A novel class of negative allosteric modulators of NMDA receptor function

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ABSTRACT

NMDA receptors mediate a slow Ca2+-permeable component of excitatory synaptic transmission, and are involved in numerous normal brain functions including learning and memory. NMDA receptor over-activation can lead to cell death and abnormal excitation in ischemia associated with stroke, traumatic brain injury, and epilepsy. We have explored a series of novel noncompetitive allosteric modulators of NMDA receptor function characterized by an iminothiazolidinone ring. Saturating concentrations of these compounds inhibit NMDA receptors to varying maximal extents, raising the possibility that they may attenuate over-activation in pathological situations while preserving some minimal receptor function, which may limit side-effects. The best in class compounds have sub-micromolar IC50 values and show modest preference for GluN2C- and GluN2D-containing receptors.

Ionotropic glutamate receptors (iGluRs) are a family of ligand-gated ion channels that mediate the majority of fast excitatory neurotransmission in the mammalian brain. The iGluRs are subdivided into three subtypes based on sequence and structural homology, function, and pharmacology. The N-methyl-D-aspartate (NMDA) subtype of glutamate receptors have slower response time course and are more permeable to calcium ions than other iGluRs.1 Aberrant NMDA receptor activation is thought to play a role in the progression of Parkinson’s disease and the biochemical neuronal damage caused by traumatic brain injury and stroke.2,3 NMDA receptor antagonists have been shown to be neuroprotective in many animal studies of these disorders.2,4 However, clinical trials with multiple classes of NMDA receptor antagonists have been unsuccessful, due in part to complications arising from on-target side-effects.5 It has been hypothesized that next generation therapeutics possessing subunit selectivity and/or submaximal inhibition might avoid these side-effects.6–7 That is, unwanted on-target side-effects could be due to drug actions on a subset of GluN2-containing NMDA receptors and could be avoided by using compounds with GluN2 subunit selectivity.6,7 Additionally, side-effects might arise from complete blockade of NMDA receptors, and therefore might be avoided by preserving a low level of activity through use of compounds that produce submaximal inhibition at saturating concentrations.6,7 This latter possibility has not been evaluated due to the lack of candidate compounds that show more than 10% submaximal inhibition at maximally effective concentrations. Recently, some NMDA receptor antagonists have been investigated which may have submaximal inhibition,8 although potency and drug-like properties of these compounds would likely need to be improved before use in pre-clinical testing.

In an attempt to new identify subunit-selective inhibitors, a medium-throughput screen was implemented to identify modulators of GluN2C- or GluN2D-containing NMDA receptors.9 Among the NMDA receptor modulators identified as hits,10–15 compound (1) (Fig. 1) was selected for further investigation due to its distinct structural and pharmacological properties. Compound 1 contains an iminothiazolidinone ring connected to the thiophene moiety through an acetamide linkage. Within the present study, we varied both heterocyclic rings of the parent compound (1) in order to understand the interaction of these compounds with NMDA receptors and the resulting pharmacological effect.

Here we report (a) the structure activity relationship [SAR] of a novel class of NMDA receptor antagonists; (b) data supporting the noncompetitive mechanism of action of a compound in this class on NMDA receptors, and (c) the effect of the prototypical compound on neuronal NMDA receptors, and (d) data supporting its therapeutic potential.

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The potency and extent of NMDA receptor inhibition by analogues were determined by activating receptors with maximally effective concentrations of glutamate and glycine and evaluating the concentration–effect relationship each of the four diheteromeric NMDA receptors expressed in Xenopus laevis oocytes. Each subtype was expressed in oocytes by co-injecting the cRNA for the rat GluN1–1a (hereafter GluN1) and one of the GluN2 subunits (GluN2A–D). After a sufficient time for robust surface expression of the rat GluN1-1a (hereafter GluN1) and one of the GluN2 subunits (GluN2A–D), voltage clamp recordings were performed as described. Briefly, functional NMDA receptors (1–3 days at 16–19 °C) were activated with maximally effective concentrations of glutamate and glycine and evaluating responses were fitted by the Hill equation, given by:

$$I = I_{0} - I_{\text{inh}} \max \left( \frac{[A]}{[A] + IC_{50}} \right)^{k}$$

where $I$ is the current amplitude, $I_{\text{inh}} \max$ is the percent response remaining in saturating ligand, $h$ is the Hill coefficient and $[A]$ is the concentration of ligand.

