6-(4-Pyridinyl)-1*H*-1,2,3-triazolo[4,5-*d*]-pyrimidin-4(5*H*)-one: A Structurally Novel Competitive AMPA Receptor Antagonist

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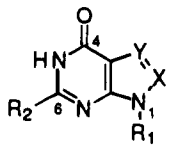
Introduction. The excitotoxic effects of the endogenous neurotransmitter glutamate are widely believed to be a major causative factor in the etiology of stroke and cerebral ischemia.[1] Also, there is considerable circumstantial evidence that implicates excessive stimulation of glutamate receptors in triggering neuronal degradation in other neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS).[2] Due to the pioneering efforts of molecular biologists and pharmacologists, three major ionotropic glutamate receptor subtypes have been identified.[3] These ionotropic receptors are named for the agonists that activate them: *N*-methyl-D-aspartic acid (NMDA), 2-amino-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA), and kainic acid (KA). These receptor subtypes offer a potential for intervention in the discovery of novel therapeutic agents for the treatment of various neurodegenerative diseases.[4]

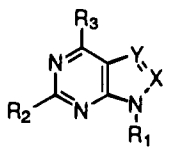
The recent discovery that NBQX (**1**) and DNQX (**2**), which are selective and potent AMPA receptor antagonists, are neuroprotective in various animal models of global and focal ischemia[5] has attracted considerable interest in the identification of other classes of AMPA antagonists.[6] Herein, we report that 6-(4-pyridinyl)-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-4(5*H*)-one (**3**) is a potent competitive antagonist of the AMPA subtype glutamate receptor.

Results and Discussion. Screening of our proprietary compound files for novel AMPA receptor antagonists, resulted in the discovery of **3** as a potent inhibitor of [^{3}H]AMPA binding to human hippocampal homogenates.[7] In an effort to better understand the structural features of **3** that are responsible for its [^{3}H]AMPA binding inhibitory activity, a substructure search of our compound library based on **3** was conducted. The compounds selected based on this search were evaluated for their ability to inhibit the binding of [^{3}H]AMPA to human hippocampal homogenates.[8] A subset of these compounds and their binding activities are shown in Table 1. The known AMPA antagonist DNQX is shown as a reference standard.

Table 1. Inhibition of [^{3}H]AMPA Binding by Compounds **3**–**10**



3,5,6,9,10



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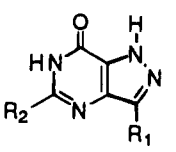

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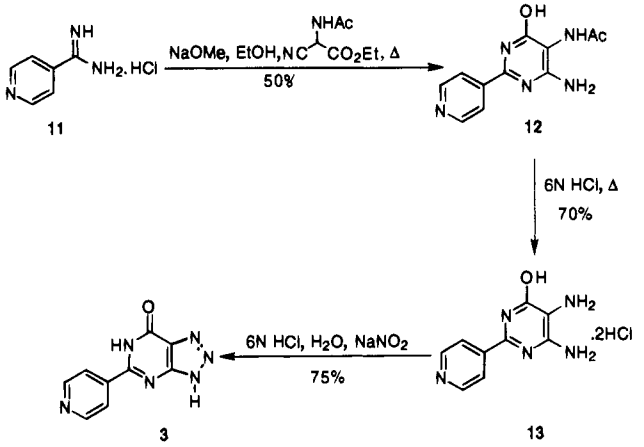
compd	R^1	R_2	R_3	X	Y	IC_{50} (μM)[a]
3	H	4-pyridyl		N	N	1.8
4	H	4-pyridyl	NH_2	N	N	*b*
5	H	4-pyridyl		CH	N	1.9
6	cyclopentyl	4-pyridyl		CH	N	2.1
7	H	4-pyridyl	NH_2	CH	N	*b*
8	cyclopentyl	4-pyridyl				*b*
9	cyclopentyl	4-pyridyl		N	CH	*b*
10	H	Me		N	CH	*b*
DNQX						0.28

[a] IC_{50} values are the geometric mean of at least two different determinations in triplicate with six dose levels of each inhibitor. The standard deviation was $<\pm 10\%$. [b] These compounds showed little (<5%) or no inhibition of [^{3}H]AMPA binding at concentrations of 5–7 μM.

