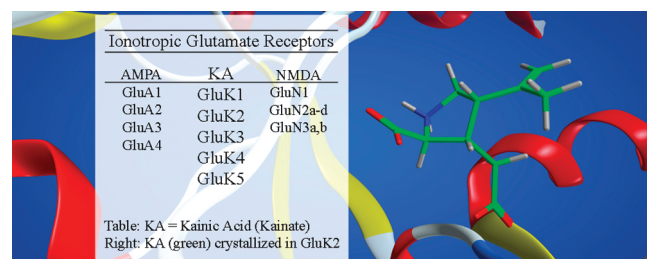# Medicinal Chemistry of Competitive Kainate Receptor Antagonists

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Abstract

Ionotropic Glutamate Receptors

AMPA	KA	NMDA
GluA1	GluK1	GluN1
GluA2	GluK2	GluN2a-d
GluA3	GluK3	GluN3a,b
GluA4	GluK4	
	GluK5	

Table: KA = Kainic Acid (Kainate)
Right: KA (green) crystallized in GluK2

Kainic acid (KA) receptors belong to the group of ionotropic glutamate receptors and are expressed throughout in the central nervous system (CNS). The KA receptors have been shown to be involved in neurophysiological functions such as mossy fiber long-term potentiation (LTP) and synaptic plasticity and are thus potential therapeutic targets in CNS diseases such as schizophrenia, major depression, neuropathic pain and epilepsy. Extensive effort has been made to develop subtype-selective KA receptor antagonists in order to elucidate the physiological function of each of the five subunits known (GluK1−5). However, to date only selective antagonists for the GluK1 subunit have been discovered, which underlines the strong need for continued research in this area. The present review describes the structure−activity relationship and pharmacological profile for 10 chemically distinct classes of KA receptor antagonists comprising, in all, 45 compounds. To the medicinal chemist this information will serve as reference guidance as well as an inspiration for future effort in this field.

Keywords: Glutamate receptors, kainic acid receptors, competitive antagonists, medicinal chemistry, structure−activity relationship studies

The common α-amino acid (*S*)-glutamate (Glu) functions as the major endogenous excitatory neurotransmitter in the central nervous system (CNS). Upon its release into the synaptic cleft, it activates the Glu receptors. These receptors have been divided into two major classes: the ligand-gated ion channels named *the ionotropic receptors* (iGluRs) and the slower acting G-protein coupled receptors named *the metabotropic receptors* (mGluRs). On the basis of ligand affinity studies, the class of iGluRs has been divided into three subgroups: the *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainate (KA) receptors (*1−3*). With focus on the KA receptors, it is believed that this subgroup of receptors are potential therapeutic targets in diseases such as schizophrenia, major depression, pain, and epilepsy. In order to elucidate the role of the KA receptors, medicinal chemists have attempted to develop subtype-selective KA receptor ligands for the last two to three decades. Although extensive effort has been made, only a limited number of subtype-selective KA receptor ligands have been reported. For a comprehensive review on KA receptor agonists, see our recent review (*4*). The aim of this review is to provide the medicinal chemists (and others) who have an interest in the structure−activity relationship (SAR) of competitive KAR antagonists with an overview of the field. This information may aid the design of new subunit-selective KA receptor antagonists.

KA Receptor Structure, Expression, and Function

The iGluRs are transmembrane-bound proteins comprising four regions: an intracellular carboxylate terminal domain (CTD), a transmembrane domain (TMD), a ligand-binding domain (LBD), and the amino-terminal domain (ATD) (Figure 1A) (*5*). To this date, five distinct KA subunits have been identified which are named GluK1, GluK2, GluK3 (formerly iGluR5−7), and GluK4 and GluK5 (formerly KA1 and KA2), in accordance with the new IUPHAR nomenclature (*6−8*). Functional KA receptors comprise four KA subunits which are assembled from two dimers of two homo- or two heteromeric subunits. In control of the assembly process, the ATD is believed to play a pivotal role (*9*). It has been shown that GluK1−3 may form functional

homomeric(*10*) or heteromeric(*11*) receptors, whereas GluK4,5 only form functional receptors in combination with subunits GluK1, GluK2, or GluK3 (Figure 1B) (*12*). However, homomeric GluK4(*13*) and homomeric GluK5 receptors have been expressed in mammalian cells for use in binding studies. Although a higher degree of symmetry (2-fold) is achieved by opposite assembly (heterotetramer B), it remains to be elucidated if uniform assembly (heterotetramer A) is also feasible (Figure 1).

KA receptors are highly expressed in the central nervous system (CNS), particularly in the hippocampal formation (mossy fiber (MF)) (*15*), and are implicated in several neurological functions (*7, 16−20*), such as long-term potentiation (LTP) (*21*) and synaptic plasticity(*5*).

Although most studies have focused on AMPA receptors (*5*), the biostructural mechanism underlying activation of the KA receptors has also been compared to the *closure of a clam shell* (Figure 2) (*22*): The ligand-binding domain (LBD) is in its apo state at which the KA agonist(*4*) may approach. This progression facilitates closing of the D1- and D2-domains, rendering the agonist-LBD in an occluded state (agonist state). During this process, the ion channel at the center of the tetrameric receptor complex undergoes a conformational change which allows for calcium ions to flow. Rapidly thereafter the agonist-bound receptor enters a desensitized

closed state in which the ion channel is again closed. It has been shown that some agonists promote desensitization at a speed so fast that an ion channel opening cannot be measured (current). This phenomenon has been named functional antagonism and is outside the scope of this review. For KA receptors, desensitization may be inhibited by addition of concanavalin A (ConA) (*23*) which is a lectin isolated from the jack-bean. Release of the agonist renders the receptor in the desensitized open state which is reorganized into the free apo state. It may be speculated whether the latter process exists in equilibrium (dotted arrow). In that case, it would be possible for a full functional antagonist to facilitate the desensitized closed state. In contrast, the mechanism underlying the functional properties of an antagonist is more straightforward. When in its apo form, the receptor recognizes the antagonist, and upon binding the closure of the clam shell (D1, D2) is blocked (antagonist state) and the ion channel is not opened.