Compounds tested were either obtained from commercial vendors at a purity greater than 90% (assessed by $^1$H NMR and/or LC/MS) or synthesized according to the procedures described below. A series of structurally-similar molecules has been shown to have favorable constituent and improve potency, selectivity, or both. We hypothesized that the increase in hydrophobic character of these compounds would help reduce the total polar surface area toward a more drug-like value common to CNS drugs. Interestingly, introduction of the tetrahydrobenzothiophene ring system so as to avoid regioisomers that could form in the condensation reaction toward the thiophene moiety provided several potent NMDA receptor inhibitors.

Comparison of both 20 and 5 showed that changing from the methyl ester to the ethyl ester did not significantly alter potency. When compared to the original screening hit (1, Table 1), analogues with the fused cyclohexyl ring demonstrated either a 2- (20) or 3-fold increase (5) in potency at GluN2D-containing receptors. To our surprise, they also exhibited varying degrees of submaximal inhibition at all receptor combinations tested. The specificity of the R1 position is highlighted by the loss of activity of the carboxylic acid 13. Interestingly, the nitrile substituted analogue (21) appears to be less potent than 1 and has reduced activity (60% maximal inhibition). This result supported the hypothesis that this portion of the molecule interacts with a pocket which prefers hydrophobicity.

Additional analogues were generated containing the bicyclic thiophene moiety in attempt to expand the SAR surrounding this favorable constituent and improve potency, selectivity, or both. Various substitutions were made exclusively at the 6-position of the tetrahydrobenzothiophene ring system so as to avoid regioisomers that could form in the condensation reaction toward the aminothiophene building blocks (Scheme 1). Introduction of a single methyl unit onto the cyclohexyl ring (4) resulted in slightly improved potency. However, exchange of the ethyl group for a methyl group at the position proximal to the sulfur atom in the thiophene ring in analogue 15 resulted in a 4-fold decrease in potency. Addition of alkyl substituents at the R2 position of the thiophene ring in compounds 16, 17, and 18 all reduced the potency. The replacement of the alkyl substituents with hydrogen atoms at both the R2 and R3 positions (19) strongly reduced potency over 10-fold. While none of these compounds offered improvements in potency, the data indicates that one or more alkyl substituents on the thiophene ring are necessary for activity, potentially by stabilizing the compound into a hydrophobic binding pocket.

The preference for lipophilic functionality on the thiophene ring was further demonstrated through the improved potency observed with several of the compounds summarized in Table 2. Specifically, we evaluated compounds containing a bicyclic thiophene as a replacement for the thiophene ring with short alkyl appendages present in compounds like 1. We hypothesized that the increase in hydrophobic character of these compounds would help reduce the total polar surface area toward a more drug-like value common to CNS drugs. Interestingly, introduction of the tetrahydrobenzothiophene ring system so as to avoid regioisomers that could form in the condensation reaction toward the thiophene moiety provided several potent NMDA receptor inhibitors. 

Scheme 1. Synthetic route toward analogues of 1. (a) $S_{8}$, morpholine, 50 °C. (b) Maleic anhydride, Et$_{2}$O, 25–35 °C. (c) HOBt, EDCI, DMF, 1 h, then thiourea, 60 °C, 24 h. (d) Acetic anhydride, sodium acetate, 75 °C, then rt, 12–18 h. (e) EtOH, rt, 24–48 h.
Table 1
Effect of alkylation substitutions to the thioephene ring on the potency of analogues of $^{1}$