Scheme 1



As evident from this data, there is a very stringent structural requirement for activity among this series of AMPA antagonists. Replacement of the carbonyl group of **3** with an amino group (compound **4**) resulted in complete loss of activity. The imidazopyrimidone **5** was equipotent to **3**. The presence of the N-1 hydrogen was not critical for activity, as evidenced from **6**, which was equipotent to both **3** and **5** in the binding assay. As seen with compound **4**, the C-4 amino analog of **5** (compound **7**) was devoid of any [^{3}H]AMPA inhibitory activity. These results could possibly be ascribed to the presence of the hydrogen bond accepting carbonyl group at C-4. Interestingly, compound **8**, where the N-1 and C-2 of **5** are juxtaposed, led to complete loss of activity. Also, the pyrazole analog of **5** (compound **9**) was devoid of binding affinity. These results suggests that a N-1(3) is needed for activity among this series. Analog **10**,[9] wherein the 4-pyridinyl group of **6** has been replaced with a methyl group, was inactive.

The synthesis of compound **3** is shown in Scheme 1. Thus, the readily available amidine **11**[10] was converted to the pyrimidine **12** via base-catalyzed condensation with ethyl acetamidocyanoacetate. Hydrolysis of **12** under acidic conditions followed by diazotization of the resulting diamine **13** gave the triazole **3** in good yield.

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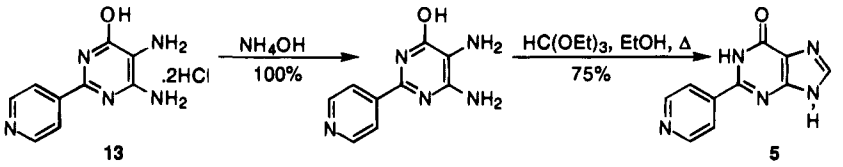
Scheme 2



Scheme 3

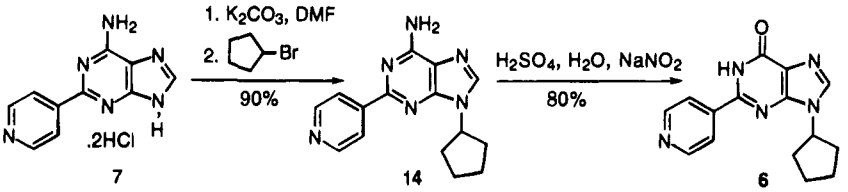


Table 2. Receptor Selectivity of **3** and **6**

compd	[^{3}H]AMPA (AMPA receptor) IC_{50} (μM)	% inhibition at 10 μM[a] [^{3}H]CGS 19755 (NMDA receptor)	[^{3}H]kainic acid (kainate receptor)
3	1.8	17	37
6	2.0	25	15

[a] Each determination of percent inhibition was performed in triplicate.

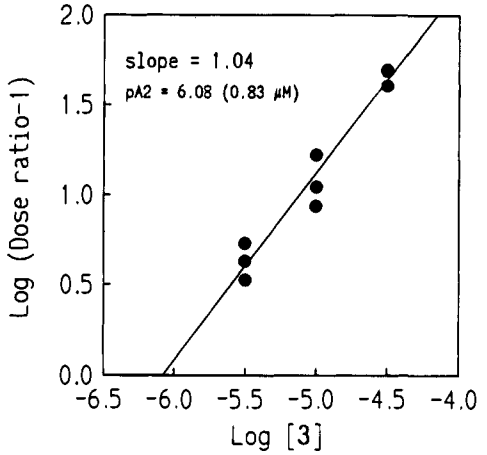


Figure 1. Effect of **3** on the AMPA-induced responses in frog oocytes.

Condensation of the free base of **13** with triethyl orthoformate afforded the imidazole **5** in 75% yield (Scheme 2). The cyclopentyl analog **6** was prepared from the known[11] imidazo pyrimidine **7** via a two-step sequence (Scheme 3). Alkylation of **7** with cyclopentyl bromide gave **15** which upon diazotization provided **6** in good yield.