Therapeutic Potential

Despite evidence that AMPA/KA receptors play a pivotal role in respiratory (*24*) and cardiovascular (*9, 25*) regulation, AMPA/KA receptor antagonists have been shown to be well-tolerated after systemic administration in animals (*26*), and in human studies (*25, 27−29*). Although AMPA/KA receptor antagonists have been shown to cause cognitive deficits in rats, such side effects are believed to be temporary and thus acceptable in the case of prevention of permanent neurological damage after, for example, stroke (*30*).
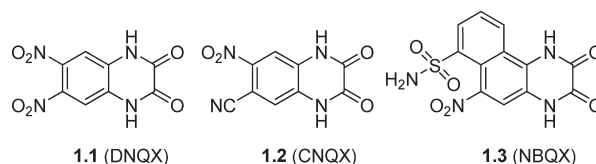
GluK2-containing KA receptors have been suggested to facilitate propagation of seizures (*31, 32*). In support of this hypothesis, it has been demonstrated that mice lacking the GluK2 gene (GRIK2 knock out) are much less sensitive to KA-induced seizures than wild type mice (*33*). GluK1 and/or GluK3 may also be involved in seizure activity, since antagonism (by compound **3.1**, see Table 3) of receptors containing these subunits provides an anticonvulsant effect in the kindling model of epilepsy. Extracellular glutamate has been shown to increase after physical brain trauma (*34, 35*), and an
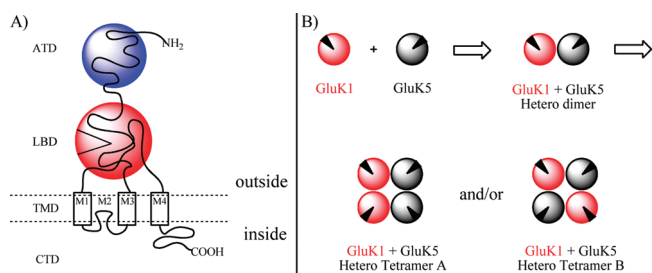
Figure 1. (A) Cartoon illustration of an iGluR showing the four regions: the carboxylate terminal domain (CTD), the transmembrane domain (TMD), the ligand binding domain (LBD), and the amino terminal domain (ATD). (B) Cartoon illustration of uniform assembly (hetero tetramer A) or opposite assembly (hetero tetramer B) of two heterodimers of GluK1 and GluK5. Black triangle illustrates the LBD.
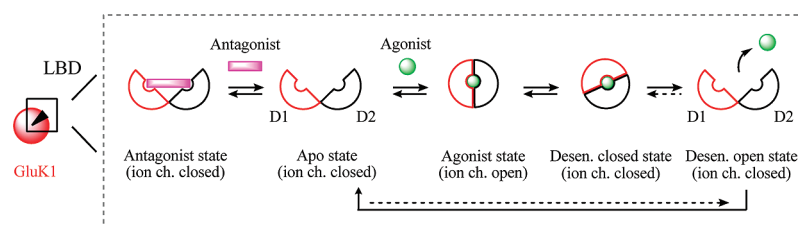
Figure 2. Schematic representation of the ligand-binding domain (LBD) of GluK1 as a *clam shell*: In its open free form (apo state), an agonist may bind, which facilitates closure of the shell and opening of the ion channel (agonist state). Depending on the nature of the agonist, the receptor may enter a desensitized closed state in which the ion channel is blocked. Subsequent release of the agonist renders the receptor available in the free apo state via an intermediate desensitized open state. An antagonist captures and stabilizes the apo state of the receptor (antagonist state), thus keeping the ion channel closed.

Table 1. Chemical Structures and Pharmacological Profile of Quinoxaline-2,3-dione Analogues **1.1**−**1.3**[a]

cmpd	AMPA func.[b]	KA func.[b]	NMDA func.[b]	GluK1 bind.[c]	GluK1 func.[d]	GluK2 bind.[e]	GluK2 func.[d]	GluK3 bind.[c]	GluK2/5 bind.[c]	GluK2/5 func.[d]
1.1 (DNQX)	0.25[f]	0.53[f]	4.1[f]	−[h]	−[h]	0.35	−[h]	−[h]	−[h]	−[h]
1.2 (CNQX)	0.40[f]	0.27[f]	13[f]	−[h]	8.0	0.53	18	−[h]	−[h]	72
1.3 (NBQX)	0.063[g]	0.078[g]	> 300[g]	12	25	0.87	21	24	0.6	87

[a] Data for KA receptor subunits were obtained in the presence of ConA. All values in [μM]. [b] K_i values for inhibition of currents activated by kainate (100 μM), AMPA (30 μM), or NMDA (30 μM/glycine (3 μM)) in *Xenopus* oocytes injected with mRNA from rat cortex. [c] K_i values for displacement of [³H]KA radioligand at human KA receptors expressed in HEK293 cells; from ref *56*. [d] IC$_{50}$ values for inhibition of 100 μM glutamate-induced Ca^{2+} influx in HEK293 cells expressing human KA receptors; from ref *50*. [e] K_i values for displacement of [³H]KA radioligand at homomeric receptors in BHK cells; from ref *51*. [f] From ref *57*. [g] From ref *58*. [h] Not tested.

increased synaptic release of glutamate has been reported during periods of severe global and focal ischemia (*36*, *37*). Accordingly, KA receptor antagonists may prove neuroprotective by inhibiting Glu release. On the other hand, KA agonists may be beneficial in accordance with the hypoglutamatergic theory of schizophrenia which builds on the finding that the expression of GluK2 and GluK5 mRNAs is reduced in the hippocampus of schizophrenic patients (*38*). Finally, activation of AMPA, KA, and NMDA receptors appears to be involved in the development of chronic pain (*39*−*42*). As an example, GluK1-selective antagonists have been shown effective in the treatment of migraine (*43*).

