Synthesis of Willardiine and 6-Azawillardiine Analogs: Pharmacological Characterization on Cloned Homomeric Human AMPA and Kainate Receptor Subtypes

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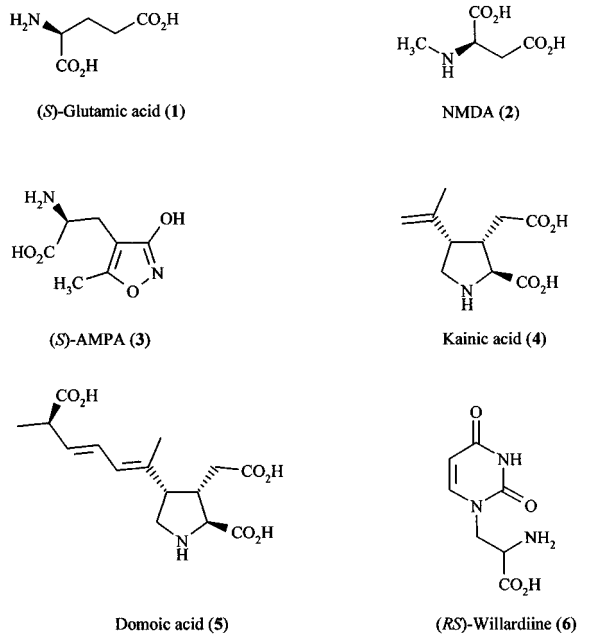
Both willardiine and azawillardiine analogs (**18**−**28**) have been reported to be potent and selective agonists for either AMPA or kainate receptors. We report here the novel synthesis and pharmacological characterization of a range of willardiine (**18**−**23**) and 6-azawillardiine (**24**−**28**) analogs on cells individually expressing human homomeric hGluR1, hGluR2, hGluR4, or hGluR5 receptors. Reaction of the sodium salts of substituted uracils (**7**−**12**) or 6-azauracils (**13**−**16**) with (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) in dry DMF, subsequent deprotection in TFA, and purification by ion-exchange chromatography gave mainly the willardiine analog in which alkylation took place on N1 of the uracil ring. We have investigated the subtype selectivity of these compounds by examining their binding affinity for homomeric hGluR1, -2, -4, or -5 (and hGluR6 in the case of 5-iodowillardiine (**22**)). From this study we have demonstrated that **22** has high affinity for hGluR5 and, compared to kainate, displays excellent selectivity for this receptor over both the AMPA receptor subtypes and the homomeric kainate receptor, hGluR6. 5-Fluorowillardiine (**19**) has higher affinity than AMPA for both homomeric hGluR1 and hGluR2 and compared to AMPA displays greater selectivity for AMPA receptor subtypes over the kainate receptor, hGluR5. Some structural features required for optimal activity at homomeric AMPA or kainate receptor subtypes have also been identified. It would appear that quite large lipophilic substituents at the 5-position of the uracil ring not only are accommodated by hGluR5 receptors but also lead to enhanced affinity for these receptors. In contrast to this, for optimal binding affinity to hGluR1, -2, or -4, smaller, electron-withdrawing substituents are required. For optimal activity at hGluR4 receptors a 6-aza-substituted willardiine is favored. The subtype-selective compounds described here are likely to be useful tools to probe the distribution and the physiological roles of the various glutamate receptor subunits in the central nervous system.

Introduction

It is widely accepted that (*S*)-glutamic acid (L-Glu, **1**) is the endogenous transmitter at a range of excitatory amino acid (EAA) receptors in the central nervous system (CNS). EAA receptors can be subdivided into two main classes, ionotropic receptors,[1] which mediate fast synaptic transmission through ligand-gated ion channels, and metabotropic glutamate receptors,[2] which are G-protein-coupled to second-messenger systems and are thought to be responsible for modulation of synaptic transmission. At present there are three main classes of ionotropic glutamate receptors named after the selective agonists that activate them: the *N*-methyl-D-aspartate (NMDA, **2**),[3] (*S*)-2-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic acid (AMPA, **3**), and kainic acid (**4**) receptors,[4,5] (see Chart 1 for agonist structures). Molecular biology has revealed that these pharmacologically distinct receptor subtypes are encoded by separate, albeit related, gene families.[4,5] Human AMPA receptors are encoded by the hGluR1−4 genes. These subunits can form functional homomeric ion channels that can be activated by both AMPA (**3**) and kainate (**4**). Human kainate receptors, hGluR5 and hGluR6, are activated by kainate and more potently by domoate (**5**), while AMPA has very little activity. Other kainate receptor assemblages (homomeric hGluR7, KA1, and KA2) bind kainate with high affinity but do not form functional ion channels.[4,5]