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>$R_1$</th>
<th>$X$</th>
<th>$R_2$</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GluN2A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>Me</td>
<td>H</td>
<td>Et</td>
<td>4.8 [4.4, 5.3] (98 ± 0.8)</td>
<td>9.3 [8.2, 10.5] (97 ± 1.4)</td>
<td>2.5 [2.1, 3.0] (97 ± 1.0)</td>
<td>2.6 [2.2, 3.1] (98 ± 0.8)</td>
<td></td>
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</tr>
<tr>
<td>14</td>
<td>Et</td>
<td>H</td>
<td>Et</td>
<td>6.2 [4.3, 9.0] (96 ± 1.3)</td>
<td>9.7 [8.2, 10.5] (96 ± 1.4)</td>
<td>3.7 [2.6, 5.1] (97 ± 0.8)</td>
<td>2.6 [1.9, 3.7] (94 ± 2.4)</td>
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<tr>
<td>15</td>
<td>Et</td>
<td>H</td>
<td>Me</td>
<td>42.8 [38.2, 48.0] (93 ± 1.8)</td>
<td>44.9 [39.5, 50.8] (91 ± 2.3)</td>
<td>11.2 [9.2, 13.5] (98 ± 1.0)</td>
<td>12.3 [11.4, 13.2] (99 ± 0.6)</td>
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<tr>
<td>16</td>
<td>Me</td>
<td>Me</td>
<td>Me</td>
<td>22.3 [19.5, 25.6] (88 ± 3.5 $^*$)</td>
<td>28.2 [21.9, 36.1] (63 ± 3.9 $^*$)</td>
<td>9.7 [8.6, 11.0] (88 ± 1.8 $^*$)</td>
<td>10.4 [9.6, 11.2] (94 ± 0.9)</td>
<td></td>
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</tr>
<tr>
<td>17</td>
<td>Et</td>
<td>Me</td>
<td>Me</td>
<td>30.2 [16.6, 55.0] (80 ± 3.5 $^*$)</td>
<td>—</td>
<td>8.1 [6.3, 10.3] $^<em>$ (77 ± 2.7 $^</em>$)</td>
<td>13.0 [7.9, 21.5] (81 ± 3.2 $^*$)</td>
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<tr>
<td>18</td>
<td>Me</td>
<td>Et</td>
<td>Me</td>
<td>26.8 [22.9, 31.3] (79 ± 2.7 $^*$)</td>
<td>43.8 [37.2, 51.4] (77 ± 3.7 $^*$)</td>
<td>6.7 [5.3, 8.6] (85 ± 1.4 $^*$)</td>
<td>9.1 [8.6, 9.6] (82 ± 0.9 $^*$)</td>
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<tr>
<td>19</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>—</td>
<td>—</td>
<td>41.0 [34.1, 49.4] (100 ± 0.4)</td>
<td>43.3 [35.8, 52.4] (99 ± 0.6)</td>
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</table>

$^*$ Indicates that the Hill coefficient held constant ($h = 1$) to obtain estimates of lower potency or less soluble compounds.

Table 2
Effect of substituted tetrahydrobenzoazepinones on the potency of analogues of $^{1}$