Quinoxaline-2,3-dione Derivatives

The quinoxaline-2,3-dione parental skeleton is a highly rigid heterocycle with an aromatic benzene ring fused with a nonaromatic heterocyclic moiety. Within the class, analogues **1.1** (DNQX) and **1.2** (CNQX) (Table 1) were the first competitive AMPA receptor antagonists to be discovered. The two compounds show micromolar affinity for AMPA/KA receptors but low receptor group and -subtype selectivity (Table 1). Furthermore, they show affinity for the glycine binding site at NMDA receptors, which may produce unwanted side effects (*44*, *45*). From X-ray structure studies in GluA2, it has been shown that it is the dione moiety of compound **1.1** (DNQX) that interacts with the receptor amino acid residues otherwise engaged in α-amino acid recognition and binding (*46*). The compounds primarily interact with domain D1, preventing agonists from forming initial contacts with the open cleft of the binding pocket (*47*).

Expanding the quinoxaline-2,3-dione scaffold by a fused benzylsulfonamide ring gave rise to **1.3** (NBQX) which was disclosed as a potent, selective antagonist at AMPA/KA receptors (Table 1) (*48*). This compound
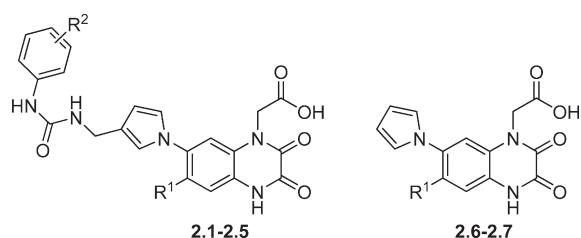
showed a modest selectivity (3-fold) for AMPA receptors versus GluK1-containing KA receptors expressed in dorsal root ganglia (K_B values: 0.3 and 0.9 μM, respectively) (*49*). However, when tested in HEK293 cells expressing GluK1 receptors, **1.3** displayed an IC$_{50}$ value of 25 μM (*50*). In a binding study, **1.3** showed micromolar affinity for GluK2 (*51*), with only modest antagonistic activity at this subtype (IC$_{50}$ = 21 μM) (*50*). In a functional study, little antagonistic effect was observed when **1.3** was applied at a concentration of up to 0.30 μM at rat GluK2 or GluK3 receptors (*52*). However, care should be taken when interpreting this latter finding, as the pharmacological profile of native human GluK3 is very different from that of the rat homologue used in this study (*53*). Although **1.3** has been shown to exhibit neuroprotective and analgesic effects in rats (*45*, *54*), the compound was discontinued as a drug candidate due to nephrotoxicity arising from low water-solubility (*55*).

By introduction of a substituent at N¹ and introduction of a pyrrolyl group in the 7-position, a third generation of quinoxalinedione analogues **2.1**−**2.7** (Table 2) and **3.1**−**3.3** (Table 3) was discovered. The class showed selective affinity for AMPA/KA receptors (*59*). In **2.1**−**5**, a phenylureamethyl group was added in the 3-position of the pyrrolyl moiety, affording high affinity for AMPA receptors but only moderate affinity for KA receptors. By furthermore introducing an ethylester group in the 3- or 4-position of the phenyl ring (**2.2** and **2.3**, respectively), both AMPA and KA receptor affinity was increased moderately (3−5-fold). Replacing this ester group with a halogen-containing group (**2.4** and **2.5**) further improved KA receptor affinity (5-fold) while maintaining the level of AMPA receptor affinity. A final attempt in this series was to omit substituents on the pyrrolyl moiety (**2.6**), which led to the loss of AMPA

affinity, decrease in KA affinity, and increase in NMDA receptor affinity. Replacement of the trifluoromethyl group in the 6-positon of **2.6** with a nitro group (compound **2.7**) amounted in loss of NMDA affinity and moderate increase of both AMPA and KA receptor affinity.

Inspired by **2.1**−**2.5**, compound **3.1** (LU 136541) was designed with a pyrrolyl group carrying a phenylurea-methyl substituent in the 3-position. A hydroxyl group was furthermore introduced at N^1 and a hexane ring was fused with the aromatic parental skeleton. The compound exhibited moderate to good affinity for subtypes
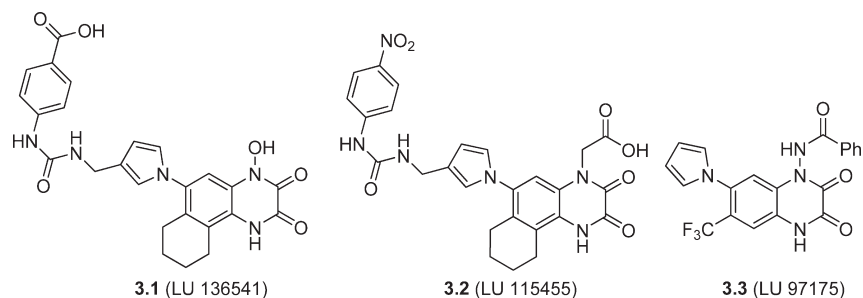
GluA2, GluK1, GluK2, and GluK3 (Table 3). Affinity for GluK2 and GluK3 was improved by replacement of the N^1-hydroxyl with a methylacetyl group, and by replacement of the distal acid with a nitro group to yield **3.2** (LU 115455). A noteworthy compound in this series, compound **3.3** (LU 97175), displayed 60-fold higher affinity for recombinant rat GluK3 receptors versus native rat AMPA receptors in radioligand binding assays and within the KA receptor subtypes a 4- and 14-fold higher affinity for GluK3 over GluK1 and GluK2, respectively (59). In *in vivo* rat models, **3.3** exhibited improved anticonvulsant activity compared to selective AMPA antagonists (63) without inducing motor impairment (59).