Chart 1. Structures of Selective Ionotropic Glutamate Receptor Agonists



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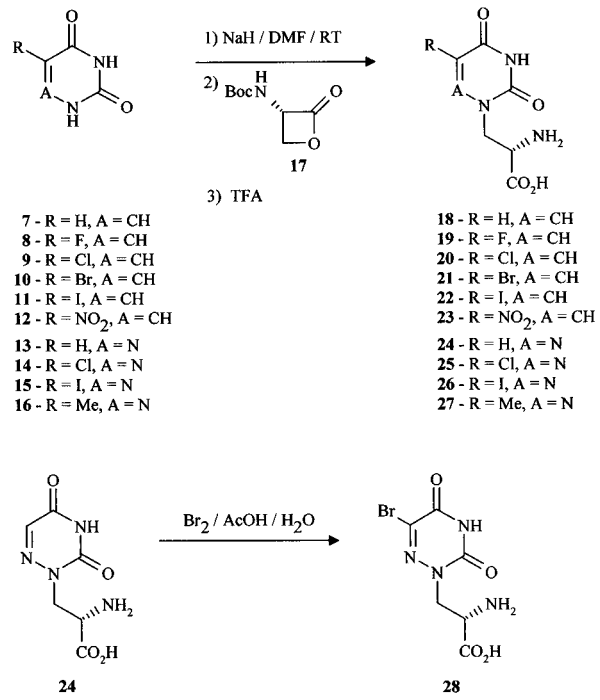
We have reported that analogs of the naturally occurring substance, (*S*)-willardiine (**18**), have potent and selective actions on AMPA and kainate receptors, the corresponding *R* enantiomers being inactive.[6−14] Thus, (*S*)-5-fluorowillardiine (**19**) is more potent and selective than AMPA as an AMPA receptor agonist in both cultured hippocampal neurons[8,9] and motoneurons in the neonatal rat spinal cord.[6] Conversely, (*S*)-5-iodowillardiine (**22**) is more potent and selective as a kainate receptor agonist than kainate in both cultured dorsal root ganglion (DRG) cells[9] and dorsal roots of the neonatal rat spinal cord.[7,13] In the case of the actions of willardiine analogs on hippocampal neurons and DRG cells, an extensive structure−activity study was undertaken.[9] It was observed that for potent and selective activity at AMPA receptors, electron-withdrawing groups at the 5-position of the uracil ring were required and that such groups should be as small as possible, because large groups were less well accommodated by the receptor. For optimal activity at kainate receptors larger electron-withdrawing groups with a high degree of lipophilicity were required. As a result of this study we decided to synthesize a range of 6-azawillardiine analogs (**24**−**28**) in an attempt to improve the potency and selectivity at both AMPA and kainate receptors. It was reasoned that this 6-aza substitution would lower the pK_a of the uracil ring, and therefore more of the ionized form would be available at physiological pH. In addition, because this substitution would not result in an increase in bulk around the uracil ring, it would be likely that such a substitution would not be detrimental to activity at either AMPA or kainate receptors. We have recently reported that these (*S*)-6-azawillardiine analogs are more potent agonists at AMPA receptors in the spinal cord than the corresponding willardiines as determined electrophysiologically in the neonatal rat spinal cord preparation[14] and by displacement of (*S*)-[^{3}H]AMPA in rat brain.[10−12] Since both willardiine and azawillardiine analogs were potent and selective ligands for either AMPA or kainate receptors, we decided to investigate whether these compounds had selective action on homomeric non-NMDA receptors. Such subtype-selective compounds would likely be useful tools to probe the distribution and the physiological roles of the various glutamate receptor subunits in the CNS. We report here the novel synthesis and pharmacological characterization of a range of willardiine (**18**−**23**) and 6-azawillardiine (**24**−**28**) analogs on cells individually expressing human homomeric hGluR1, hGluR2, hGluR4, or hGluR5 receptors (and hGluR6 in the case of 5-iodowillardiine (**22**)). In addition, the structural features for selective action at such homomeric glutamate receptors are discussed.

Chemistry

Racemic willardiine (**6**) has been previously synthesized in a 62% yield utilizing the Strecker amino acid synthesis with 1-(formylmethyl)pyrimidine-2,4-dione as the substrate.[15] Yields in the first two stages involving the synthesis of 1-(formylmethyl)pyrimidine-2,4-dione have been improved.[16−17] However, this approach to the synthesis of willardiines has a number of disadvantages: it involves a multistep synthesis, cannot be applied to a range of substituted analogs, and leads to racemic mixtures, which require resolution in order to obtain the individual enantiomers. We have attempted

Scheme 1



the synthesis of a range of 5-halo-substituted 1-(formylmethyl)pyrimidine-2,4-diones using the procedures previously described for the synthesis of the unsubstituted aldehyde[16,17] but obtained only poor yields of impure aldehydes. Arnold and co-workers have reported that (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (readily prepared from (*S*)-serine) can act as a chiral electrophilic alanine cation equivalent, which reacts with nucleophiles to give optically pure β-substituted alanines.[18−21] We have applied this reaction to the synthesis of a range of willardiine analogs.