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>$R_1$</th>
<th>$X$</th>
<th>$R_2$</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
<th>$IC_{50}$ (μM)</th>
<th>(% maximal inhibition)</th>
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<td>GluN2A</td>
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<tr>
<td>20</td>
<td>CO$_2$Me</td>
<td>CH$_2$</td>
<td>3.4 [2.9, 3.9] (81 ± 4.0)</td>
<td>4.2 [3.6, 4.9] (86 ± 2.0)</td>
<td>1.0 [0.8, 1.2] (88 ± 1.3)</td>
<td>1.1 [0.8, 1.2] (86 ± 1.3)</td>
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<td>5</td>
<td>CO$_2$Et</td>
<td>CH$_2$</td>
<td>2.3 [1.5, 3.6] (61 ± 3.0 $^*$)</td>
<td>4.2 [3.0, 6.0] (35 ± 1.5 $^*$)</td>
<td>1.2 [0.7, 1.9] (53 ± 2.4 $^*$)</td>
<td>0.6 [0.4, 0.9] (41 ± 1.6 $^*$)</td>
<td>54</td>
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<tr>
<td>13</td>
<td>CO$_2$Et</td>
<td>CH$_2$</td>
<td>—</td>
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<tr>
<td>21</td>
<td>CN</td>
<td>CH$_2$</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.9 [2.4, 3.5] $^<em>$ (59 ± 2.4 $^</em>$)</td>
<td>4.3 [2.5, 7.5] $^<em>$ (63 ± 4.2 $^</em>$)</td>
<td>19</td>
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<tr>
<td>4</td>
<td>CO$_2$Et</td>
<td>CHMe</td>
<td>0.9 [0.7, 1.2] (88 ± 3.1)</td>
<td>2.2 [1.0, 4.5] (70 ± 7.1)</td>
<td>0.7 [0.4, 1.2] (80 ± 1.5)</td>
<td>0.4 [0.3, 0.5] (76 ± 2.9)</td>
<td>36</td>
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<tr>
<td>22</td>
<td>CO$_2$Me</td>
<td>CHMe</td>
<td>0.7 [0.6, 0.8] (80 ± 3.4)</td>
<td>0.6 [0.4, 0.8] (74 ± 5.0)</td>
<td>0.3 [0.3, 0.4] (95 ± 1.5)</td>
<td>0.3 [0.2, 0.4] (94 ± 1.6)</td>
<td>43</td>
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<tr>
<td>10</td>
<td>CO$_2$Et</td>
<td>CHEt</td>
<td>3.4 [2.3, 5.1] (58 ± 6.5 $^*$)</td>
<td>2.2 [1.8, 2.7] (59 ± 3.8 $^*$)</td>
<td>0.9 [0.6, 1.3] (80 ± 3.2)</td>
<td>1.5 [1.0, 2.3] (73 ± 3.7 $^*$)</td>
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<tr>
<td>8</td>
<td>CO$_2$Et</td>
<td>CMe$_2$</td>
<td>16.1 [5.6, 46.3] $^*$ (72 ± 7.5)</td>
<td>9.2 [2.9, 29.0] (44 ± 8.0 $^*$)</td>
<td>9.3 [5.4, 16.1] (89 ± 4.0)</td>
<td>8.8 [6.0, 12.9] (90 ± 4.2)</td>
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<td>—</td>
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<tr>
<td>11</td>
<td>CO$_2$Et</td>
<td>CF$_2$</td>
<td>5.2 [3.2, 8.2] (84 ± 2.0)</td>
<td>8.0 [5.4, 11.9] (74 ± 3.8)</td>
<td>1.4 [0.8, 2.4] (75 ± 1.8)</td>
<td>1.5 [0.9, 2.8] (65 ± 3.8 $^*$)</td>
<td>37</td>
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<tr>
<td>6</td>
<td>CO$_2$Et</td>
<td>S</td>
<td>6.2 [4.9, 7.8] (82 ± 1.7)</td>
<td>12.2 [9.9, 15.2] (78 ± 1.4)</td>
<td>0.8 [0.7, 1.1] (88 ± 1.2)</td>
<td>0.8 [0.6, 1.1] (84 ± 1.2)</td>
<td>48</td>
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<tr>
<td>7</td>
<td>CO$_2$Et</td>
<td>O</td>
<td>3.0 [1.8, 5.0] (74 ± 2.0)</td>
<td>5.6 [3.5, 8.9] (69 ± 3.7)</td>
<td>2.9 [2.1, 4.0] (59 ± 2.6 $^*$)</td>
<td>2.1 [1.1, 3.9] (41 ± 3.9 $^*$)</td>
<td>35</td>
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</table>

$^*$ Indicates that the Hill coefficient held constant ($h = 1$) to obtain estimates of lower potency or less soluble compounds.

$^*$ Indicates that the maximal inhibition was significantly different than the maximal inhibition of 1 for the same GluN2 subunit (1 way ANOVA performed on the log transformed $IC_{50}$, Dunnett’s post hoc test, p < 0.05).
Proliferator-activated receptor (PPAR) activators. We suspect racemization with the structurally-related glitazone class of peroxisome racemization. This observation is consistent with reports of racemization with HPLC, we found that the molecules were prone to rapid racemization. While the separation of enantiomers for compounds in electrophysiological solutions (3.25–100 μM) was successfully accomplished using chiral semi-preparative HPLC, we found that the molecules were prone to rapid racemization. This observation is consistent with reports of racemization with HPLC, we found that the molecules were prone to rapid racemization. Taken together, the data suggest that the presence and position of hydrogen bond donating nitrogen atoms in this ring are important for activity.