The pyrrolylquinoxaline-2,3-dione analogues **4.1**−**4.6** were characterized in binding assays at homomeric GluK1−3 and homomeric GluK5 receptors (14) (Table 4) (64). The *N*-benzyl-*N*-methylamine **4.1** (BSF-84077) was found to bind selectively to GluK1 (Table 4) with low affinity for AMPA receptors ($> 23 \mu$M). In *in vivo* models, this analogue protected animals from NMDA-induced convulsions with a median effective dose (ED_{50}) of 14 mg/kg. In **4.2**, the *N*-benzyl-*N*-methylamine was substituted for a piperidine ring. This retained the same binding profile at KA receptors as compared to **4.1**, but induced an increased affinity at the AMPA receptors (> 10-fold). In analogue **4.3**, a phenyl group was included at the 4-position of the piperidine ring which improved the GluK3 affinity by 10-fold. This phenyl substituent was linked by an ethylene in **4.4** and the piperidine ring replaced by a piperazine, improving GluK1 affinity 19-fold compared to **4.3**. However, the optimal linker for high GluK1-affinity appeared to be a methylene group as in analogue **4.5** (BSF-91594), which showed an impressive > 170-fold selectivity for human GluK1 (K_i value 3.8 nM) over GluK2, GluK3, and

Table 2. Chemical Structures and Binding Affinities of Quinoxaline-2,3-dione Analogues **2.1**−**2.7** at Native iGluRs (Rat Synaptosomes)[a]

2.1-2.5   **2.6-2.7**

cmpd	R^1	R^2	KA	AMPA	NMDA/Gly
2.1[b]	CF_3	H	3.4	0.015	> 30
2.2[c]	CF_3	3-COOEt	1.2	0.004	> 30
2.3[c]	CF_3	4-COOEt	0.7	0.004	> 30
2.4[b]	CF_3	4-Br	0.11	0.004	> 30
2.5[b]	CF_3	4-CF_3	0.11	0.005	> 30
2.6[d]	CF_3		1.5	0.17	8.0
2.7[d]	NO_2		0.41	0.070	> 25

[a] All values are K_i in [μM]. Radioligands used: AMPA, [^{3}H]AMPA; GluK1-3,5, [^{3}H]KA radioligand. [b] From ref 60. [c] From ref 61. [d] From ref 62.

Table 3. Chemical Structures and Binding Affinities (K_i) of Quinoxaline-2,3-dione Analogues **3.1**−**3.3** at Homomeric iGluR Subtypes (HEK293 Cells)[a]
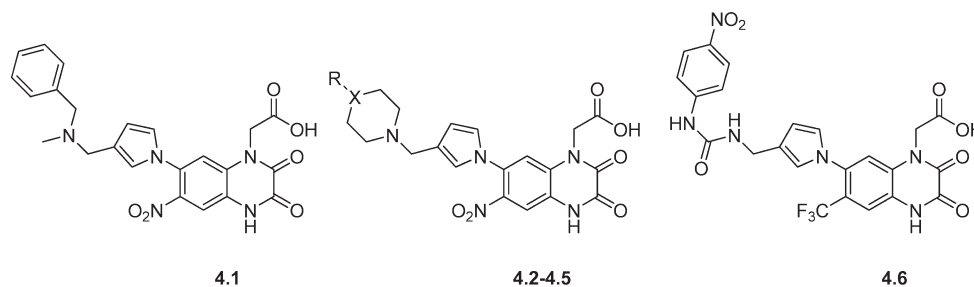

3.1 (LU 136541)   **3.2** (LU 115455)   **3.3** (LU 97175)

cmpd	GluA2	GluK1	GluK2	GluK3
3.1 (LU 136541)[b]	0.06	1.1	1.2	0.26
3.2 (LU 115455)[b]	0.018	1.3	0.37	0.21
3.3 (LU 97175)[b]	1.3	0.088	0.31	0.022

[a] All values in [μM]. Radioligands used: GluA2, [^{3}H]AMPA; GluK1-3, [^{3}H]KA. [b] From ref 59.

Table 4. Chemical Structures and Binding Affinities (K_i) of Quinoxaline-2,3-dione Derivatives **4.1−4.6** at Native AMPA Receptors[a]

cmpd	X	R	AMPA	GluK1	GluK2	GluK3	GluK5
4.1 (BSF-84077)[c]			> 23	0.43	23	—[b]	> 30
4.2[c]	CH_2		3.0[d]	0.33	28	5.7	> 30
4.3[c]	CH	Ph	—[b]	0.23	3.6	0.53	18
4.4[c]	N	PhCH2CH2-	—[b]	0.012	1.4	1.4	25
4.5 (BSF-91594)[c]	N	Ph−CH2-	0.25	0.0038	4.1	0.68	20
4.6[c]			0.005[d]	0.10	0.61	0.046	12

[a] All values in [μM]. Radioligands used: AMPA, [³H]AMPA; GluK1-3,5, [³H]KA radioligand. [b] Not tested. [c] From ref *64*. [d] From ref *60*.

GluK5. Furthermore, **4.5** showed only moderate binding affinity at native AMPA receptors (K_i value 0.25 μM), and no binding to the glycine coagonist site of the NMDA receptors was observed. In an *in vivo* model, **4.5** also protected animals from NMDA-induced convulsions with a median effective dose (ED_{50}) of 3.3 mg/kg (*64*). The basic nature of the distal amine was depleted by its inclusion in an aromatic ureyl moiety, compound **4.6**. This analogue displayed decreased affinity for GluK1, but higher affinity for GluK2, GluK3 (*64*), and native AMPA (*60*) receptors.

Isatinoxime Derivatives

Oxime **5.1** (NS102) has been reported to be a selective GluK2 antagonist with little effect on GluA2/GluA4 receptors (*65*). However, **5.1** has also been shown to block GluK1-containing KA receptors on DRG neurons with weaker effects at native AMPA receptors expressed in the cortex (Table 5) (*49*). In mice, **5.1** (10 mg/kg i.p.) (*66*) antagonized behavioral effects including seizures induced by systemic domoate administration and blocked domoate-induced neurodegeneration. However, **5.1** only offered partial protection of the negative behavioral responses to KA and could not block KA-induced neurodegeneration. The lack of discrimination between KA receptor subunits and low water solubility limits the usefulness of **5.1** (NS-102) as a pharmacological tool.