Reaction of the sodium salts of substituted uracils (**7**−**12**) or 6-azauracils (**13**−**16**) with (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) in dry DMF (Scheme 1) gave mainly the willardiine analog in which alkylation took place on N1 of the uracil ring; none of the product alkylated on N3 was observed (due to N1 being more acidic than N3 (i.e., p$K_{a_{N1}}$ < p$K_{a_{N3}}$)[22]). However, in some cases small amounts of the N1,N3-dialkylated compound were formed, but this could be minimized by using excess of the uracil and by adding the (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) portionwise. Removal of the *N*-Boc protecting group by treatment with trifluoroacetic acid gave the crude willardiines, which were purified by ion-exchange chromatography.

Contamination by the starting uracils was removed by applying a suspension of the crude product in water to a bed of Dowex 50WX8-400 H+ resin. Elution of this column with water/ethanol (50:50) completely removed the contaminating uracil. Willardiine analogs were then isolated by elution of the column with 10% aqueous pyridine solution. Crystallization of the crude amino acid from water gave the corresponding willardiine analog free from contamination by either serine or the N1,N3-dialkylated compound.

This method therefore is the method of choice for preparing a range of willardiine analogs with a variety of substituents at the 5-position of the uracil ring. The 5-bromo-substituted compound (**28**) was obtained by treatment of **24** with a solution of bromine in aqueous

Table 1. Data for Displacement of Either [^{3}H]AMPA or [^{3}H]Kainate Binding from Homomeric hGluRl, hGluR2, hGluR4, or hGluR5 Receptors by Willardiine Analogs (**18**−**28**)

	K_i (nM)[a]			
compd	hGluR1	hGluR2	hGluR4	hGluR5
1	1360 ± 257	940 ± 93	868 ± 219	701 ± 46
3	103 ± 13	107 ± 16	155 ± 10	2144 ± 416
4	7450 ± 2020	12200 ± 2740	1710 ± 170	177 ± 22
18	386 ± 92	898 ± 86	8850 ± 923	28900 ± 7350
19	14.7 ± 1.3	25.1 ± 5.2	305 ± 107	1820 ± 149
20	65 ± 11	53.1 ± 4.4	451 ± 65	57.1 ± 23.5
21	92 ± 25	101 ± 19	457 ± 49	9.1 ± 2.4
22	163 ± 42	176 ± 29	972 ± 155	0.24 ± 0.06
23	279 ± 102	115 ± 22	207 ± 59	393 ± 217
24	163 ± 14	461 ± 57	189 ± 22	1980 ± 456
25	7.1 ± 2.2	16.7 ± 6.6	3.6 ± 1.4	30.9 ± 5.4
26	73.9 ± 4.8	101 ± 37	19.9 ± 8.2	19.8 ± 6.0
27	240 ± 39	372 ± 61	89 ± 8	1150 ± 172
28	29.0 ± 6.8	19.3 ± 8.3	7.9 ± 3.8	2.9 ± 0.3

[a] Versus [^{3}H]AMPA (hGluRl, -2, or -4) or [^{3}H]kainate (hGluR5) binding at homomeric non-NMDA receptor subtypes. Values represent a minimum of three determinations ± SEM.

acetic acid (Scheme 1) followed by purification by ion-exchange resin chromatography using Dowex 50WX8-400 resin.

Discussion

The pharmacological characterization of willardiine analogs (**19**−**28**) as potent agonists at either AMPA or kainate receptors has been previously reported;[6−14] however, these compounds have not been characterized on cloned homomeric AMPA or kainate receptor subtypes. We have investigated the subtype selectivity of these compounds by examining their binding affinity for homomeric hGluR1, -2, -4, or -5. Examination of the binding data in Table 1 reveals that in general substitution at the 5-position of the uracil ring of willardiine with either halogen or nitro substituents or 6-aza substitution increases affinity for all the homomeric receptors (hGluR1, -2, -4, or -5). As discussed in detail below, 5-halo substitution leads to some selectivity between the various cloned homomeric subtypes; however, in the case of 5-nitro substitution very little selectivity is observed. In comparison, glutamate (**1**) shows very little selectivity between the various subtypes, while AMPA (**3**) is 14−20-fold more selective for AMPA receptor subtypes (but does not show selectivity between hGluR1, -2, or -4) over hGluR5. Kainate (**4**) shows 10−40-fold selectivity for hGluR5 over the AMPA receptor subtypes (hGluR1, -2, and -4).

