendipine (0.1 nM)	Nifendipine	50 mM Tris HCl, 50 mM NaCl, 1 mM CaCl <sub>2</sub>	7.4
Oxytocin	[ <sup>3</sup> H]Oxytocin	Oxytocin	50 mM HEPES, 10 mM MnCl <sub>2</sub>	7.4
V <sub>1A</sub>	[ <sup>3</sup> H]Vasopressin (1 nM)	Vasopressin	20 mM Tris HCl, 100 mM NaCl, 10 mM MgCl <sub>2</sub> , 0.1 mg/ml bacitracin, 1 mg/ml BSA	7.4