Compounds **3** and **6** were further evaluated against other glutamate receptors and were found to be selective for the inhibition of [^{3}H]AMP binding (Table 2). Also, up to a concentration of 30 μM, these compounds did not significantly inhibit NMDA-induced current in frog oocytes.[12]

In further studies, a representative of this new class of AMPA antagonists (compound **3**) was examined for its ability to antagonize AMPA- and kainate-induced responses in frog oocytes.[13,14] The results are shown in the accompanying Schild plots.[15,16] Consistent with a competitive mode of antagonism of the AMPA receptor, compound **3** produced rightward shifts in the AMPA concentration curves with a pA_2 value of 6.1 (Figure 1).

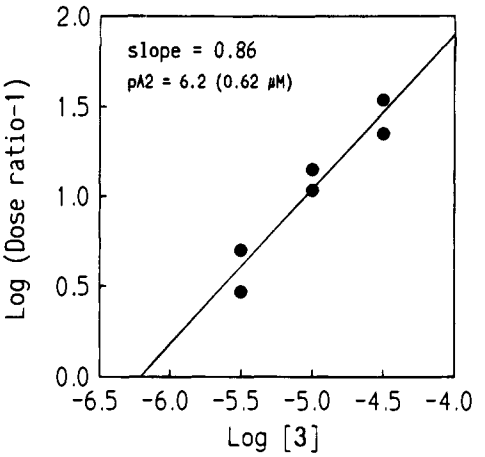
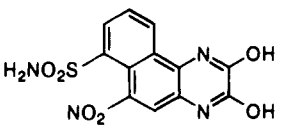


Figure 2. Schild plot of **3** vs kainate-induced responses in frog oocytes.



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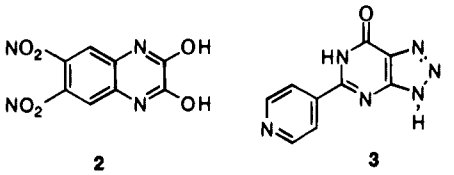

2 **3**

Similar results were obtained (pA_2 = 6.2) when kainate was used as the agonist in this assay (Figure 2).[17,18]

In summary, triazolopyrimidone **3** is a structurally novel, selective, and potent competitive AMPA receptor antagonist. Work is currently underway to evaluate the effect of **3** in various animal models of neuroprotection. Results of these studies and our efforts to improve the potency and selectivity among this class of AMPA antagonists will be the subject of future publications.

Acknowledgment. We thank Drs. John Mallamo, Matthew Miller, and Susan Ward for helpful discussions.

Supplementary Material Available: Methods for the receptor expression in frog oocyte, electrophysiology, and detailed synthetic procedures for **3**, **5**, and **6** (3 pages). Ordering information is given on any current masthead page.

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(12) At a concentration of 30 μM, compounds **3** and **5** showed <20% inhibition of NMDA (100 μM)-induced current in frog oocytes.

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(16) The data from electrophysiological recording were analyzed as follows. The ED_{50} (the concentration producing a half maximal effect) were calculated by fitting curves derived from the equation fractional response = $1/[1 + [ED_{50}/(\text{agonist})]]$ to dose–response data. Dose ratios (ED_{50} in presence of antagonist divided by control ED_{50}) were calculated for each antagonist concentration and a Schild plot (Figures 1 and 2) of log(dose ratio − 1) vs log(antagonist dose) generated. Linear regression of these points provided the pA_2 value, the antilog of which represents the apparent dissociation constant for the antagonist if the slope is unity.

(17) Kainate is a known agonist for AMPA receptor and has been used to study the effects of various agonists/antagonists that act at the AMPA receptor (see ref 18). Also, Oocytes injected with brain RNA preferentially express AMPA receptors as compared to kainate receptors (see ref 13). Hence, the results obtained with the use of kainate as an agonist in this study are due to the antagonism of AMPA receptors by **3**.

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