The binding profiles for willardiine and its analogs (**18**−**28**) are broadly similar for homomeric hGluR1 and hGluR2 receptors with the rank order of potencies being: (hGluR1) 5-Cl-6-azawil > 5-F-wil > 5-Br-6-azawil > 5-Cl-wil ≥ 5-I-6-azawil ≥ 5-Br-wil > 5-I-wil = 6-azawil ≥ 5-NO_2-wil ≥ 5-Me-6-azawil ≥ wil; (hGluR2) 5-Cl-6-azawil ≥ 5-Br-6-azawil ≥ 5-F-wil > 5-Cl-wil > 5-I-6-azawil = 5-Br-wil ≥ 5-NO_2-wil > 5-I-wil > 5-Me-6-azawil ≥ 6-azawil > wil. For 5-halowillardiines (**19**−**22**) the rank order of potency on both homomeric hGluR1 and hGluR2 receptors is 5-F > 5-Cl > 5-Br > 5-I, a trend that is also observed with the 5-halo-6-aza analogs (**25**, **26**, **28**). This order of potency is in agreement with that observed for **19**−**22** in electrophysiological studies on hippocampal cells[8,9] and neonatal rat spinal cord.[6] A similar rank order of potency for **25**, **26**, and **28** was observed in an electrophysiological study on neonatal rat motoneurons.[14] Furthermore, on the cloned receptors, the 6-aza analogs (**24**−**27**) are more potent than the corresponding non-aza analogs (**18**, **20**−**22**), with the 5-chloro-6-aza analog (**25**) being 9 times more potent on hGluR1 and 3 times more potent on hGluR2 than 5-chlorowillardiine (**20**).

Hansch analysis of the electrophysiological data for **19**−**22** obtained in hippocampal cells revealed a strong relationship between size and electron-withdrawing ability of the 5-substituent and agonist potency for AMPA receptors, with small, electron-withdrawing substituents being required for optimal activity.[9] As previously reported,[12,14] 6-aza substitution results in an increase in agonist potency at AMPA receptors, which may be explained by the resultant lowering of the pK_a of the uracil ring upon 6-aza substitution.[23] The binding profile for **18**−**28** on homomeric hGluR4 receptors is markedly different from that observed for homomeric hGluR1 or hGluR2 receptors. There is an approximately 2500-fold range in binding affinities for this series of compounds, suggesting that the homomeric hGluR4 receptor binding site is more sensitive to changes in substitution pattern on the uracil ring than either homomeric hGluR1 or hGluR2 receptors. The rank order of potency on homomeric hGluR4 receptors is 5-Cl-6-azawil ≥ 5-Br-6-azawil ≥ 5-I-6-azawil > 5-Me-6-azawil > 6-azawil ≥ 5-NO_2-wil ≥ 5-F-wil ≥ 5-Cl-wil = 5-Br-wil > 5-I-wil > wil. For optimal binding affinity to hGluR4 small electron-withdrawing substituents are required, with 5-chloro-6-azawillardiine (**25**) being 25 times more potent than the 5-methyl-6-aza analog (**27**) even though the 5-substituent is of similar size. For the 5-halo-substituted analogs (**20**−**22**, **25**, **26**, **28**) the rank order of potency is Cl > Br > I, in parallel to that found for hGluR1 and -2. The 6-aza analogs (**24**−**28**) have higher affinity for homomeric hGluR4 receptors than the corresponding non-aza analogs (**18**−**23**), with the 5-chloro-6-aza analog (**25**) being the most potent (K_i = 3.6 ± 1.4 nM).

In the case of 5-iodowillardiine (**22**), 6-aza substitution leads to a 50-fold increase in affinity for hGluR4. The improvement in potency on hGluR4 receptors upon 6-aza substitution is not likely to be due solely to a decrease in the pK_a of the uracil ring, as the 5-methyl-6-aza analog (**27**) is more potent than 5-nitrowillardiine (**23**) even though it is likely that **23** has a lower pK_a. Alternative explanations may be that the lone pair of the nitrogen atom at the 6-position of the uracil ring may be involved in extra hydrogen bonding with the receptor, or perhaps a $\pi-\pi$ interaction between the uracil ring and an aromatic group on the receptor is involved and this interaction is enhanced upon 6-aza substitution. There is an approximately 120000-fold range in affinity for the willardiine analogs (**18**−**28**) on homomeric hGluR5 receptors, demonstrating the remarkable sensitivity of these receptors to small changes in chemical structure. For example, 5-iodowillardiine (**22**) is approximately 7600 times more potent on hGluR5 than 5-fluorowillardiine (**19**). The rank order of potency of willardiine analogs (**18**−**28**) on homomeric hGluR5 receptors is 5-I-wil > 5-Br-6-azawil > 5-Br-wil > 5-I-6-azawil > 5-Cl-6-azawil ≥ 5-Cl-wil > 5-NO_2-wil > 5-Me-6-azawil > 5-F-wil ≥ 6-azawil ≫ wil. For the 5-halo-substituted compounds (**19**−**22**) the rank order of potency on hGluR5 receptors (5-I > 5-Br > 5-Cl > 5-F)