Compound **5.2** (NS257) exhibited an IC_{50} value of 1.05 μM estimated from inhibition of KA-induced currents recorded from oocytes injected with total mouse brain mRNA. The compound was shown to have a slight preference (2-fold) for GluA2 antagonism over GluK2 antagonism (Table 5) (*67*).

Analogue **5.3** ((R/S)-NS1209) showed medium range nanomolar affinity for homomeric GluA1, GluA2, and GluK1 and low micromolar affinity for GluK2 (Table 5). In a functional study, **5.3** was 100-fold more potent at GluK1 versus GluK2 receptors (Table 5) (*68, 69*). The (S)-stereoisomer stabilizes the GluA2 ligand-binding domain in a hyper-extended conformation compared to the apo state (*69*), but whether this flexibility is also true for KA receptors is unknown. Racemate **5.3** has shown promising results in animal models of status epilepticus (SE) (*70*), neuropathic pain, and allodynia in humans (*71*) and as a neuroprotectant against status-induced hippocampal neurodegeneration in a rodent (rat) animal model (*70*).

Decahydroisoquinoline Derivatives

Decahydroisoquinolines **6.1−6.6** constitute a class of AMPA/KA receptor antagonists which comprise an α-amino acid moiety as part of a rigid skeleton The analogues possess a flexible linker with a distal tetrazolyl group as a carboxylic acid bioisoster which forms hydrogen bonding interactions with the D2 domain of the KA receptor. On the chemical variation of this distal functionality, several interesting pharmacological results were observed. Analogue **6.1** (LY293558 or LY326325 for the HCL salt), which contains a tetrazolyl group as a bioisosteric substitution for a carboxylic acid, is a nonselective AMPA/KA receptor antagonist (*72−74*). The compound displayed only a 10-fold selectivity for GluK1 receptors versus NMDA receptors (*17*). Nonetheless **6.1** has been used in the identification and characterization of GluK1 receptors in neurons (*75*). Activity at neuronal AMPA receptors in acutely isolated cerebellar

Table 5. Chemical Structures and Pharmacological Profile of Isatin Oxime Analogues **5.1−5.3**[a]

5.1 (NS 102)   **5.2** (NS 257)   **5.3** ((R/S)-NS 1209)
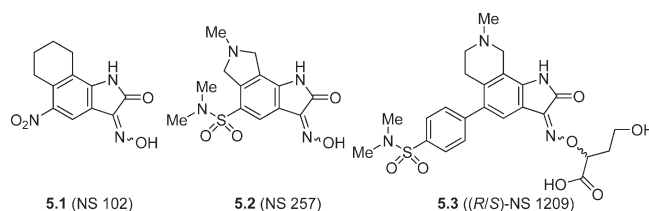
cmpd	GluA1		GluA2	GluK1		GluK2	
	bind.	func.	bind.	bind.	func.	bind.	func.
5.1 (NS 102)	—[b]	—[b]	—[b]	—[b]	6[c]	1.4[d]	—[b]
5.2 (NS 257)[e]	—[b]	0.97	—[b]	—[b]	—[b]	—[b]	2.3
5.3 ((R/S)-NS 1209)	0.12[g]	—[b]	0.030[g]	0.74[g]	0.63[f]	6.2[g]	65[f]

[a] All values in [μM]. [b] Not tested. [c] K_B value for inhibition of Ca^{2+} influx in rat dorsal root ganglion (DRG) neurons; from ref 49. [d] Displacement of [^{3}H]KA radioligand; from ref 65. [e] IC_{50} values for inhibition of KA-induced depolarization in the appropriate mRNA-injected *Xenopus* oocytes; from ref 67. [f] IC_{50} values for inhibition of domoate-induced currents in HEK293 cells; from ref 68. [g] Displacement of [^{3}H]AMPA radioligand; from ref 69.

Purkinje neurons has been previously reported (*75*) for **6.1** giving an estimated IC_{50} of 0.5 μM (vs 100 μM KA). The compound was shown to be neuroprotective in a cat focal ischemia model and demonstrated anticonvulsant activity in mice (*73*) and analgesic activity in rats (*54*). Furthermore, it has recently been proven effective in preclinical models for acute migraine, which is believed to be due to its GluK1 antagonistic acitivty (*76*). In rat models, the monohydrate has proven to be antipsychotic with no extra-pyramidal side effects (*77*) and anxiolytic with no negative locomotor side effects (*78*). Finally, **6.1** reduced hyperalgesia in a human study on skin (*27*).
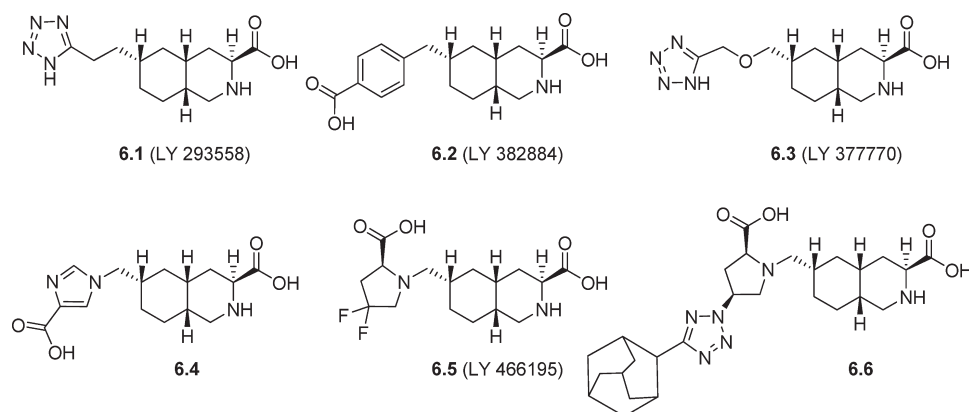
Reverting to a carboxylic acid functionality, compound **6.2** (LY382884) showed good affinity and antagonistic activity at human GluK1 receptors (Table 6) with no significant activity at AMPA or other KA receptor subunits at a concentration of 100 μM (*79*). However, the quantity of compound required to fully block KA receptors (10 μM) *in vivo* also weakly antagonized AMPA receptor-mediated synaptic transmission at CA3−CA1 synapses (*80*). Compound **6.2** displayed only weak activity at NMDA receptors (*80*), and a number of observations suggest that the compound is also effective as an antagonist of heteromeric compositions of GluK1 and GluK2 (*17*, *50*, *79*, *80*). **6.2** prevented induction and maintenance of seizures in multiple models of epilepsy (*81*), and it has been shown to have analgesic effects when tested *in vivo* in rats (*54*). This suggests that the GluK1 subunit has a central role in epilepsy and pain sensation. Furthermore, **6.2** produced anxiolytic type behavior in the Vogel conflict test (*82*).