Since all compounds were tested as the racemic mixture, we sought to determine whether the enantiomers had different degrees of potency. While the separation of enantiomers for compound 11 was successfully accomplished using chiral semi-preparative HPLC, we found that the molecules were prone to rapid racemization. This observation is consistent with reports of racemization with the structurally-related glitazone class of peroxisome proliferator-activated receptor (PPAR) activators. We suspect that this facile process occurs via enolization at the stereogenic center. Consequently, this rapid racemization prohibited biological evaluation of the enantiomers.

A key feature of this class of inhibitors is their ability to exert submaximal inhibition at saturating concentrations. This could reflect low solubility, which can lead to precipitation of compound at higher concentrations, setting an effective ceiling on the maximal concentration observed for a given compound in aqueous solution. We therefore determined the aqueous solubility of the most active compounds using nephelometry. Briefly, solubility was determined by measuring the forward light scattering of compounds in electrophysiological solutions (3.25–100 μM) and fitting the data with a linear segmental regression fit, where the intersection point was deemed to be the maximum solubility (NEPHELOstar, BMG Labtech, Cary, NC). The estimated maximal solubility for all active compounds was greater than 20 μM (except for 10 and 21), and was 10 times higher than the IC50 (except for 10, 8, 7 and 21), suggesting that the submaximal inhibition observed by saturating concentration of several analogues in this series does not reflect loss of material from solution (Table 2).

We next examined the mechanism of action for the prototypical compound 4, which showed in separate experiments 73–81% inhibition at saturating concentrations against all GluN2 subunits (n = 4–6, p <0.05 vs complete inhibition, extra sum-of-squares F test). We first evaluated whether the inhibition was derived from interference with the binding of either co-agonist. We tested whether the extent of 4 inhibition was reduced in the presence of increased concentrations of glutamate (1000 μM) or glycine (300 μM), which would be predicted if 4 acted as a competitive antagonist at the glutamate or glycine binding sites. Submaximal inhibition of the diheteromeric NMDA receptor responses by 5 μM 4 could not be surmounted by a 10-fold increase in glutamate or glycine concentration, indicating that the compound acts by a noncompetitive mechanism (Fig. 2).

We also examined whether the inhibition observed with 4 was voltage-dependent. Two electrode voltage clamp recordings of NMDA receptor current responses to a maximally effective concentration of glutamate and glycine with or without 4 were made at different membrane potentials between −60 and +60 mV and in the absence of Mg2+. The degree of inhibition produced by 3 μM 4 was the same at all voltages tested (n = 5, ANOVA p = 0.99). In addition, the mean reversal potential (Vrev) was not significantly different between control response to glutamate and glycine and the response to glutamate and glycine plus 3 μM 4.

**Table 3**

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>R1</th>
<th>R2</th>
<th>IC50 (μM)</th>
<th>% maximal inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>NH</td>
<td>NH</td>
<td>S</td>
<td>CH3(CH2)2CH3</td>
<td>-</td>
<td>2.3 [1.5, 3.6]</td>
<td>1.6 [0.7, 1.9]</td>
</tr>
<tr>
<td>24</td>
<td>NH</td>
<td>O</td>
<td>S</td>
<td>CH3(CH2)2CH2</td>
<td>-</td>
<td>4.2 [3.0, 6.0]</td>
<td>2.3 [1.5, 3.6]</td>
</tr>
<tr>
<td>25</td>
<td>NH</td>
<td>O</td>
<td>S</td>
<td>CH3(CH2)2CH2</td>
<td>-</td>
<td>1.2 [0.7, 1.9]</td>
<td>1.2 [0.7, 1.9]</td>
</tr>
<tr>
<td>26</td>
<td>NMe</td>
<td>NMe</td>
<td>S</td>
<td>CH3(CH2)2CH2</td>
<td>-</td>
<td>0.6 [0.4, 0.9]</td>
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</tr>
<tr>
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<td>NH</td>
<td>O</td>
<td>NH</td>
<td>CH3(CH2)2CH2</td>
<td>-</td>
<td>0.6 [0.4, 0.9]</td>
<td>0.6 [0.4, 0.9]</td>
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*IC50 values were obtained by least squares fitting the Hill equation to individual experiments, the Hill slope was allowed to vary between 0 and 1.5.*