is the reverse of that observed for AMPA receptors (hGluR1 and hGluR2 receptors), which is in agreement with earlier reports on the electrophysiological characterization of **19**−**22** in dorsal root ganglion cells[9] and immature rat dorsal roots.[7,13] The 6-aza analogs (**25**, **26**, **28**) are no more potent than the 5-halo-substituted willardiines (**20**−**22**), and in fact 6-aza substitution of (*S*)-5-iodowillardiine (**22**) lowers affinity for hGluR5 30-fold, again in agreement with an earlier report on the electrophysiological characterization of **25**, **26**, and **28**.[13] The most potent ligand reported to date for homomeric hGluR5 receptors, (*S*)-5-iodowillardiine (**22**) ($K_i = 0.24 \pm 0.06$ nM), is approximately 700-fold more selective for hGluR5 receptors over either hGluR1 or hGluR2 receptors and 4000-fold more selective for hGluR5 over hGluR4 receptors. In addition, (*S*)-5-iodowillardiine (**22**) (100 μM) did not significantly displace [^{3}H]kainate binding from homomeric hGluR6 receptors and thus displays >400000-fold selectivity for hGluR5 over hGluR6 receptors. The selectivity of **22** for kainate receptors is in agreement with work on the electrophysiological characterization, where this compound was found to be 137 times more potent on kainate receptors in dorsal root ganglion cells ($EC_{50} = 0.14$ μM) than on AMPA receptors in hippocampal neurons ($EC_{50} = 19.2$ μM).[9] In contrast to this, and in agreement with previous work,[6−12] 5-fluorowillardiine (**19**) is highly selective for AMPA receptors (hGluR1, hGluR2, and hGluR4) over kainate receptors (hGluR5), being over 120-fold more potent on hGluR1 than hGluR5.

In conclusion, it would appear that quite large lipophilic substituents at the 5-position of the uracil ring not only are accommodated by hGluR5 receptors but also lead to enhanced affinity for these receptors. In contrast to this, for optimal binding affinity to hGluR1, -2, or -4, smaller, electron-withdrawing substituents are required. For optimal activity at hGluR4 receptors a 6-aza substituent is favored. We have identified that **22** has high affinity for hGluR5 and, compared to kainate, displays excellent selectivity for this receptor over both the AMPA receptor subtypes and the homomeric kainate receptor, hGluR6. 5-Fluorowillardiine (**19**) has higher affinity than AMPA for both homomeric hGluR1 and hGluR2 and compared to AMPA displays greater selectivity for AMPA receptor subtypes over the kainate receptor, hGluR5. Thus, from this study some structural features required for optimal activity at homomeric AMPA or kainate receptor subtypes have been identified. It is hoped that these can be used to design analogs with greater subtype selectivity in the future and that such compounds will be useful tools to probe not only the involvement of receptor subtypes in physiological processes but also their distribution in the CNS.

Experimental Section

Chemistry: General Procedures. Melting points were taken on a digital electrical melting point apparatus. Proton and carbon NMR spectra were measured with a 300.4 MHz Jeol spectrometer in D_2O and 20% aqueous DCl, using the sodium salt of 3-(trimethylsilyl)propanesulfonic acid as an internal standard. Thin layer chromatography was performed on 60 Å Whatman precoated silica gel plates. Ion-exchange chromatography was carried out using Dowex 50WX8-400 resin (obtained from Aldrich Chemical Co., U.K.). Elemental analyses were performed at the Microanalytical Laboratory of the University of Bristol. (*S*)-3-[(*tert*-Butoxycarbonyl)amino]-oxetan-2-one (**17**) was synthesized by the reported procedure.[18,19] The 6-azauracils (**14**, **15**) were synthesized using a literature method.[24] All other reagents were obtained from Aldrich Chemical Co. All uracils (**7**−**16**) were dried *in vacuo* (0.1 mmHg) over P_2O_5 for 3 days before use.