Compound **6.3** (LY377770), the active stereoisomer of racemate LY294486, contains a one-atom longer ether linker at the distal region, as compared to **6.1**. The compound was used as a pharmacological probe for GluK1-containing KA receptors in studies of synaptic transmission in the rat hippocampus and in studies of cerebral ischemia in the gerbil middle cerebral artery occlusion model (*83*, *84*). This suggested a preference for GluK1-containing KA receptors with an approximate 10-fold selectivity for GluK1 receptors over AMPA receptors. Consistent with this, **6.3** was found to be a potent and selective antagonist at homomeric GluK1 receptors (Table 6) with no activity at GluK2 homomeric receptors. Furthermore, NMDA receptor mediated currents were not affected (*85*). The imidazolyl analogue **6.4** inhibited glutamate-evoked calcium influx in the human GluK1-(Q) receptor transfected in HEK293 cell membranes, with an estimated K_B value of 0.26 μM (Table 6) (*43*). In addition, **6.4** was evaluated at GluK1 receptors isolated from rat neonatal DRG neurons, giving an IC_{50} value of 0.85 μM (30 μM KA) (*72*).

Compound **6.5** (LY466195) exhibited a K_B value of 0.024 μM for antagonism of human recombinant GluK1 and an estimated K_B value of 13 nM for antagonism of GluK1-containing KA receptors expressed in dorsal root ganglion cells (*86*, *87*). The compound displayed only weak competitive antagonist activity at NMDA receptors (IC_{50} = 2.5 μM) and AMPA receptors (IC_{50} = 54 μM) expressed in hippocampal neurons. It is currently being evaluated as an antimigraine drug further highlighting the involvement of GluK1 in the pathophysiology of pain (*88*).

Compound **6.6** was reported as the most potent GluK1 antagonist of the decahydroisoquinoline series with a K_B value of 3 nM for antagonism of human recombinant GluK1. It is clear that introduction of the bulky hydrophobic tricyclic moiety contributes significantly to this by reducing the water solubility properties of the ligand. Analogue **6.6** exhibited no activity at GluA2 or GluK2 receptors up to a concentration of 100 μM (*86*).

Table 6. Chemical Structures and Pharmacological Profile of Decahydroisoquinolines **6.1−6.6**[a]

6.1 (LY 293558) **6.2 (LY 382884)** **6.3 (LY 377770)**

6.4 **6.5 (LY 466195)** **6.6**

cmpd	GluA1 bind.	GluA2 bind.	GluA2 funct.	GluA3 bind.	GluA4 bind.	GluK1 bind.	GluK1 funct.	GluK3 bind.
6.1 (LY293558)[c]	9.2	3.2	0.40[b]	32	51	4.2	0.5[b]	>100
6.2 (LY382884)[c]	>100	>100	>100[b]	>100	>100	4.0	1.1[b]	>100
6.3 (LY377770)[d]	>100	35	—[f]	48	32	3.1	0.09[b]	—[f]
6.4[e]	130	120	—[f]	250	260	0.16	1.2	50
6.5 (LY466195)[c]	75	270	>100[b]	310	430	0.05	0.024	8.9
6.6	—[f]	—[f]	>100[b]	—[f]	—[f]	—[f]	0.003[b]	—[f]

[a] Binding affinities (K_i) were determined using [^{3}H]AMPA radioligand at AMPA subtypes and [^{3}H]KA radioligand at KA subtypes. No affinity or activity was observed below 100 μM at homomeric GluK2 and GluK5 receptors. All values in [μM]. [b] K_B values for inhibition of 100 μM glutamate-induced Ca^{2+} influx in HEK293 cells expressing human KA receptors. Data for KA receptor subunits were obtained in the presence of ConA; from ref *86*, additional experimental data may be found in ref *50*. [c] From ref *87*: Binding data in K_i; functional data as K_B inhibition of 100 μM Glu induced Ca^{2+} influx in HEK293 cells. [d] From ref *89*: Binding data (K_i) obtained for racemate (LY294486). [e] From ref *43*: Binding data as K_i at homomeric receptors in HEK293 cells; functional data as IC$_{50}$ inhibition of 200 μM Glu invoked Ca^{2+} influx in HEK293 cells. [f] Not tested.