**Figure 2.** Noncompetitive inhibition of recombinant diheteromeric NMDA receptor by 4. The mean inhibition by 5 μM 4 of NMDA receptor current responses to saturating concentrations of glutamate and glycine (100 μM and 30 μM, respectively) is compared to inhibition in elevated concentrations of either (A) glutamate (1000 μM) or (B) glycine (300 μM). Data shown are the mean inhibition (±SEM) from 4 to 6 oocytes, there are no significant differences at different co-agonist concentrations.
(V_{\text{REV-Control}} = -13.0 \pm 0.6, V_{\text{REV-1794-2}} = -11.9 \pm 0.5, n = 5, t\text{-test } p = 0.22, \text{ Fig. 3}). These data suggest that 4 is a negative allosteric modulator of NMDA receptors, since its effects are noncompetitive and voltage-independent.

To examine if this compound class can inhibit native NMDA receptors, we prepared primary cultures of p6–8 rat cerebellar granule cells as previously described,19 and performed patch clamp recordings of NMDA receptor responses as previously described.20 NMDA receptors in granule cells were activated with 100 \mu M NMDA and 30 \mu M glycine and then inhibited by co-applying NMDA and glycine with different concentrations of 4. The mean inhibitory responses of the varying concentrations of 4 were fit by the Hill equation (Fig. 4). The IC_{50} of 4 in this preparation was similar to the IC_{50} of the compound on GluN2B-containing diheteromeric receptors expressed in oocytes. This result confirms that this series of negative allosteric modulator is active at native receptors.

We also investigated the potential neuroprotective effects of this class of inhibitors. Cultured primary hippocampal neurons were prepared as previously described,21 maintained for 7–10 days in vitro at 37 \degree C in 5/95% CO_{2}/O_{2}. Neurons were challenged by replacing the cell’s media with a similar media but lacking Mg^{2+}, and in the absence and presence of 3 \mu M 4. Data are averaged from 5 oocytes and are shown ±SEM. The smooth curve shows a 3rd order polynomial fit to the data.

![Figure 3](image1.png)

**Figure 3.** Voltage-independent mechanism of GluN1/GluN2C receptor inhibition by 4. The mean current–voltage relationship is shown for activated GluN1/GluN2C receptor responses activated by maximally effective concentration of glutamate and glycine. Current responses were recorded from oocytes in the absence of extracellular Mg^{2+}, and in the absence and presence of 3 \mu M 4. Data are averaged from 5 oocytes and are shown ±SEM. The smooth curve shows a 3rd order polynomial fit to the data.

![Figure 4](image2.png)

**Figure 4.** Negative allosteric modulation by 4 of NMDA receptors expressed on cultured primary granule cells from rat cerebellum. (A) Representative patch clamp recording of the current response under voltage clamp from a primary cerebellar granule cell (V_{\text{HLCD}} = -70 \text{ mV}). The steady-state current response was attenuated by 30 \mu M 12 during the co-application of 100 \mu M NMDA plus 50 \mu M glycine. (B) Concentration–effect relationship of 4 on NMDA-induced currents in primary cerebellar granule cell cultures with an IC_{50} = 3.4 \mu M. Data are mean ± SEM (n = 5), data were fitted by the Hill equation.

![Figure 5](image3.png)

**Figure 5.** Neuroprotection by 4 against NMDA-induced excitotoxicity of cultured primary hippocampal neurons. Attenuation of excitotoxicity produced by 100 \mu M NMDA plus 30 \mu M glycine is shown for 30 \mu M 4 and the open channel blocker MK-801 (10 \mu M). Cell death was assessed by release of the intracellular enzyme lactate dehydrogenase (22), and expressed relative to the concentration of LDH released into culture media upon in vehicle treated cells (’p < 0.05 as compared to NMDA/Gly response and ’p < 0.05 as compared to control). Measurements were made in triplicate from 6 independent experiments, and are shown as the mean ± SEM.
receptor block at saturating concentrations may allow development of analogues that are effective neuroprotectants with a reduced side-effect profile.

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Supplementary data

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References and notes