(*S*)-2-(2-Amino-2-carboxyethyl)-1,2,4-triazine-3,5-dione (24). To a vigorously stirred solution of 1,2,4-triazine-3,5-dione (**13**) (5.45 g, 48.2 mmol) in dry dimethylformamide (DMF; 150 mL) was added a 60% suspension of sodium hydride in mineral oil (1.9 g, 48 mmol). The mixture was stirred at room temperature overnight. The next day, (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (3.00 g, 16.1 mmol) was added portionwise over 1 h. Stirring was continued overnight. The next day, the mixture was evaporated under reduced pressure (0.5 mmHg). TFA (40 mL) was added to the residue with ice-cooling, and the mixture stirred at room temperature overnight. Excess TFA was removed under reduced pressure, and the residue was applied to a column of Dowex 50WX8-400 resin (0.5 mmol of cation/mL of resin). The column was eluted with 50% aqueous ethanol to remove the excess unbound uracil and then 1 M aqueous pyridine. The ninhydrin-positive fractions of the 1 M aqueous pyridine eluate were combined and evaporated to dryness under reduced pressure. Crystallization of the residue from water yielded (*S*)-2-(2-amino-2-carboxyethyl)-1,2,4-triazine-3,5-dione (**24**) (0.94 g, 29%) as a white solid: mp 225−226 °C dec; $[\alpha]^{25}_D$ +22.38° (*c* 0.36, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.42−4.58 (m, 3H), 7.59 (s, 1H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 41.8, 53.9, 138.1, 153.1, 160.4, 171.6. Anal. ($C_6H_8N_4O_4$) C, H, N.

(*S*)-2-(2-Amino-2-carboxyethyl)-6-chloro-1,2,4-triazine-3,5-dione (25) was obtained similarly from 6-chloro-1,2,4-triazine-3,5-dione (**14**) (6.94 g, 47.0 mmol), a 60% suspension of sodium hydride in mineral oil (0.9 g, 24 mmol) in dry DMF (100 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (3.00 g, 16.1 mmol). Deprotection in TFA (40 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-2-(2-amino-2-carboxyethyl)-6-chloro-1,2,4-triazine-3,5-dione (**25**) (2.26 g, 52%) as an off-white solid: mp 200.8−201.4 °C dec; $[\alpha]^{25}_D$ +18.97° (*c* 0.485, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.04 (dd, 1H, $J = 7.7, 7.7$ Hz), 4.37−4.51 (ABX system, 2H, $J = 14.5, 4.1, 6.7$ Hz); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 44.2, 55.4, 139.4, 152.7, 157.3, 172.8. Anal. ($C_6H_7N_4O_4$-Cl·0.5H_2O) C, H, N.

(*S*)-2-(2-Amino-2-carboxyethyl)-6-iodo-1,2,4-triazine-3,5-dione (26) was obtained similarly from 6-iodo-1,2,4-triazine-3,5-dione (**15**) (3.20 g, 13.4 mmol), a 60% suspension of sodium hydride in mineral oil (0.5 g, 13 mmol) in dry DMF (75 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (1.25 g, 6.7 mmol). Deprotection in TFA (20 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-2-(2-amino-2-carboxyethyl)-6-iodo-1,2,4-triazine-3,5-dione (**26**) (0.58 g, 27%) as yellow crystals: mp 222−223 °C dec; $[\alpha]^{25}_D$ +18.09° (*c* 0.29, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.44−4.63 (m, 3H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 43.4, 53.8, 111.4, 152.3, 158.3, 171.4. Anal. ($C_6H_7N_4O_4I$) C, H, N.

(*S*)-2-(2-Amino-2-carboxyethyl)-6-methyl-1,2,4-triazine-3,5-dione (27) was obtained similarly from 6-methyl-1,2,4-triazine-3,5-dione (**16**) (10.0 g, 88.4 mmol), a 60% suspension of sodium hydride in mineral oil (1.2 g, 30 mmol) in dry DMF (200 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (5.51 g, 29.5 mmol). Deprotection in TFA (80 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-2-(2-amino-2-carboxyethyl)-6-methyl-1,2,4-triazine-3,5-dione (**27**) (0.89 g, 10%) as white crystals: mp 204−205 °C dec; $[\alpha]^{25}_D$ +20.95° (*c* 0.525, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 2.22 (s, 3H), 4.44−4.58 (m, 3H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 18.6, 42.1, 53.9, 147.0, 153.3, 160.8, 171.6. Anal. ($C_7H_{10}N_4O_4$) C, H, N.