Based on **6.1** and **6.2** as lead structures, a small series of 6-tetrazolyl decahydroisoquinoline analogues were prepared (Table 7). These compounds were designed with an aromatic linker minimizing the number of possible conformations - i.e. potential loss of entropy upon binding. The aryl ether derivative **7.1** antagonized KA-evoked currents in rat DRG neurons with an IC$_{50}$ value of 0.15 μM and AMPA- and NMDA-evoked currents in hippocampal neurons with IC$_{50}$ values of 2.1 μM and 90 μM, respectively (*86*). In the subtype-specific HEK 293 cell assay, **7.1** was shown to be a potent GluK1 antagonist and a moderate GluA2 antagonist (Table 7). In compound **7.2** the aryl ether functionality was replaced by an aniline moiety. This analogue blocked KA-, AMPA-, and NMDA-evoked currents with IC$_{50}$ values of 0.18, 1.4, and 1.9 μM, respectively (*86*). In HEK 293 cells, **7.2** was shown to be a slightly more potent (13-fold) GluK1 over GluA2 antagonist. In analogue **7.3**, a chlorine substituent was added in the ortho position. This compound acted as a very potent GluK1 antagonist with a 400-fold selectivity over GluA2 (*86*).
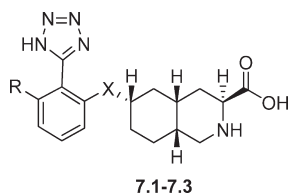
A major focus of recent research has been to improve the oral bioavailability of this class of GluK1 antagonists

by formation of ester prodrugs (*43, 86, 87, 90*). A number of successful prodrugs have been prepared: The diethyl ester prodrug of **6.4** was orally active in two animal models of migraine pain (*43*). The 2-iso-butylester prodrug of compound **7.1** showed only moderate affinity for GluK1 receptors (K_i of 7.4 μM) (*90*). However, when administered orally, this prodrug produced dose-related antihyperalgesic effects in the Carrageenan test and formalin-induced paw-licking and capsaicin-induced allodynia was reversed (*86, 90*), at doses that did not show performance deficits in the rotarod test (*90*). The 2-ethylbutylester prodrug of compound **7.2** was largely devoid of affinity for GluK1 receptors (K_i of 32 μM) (*90*), but upon oral administration this prodrug produced dose-related antihyperalgesic effects in the Carrageenan test, reversed formalin-induced paw-licking and reversed capsaicin-induced allodynia (*86, 90*). These effects occurred at doses that did not show performance deficits in the rotarod test as well (*90*). The 2-ethylbutylester prodrug of compound **7.3** produced dose-related antihyperalgesic effects in carrageenan-induced thermal hyperalgesia and reversal of capsaicin-induced allodynia, although the prodrug was largely devoid of affinity for GluK1 receptors (*86*).

Willardiine Derivatives

A series of selective GluK1 antagonists were developed by addition of substituents at the N^3 position of the uracil ring of the natural product willardiine (91−95). The lead compound **8.1** (3-CBW or UBP282) contains a 4-carboxybenzyl substituent in the N^3 position and was shown to be a competitive antagonist of AMPA receptors expressed on motor neurons and of GluK1-containing KA receptors (Table 8) expressed in dorsal root C-fibers (96). The X-ray structure of **8.1** in complex with the LBD of GluA2 revealed a hyper-extended conformation of the protein compared to its apo state (97). The 2-carboxybenzyl analogue **8.2** (UBP296) was found to be 100-fold selective for GluK1 versus AMPA receptors when tested on cloned human and native rat AMPA receptors (93, 95). In addition, **8.2** was >300-fold selective for homomeric GluK1 versus homomeric GluK2 and GluK3 and displayed similar potency on the heteromeric GluK1 receptors, GluK1/GluK2 and GluK1/GluK5, compared to that observed for GluK1 alone (Table 8) (93, 95). The antagonist activity was found to reside in the S-enantiomer **8.3** (UBP302) which is in agreement with other amino acid-based KA receptor antagonists (Tables 7−9) (98). Compound **8.3** was the only compound in the UBP series to be tested in a binding assay on human GluK3 (K_i value > 100 μM). The 5-iodo-substituted analogue of **8.3** (structure not shown) was also tested at GluK1 receptors, and it was found to have slightly increased antagonism of KA-induced responses in the dorsal root preparation (K_D = 0.21 μM)(95), but the compound was not evaluated further. By replacement of the benzene ring in **8.3** with a thiophene ring, to give **8.4** (UBP304), potency and selectivity for the GluK1 subtype was further increased (92, 99). Variation of the substituent in the 5-position of the uracil ring revealed that a methyl group, as in compound **8.5** (UBP310), led to low nanomolar potent GluK1 antagonist activity (10-fold increase) while also increasing selectivity for GluK1 versus GluK2

Table 7. Chemical Structures and Pharmacological Profile of Decahydroisoquionoline Derivatives **7.1**−**7.3** at Recombinant AMPA/KA Receptors[a]


7.1-7.3

cmpd	X	R	GluA2 bind.[b]	GluA2 funct.[c]	GluK1 bind.[b]	GluK1 funct.[c]	GluK1/2 bind.[b]	GluK1/5 bind.[b]
7.1 (LY458545)	O	H	8.3	1.1	1.7	0.04	1.0	7.9
7.2 (LY457691)	NH	H	5.5	0.4	1.6	0.03	1.4	9.9
7.3	NH	Cl	−[d]	2.0	−[d]	0.005	−[d]	−[d]

[a] Functional data represent K_B values for inhibition of 100 μM glutamate-induced Ca^{2+} influx in HEK 293 cells expressing human homomeric AMPA or KA receptor subtypes. Binding data represent K_i values. [^{3}H]AMPA was used as radioligand for GluA2; others, [^{3}H]KA. The compounds showed no affinity for GluK2 or GluK2/5. All values in [μM]. [b] From ref 90. [c] From ref 86. [d] Not tested.