(*S*)-1-(2-Amino-2-carboxyethyl)pyrimidine-2,4-dione (18) was obtained similarly from pyrimidine-2,4-dione (**7**) (5.39 g, 48.1 mmol), a 60% suspension of sodium hydride in mineral

oil (1.8 g, 48 mmol) in dry DMF (100 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (3.00 g, 16.1 mmol). Deprotection in TFA (40 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-1-(2-amino-2-carboxyethyl)pyrimidine-2,4-dione (**18**) (2.24 g, 63%) as a white solid: mp 207−208 °C dec (lit.[15] mp 206−211 °C); $[\alpha]^{25}_D$ −24.4° (*c* 1.16, 6 M HCl) (lit.[15] $[\alpha]^{20}_D$ −20° (*c* 2.0, 1 M HCl)); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.33−4.54 (ABX system, 2H, J = 15.4, 4.5, 6.5 Hz), 4.61 (dd, 1H, J = 4.6, 4.6 Hz), 5.88 (d, 1H, J = 7.9 Hz), 7.70 (d, 1H, J = 7.9 Hz); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 50.5, 54.6, 104.6, 149.4, 155.3, 168.8, 171.0. Anal. ($C_7H_9N_3O_4$·1.25H_2O) C, H, N.

(*S*)-1-(2-Amino-2-carboxyethyl)-5-fluoropyrimidine-2,4-dione (19) was obtained similarly from 5-fluoropyrimidine-2,4-dione (**8**) (3.13 g, 24.1 mmol), a 60% suspension of sodium hydride in mineral oil (1.0 g, 24 mmol) in dry DMF (80 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (1.5 g, 8.0 mmol). Deprotection in TFA (30 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-1-(2-amino-2-carboxyethyl)-5-fluoropyrimidine-2,4-dione (**19**) (0.78 g, 43%) as white crystals: mp 221−222 °C dec; $[\alpha]^{25}_D$ −28.2° (*c* 1.24, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.3−4.51 (ABX system, 2H, J = 15.2, 4.6, 6.3 Hz), 4.59 (dd, 1H, J = 4.6, 4.6 Hz), 7.93 (d, 1H, J = 6.1 Hz); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 50.8, 54.6, 133.4 (d, J = 139 Hz), 140.3, 143.5, 154.4, 168.4. Anal. ($C_7H_8N_3O_4F$·0.4H_2O) C, H, N.

(*S*)-1-(2-Amino-2-carboxyethyl)-5-chloropyrimidine-2,4-dione (20) was obtained similarly from 5-chloropyrimidine-2,4-dione (**9**) (7.05 g, 48.1 mmol), a 60% suspension of sodium hydride in mineral oil (1.9 g, 48 mmol) in dry DMF (60 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (3.00 g, 16.1 mmol). Deprotection in TFA (60 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-1-(2-amino-2-carboxyethyl)-5-chloropyrimidine-2,4-dione (**20**) (2.30 g, 61%) as an off-white solid: mp 228−229 °C dec; $[\alpha]^{25}_D$ +2.9° (*c* 1.39, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.35−4.54 (ABX system, 2H, J = 15.2, 4.6, 6.5 Hz), 4.61 (dd, 1H, J = 4.6, 4.5 Hz), 8.05 (s, 1H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 50.6, 54.5, 111.3, 145.9, 154.0, 164.1, 170.5. Anal. ($C_7H_8N_3O_4$Cl) C, H, N.

(*S*)-1-(2-Amino-2-carboxyethyl)-5-bromopyrimidine-2,4-dione (21) was obtained similarly from 5-bromopyrimidine-2,4-dione (**10**) (9.19 g, 48.1 mmol), a 60% suspension of sodium hydride in mineral oil (1.9 g, 48 mmol) in dry DMF (60 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (3.00 g, 16.1 mmol). Deprotection in TFA (60 mL), followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-1-(2-amino-2-carboxyethyl)-5-bromopyrimidine-2,4-dione (**21**) (3.00 g, 67%) as a white solid: mp 227−228 °C dec; $[\alpha]^{25}_D$ +9.1° (*c* 1.76, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.35−4.53 (ABX system, 2H, J = 15.2, 4.6, 6.4 Hz), 4.59 (dd, 1H, J = 4.6, 4.6 Hz), 8.14 (s, 1H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 50.7, 54.6, 99.2, 148.6, 154.6, 164.8, 171.2. Anal. ($C_7H_8N_3O_4$Br) C, H, N.

(*S*)-1-(2-Amino-2-carboxyethyl)-5-iodopyrimidine-2,4-dione (22) was obtained similarly from 5-iodopyrimidine-2,4-dione (**11**) (7.64 g, 32.1 mmol), a 60% suspension of sodium hydride in mineral oil (1.3 g, 32 mmol) in dry DMF (40 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (2.00 g, 10.7 mmol). Deprotection in TFA (40 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-1-(2-amino-2-carboxyethyl)-5-iodopyrimidine-2,4-dione (**22**) (2.02 g, 58%) as a white solid: mp 222−224 °C dec; $[\alpha]^{25}_D$ +15.1° (*c* 1.19, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.33−4.51 (ABX system, 2H, J = 15.2, 4.6, 6.4 Hz), 4.56 (dd, 1H, J = 4.6, 4.8 Hz), 8.19 (s, 1H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 50.6, 54.7, 72.0, 153.5, 154.9, 165.9, 170.6. Anal. ($C_7H_8N_3O_4$I) C, H, N.

(*S*)-1-(2-Amino-2-carboxyethyl)-5-nitropyrimidine-2,4-dione (23) was obtained similarly from 5-nitropyrimidine-2,4-dione (**12**) (5.04 g, 32.1 mmol), a 60% suspension of sodium hydride in mineral oil (1.3 g, 32 mmol) in dry DMF (50 mL), and (*S*)-3-[(*tert*-butoxycarbonyl)amino]oxetan-2-one (**17**) (2.00 g, 10.7 mmol). Deprotection in TFA (40 mL) followed by purification by ion-exchange chromatography and crystallization from water as described for **24** gave (*S*)-1-(2-amino-2-carboxyethyl)-5-nitropyrimidine-2,4-dione (**23**) (1.41 g, 54%) as a yellow powder: mp 222−223 °C dec; $[\alpha]^{25}_D$ +15.4° (*c* 1.62, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.42−4.53 (ABX system, 2H, J = 7.8, 3.9, 5.5 Hz), 4.58 (dd, 1H, J = 4.7, 1.6 Hz), 9.26 (s, 1H); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 52.3, 55.6, 130.4, 154.6, 156.4, 161.6, 172.4. Anal. ($C_7H_8N_4O_6$) C, H, N.

(*S*)-2-(2-Amino-2-carboxyethyl)-6-bromo-1,2,4-triazine-3,5-dione (28). To a stirred solution of (*S*)-2-(2-amino-2-carboxyethyl)-1,2,4-triazine-3,5-dione (**24**) (0.10 g, 0.5 mmol) in acetic acid (20 mL) and water (5 mL) was added a 1 M solution of bromine in acetic acid (1.5 mL, 1.5 mmol). The mixture was stirred in a stoppered container overnight. The next day, the solution was evaporated under reduced pressure. The residue was dissolved in water (5 mL) and applied to a column of Dowex 50WX8-400 resin. The column was eluted with water and then 1 M aqueous pyridine. Ninhydrin-positive fractions of the 1 M aqueous pyridine eluate were evaporated under reduced pressure. Crystallization of the residue from water yielded (*S*)-2-(2-amino-2-carboxyethyl)-6-bromo-1,2,4-triazine-3,5-dione (**28**) (0.09 g, 63%) as a white solid: mp 208−210 °C dec; $[\alpha]^{25}_D$ +7.5° (*c* 0.08, 6 M HCl); ^{1}H NMR (300.4 MHz, DCl/D_2O, pH 1) δ 4.18 (dd, 1H, J = 7.6, 7.5 Hz), 4.4−4.54 (ABX system, 2H, J = 14.5, 5, 7.3 Hz); ^{13}C NMR (300.4 MHz, DCl/D_2O, pH 1) δ 44.2, 55.3, 132.7, 152.4, 157.8, 172.9. Anal. ($C_6H_7N_4O_4$Br) C, H, N.

Methodology for Binding Studies. Cell membranes were prepared from frozen HEK293 cells expressing either recombinant AMPA[27,28] or kainate receptors[25,26] by resuspending the cells in ice-cold distilled water, sonicating, and centrifuging at 50000*g* for 10 min. The membrane pellets were then washed in >100× volumes of 50 mM Tris-HCl buffer, pH 7.5, and centrifuged to remove endogenous glutamate. Binding reactions were performed at 4 °C for 60 min in a total volume of 250 μL containing 50 μL of membrane suspension (100−150 μg of protein). For kainate receptor binding (hGluR5[25] or hGluR6[26]), the reaction mixture consisted of 150 μL of 50 mM Tris-HCl, pH 7.5, 25 μL of [^{3}H]kainate (DuPont NEN), and 25 μL of unlabeled competitor (10^{-12}−10^{-3}). The final [^{3}H]-kainate concentrations used in the competitive inhibition experiments were 20 nM. For AMPA receptor binding (hGluRl,[27] hGluR2,[27] or hGluR4[28]) 20 nM [^{3}H]AMPA (DuPont NEN) was used for each receptor subtype and 100 mM KSCN was added to the Tris-HCl buffer. Following the 60 min incubation, the membranes were centrifuged at 50000*g* for 20 min to separate bound from free ligand, and the pellets were washed three times in cold assay buffer. Nonspecific binding was determined by incubation in the presence 10 μM glutamate. All data was analyzed by GRAFIT 2.0 software.

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