Table 8. Chemical Structures and Functional as Well as Binding Data for Willardiine Analogues **8.1**−**8.6**[a]
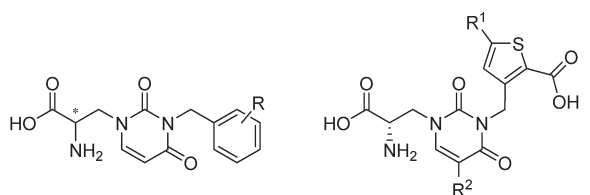

8.1 (UBP282), R = 4-COOH (S)
8.2 (UBP296), R = 2-COOH (R/S)
8.3 (UBP302), R = 2-COOH (S)

8.4 (UBP304), R^1 = H, R^2 = H
8.5 (UBP310), R^1 = H, R^2 = CH_3
8.6 (ACET), R^1 = Phe, R^2 = CH_3

cmpd	GluA2	GluK1	GluK2	GluK3	GluK1/2	GluK1/5	GluK2/5
8.1 (UBP282)[b]	−[h]	9.3	> 1000[d]	−[h]	−[h]	−[h]	> 1000[d]
8.2 (UBP296)[c]	> 300	0.6	> 1000[d]	> 100[d]	0.8	1.0	> 100[d]
8.3 (UBP302)[e]	> 300	0.6	> 100	4.0[f]	0.8	1.0	> 300
8.4 (UBP304)[e]	> 100	0.12	> 100	−[h]	0.12	0.18	> 100
8.5 (UBP310)[e]	> 100	0.010	> 100	0.023[f]	−[h]	0.008	> 100
8.6 (ACET)[e]	> 100	0.007[g]	> 100	0.092[f]	−[h]	0.005	> 100

[a] All values in [μM]. [b] From ref 93: K_D values for inhibition of KA-induced currents in neonatal rat dorsal root C fibers. [c] K_B values for inhibition of 10−100 μM glutamate-induced Ca^{2+} influx in HEK 293 cells expressing human homomeric AMPA or KA receptor subtypes; from ref 95. [d] From ref 93: Binding study K_i or IC_{50} from displacement of [^{3}H]KA at human homomeric KA receptors expressed in HEK 293 cells. [e] From ref 91: K_i or IC_{50} value for inhibition of glutamate-evoked currents in HEK 293 cells expressing homomeric KA receptors. [f] From ref 52: IC_{50} values in an *Xenopus* oocyte assay. [g] In another study, this value was measured to be 0.0014l; ref 100. [h] Not tested.

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Table 9. Chemical Structures and Binding Affinities of **9.1**−**9.5** at Native AMPA, KA, and NMDA Receptors (Rat Synaptosomes) and at Cloned Homomeric Rat GluA2 and GluK1-3 Receptors[a]

9.1   **9.2** ((S)-ATPO)   **9.3** R = H (racemate)
9.4 R = H (2S)
9.5 R = Cl (racemate)
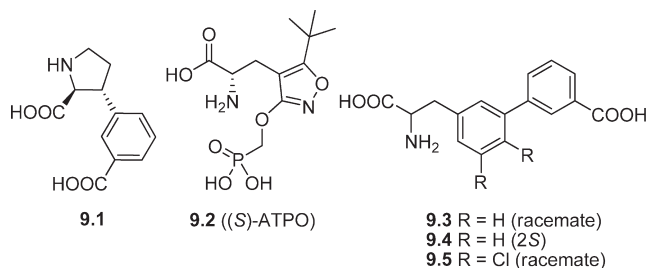
cmpd	AMPA[b]	KA[b]	NMDA[c]	GluA2[d]	GluK1[d]	GluK2[d]	GluK3[d]
9.1[f]	51	22	6.0	67	3.0	>100	8.1
9.2 ((S)-ATPO)[g]	16	>100	>100[e]	−[i]	−[i]	−[i]	−[i]
9.3[h]	>100	>100	>100	−[i]	7.9	>100	>100
9.4[h]	−[i]	>100	>100	−[i]	4.1	>100	>100
9.5[h]	>100	>100	>100	−[i]	2.8	>100	>100

[a] All values in [μM]. [b] IC$_{50}$ values and radioligands used: AMPA, [^{3}H]AMPA; KA, [^{3}H]KA. [c] K_i values and radioligand used: [^{3}H]CGP 39653. [d] K_i values and radioligands used: [^{3}H](2S,4R)-4-methyl Glu (SYM2081). [e] Radioligand used: [^{3}H]CPP. [f] From ref *102*. [g] From ref *104*. [h] From ref *106*. [i] Not tested.

and AMPA receptors (*91, 94, 98*). However, this analogue was furthermore a potent antagonist at GluK3 (IC$_{50}$ = 0.023 μM) (*52*).

X-ray crystal structures were obtained of the LBD dimer of GluK1 complexed with **8.3** and 5-methyl analogue **8.5**, respectively (*94*). The X-ray crystal structure of **8.3** revealed the largest domain opening reported so far for the iGluRs, where the α-amino moiety of the antagonist was not able to form a direct contact with Glu723, as it has been seen in all previously solved AMPA and KA receptor agonist and antagonist complexes (*94*). The improved selectivity for GluK1 versus AMPA receptors and other KA receptor subunits could be explained by the steric clashes imposed by substitution of Val670 for Leu650 and Ser720 for Met708 in GluA2. The low affinity of N^3-substituted willardiines for GluK2 could be explained by the replacement of Thr503 and Ser674 for two alanine groups in this particular subunit, preventing favorable interaction with the α-ammonium and terminal carboxyl groups of the antagonist. Similarly, replacement of Ser674 in GluK1 by an Ala residue in GluK3 may explain the low affinity of **8.2** and **8.3** for GluK3. Furthermore, Ser760 in GluK1 is replaced with an Asp residue in GluK3, possibly hindering the placement of the benzene or the thiophene ring bearing the carboxyl group in the optimal orientation for interaction with a Thr residue in D2. The residues Val670 and Ser726 in GluK1 are replaced by the more bulky Ile and Met side chains in GluK4 and GluK5 explaining the lower affinity for these subunits.

With inspiration from the X-ray crystal structure of the LBD of GluK1 in complex with **8.5**, the new antagonist **8.6** (UBP316 or ACET), was designed.

A phenyl group was introduced at the 2-position of the thiophene ring of **8.5** to increase the hydrophobic interaction with Val670 and the CH$_2$ group of Asn705. The improved selectivity for native GluK1 over AMPA receptors is likely due to the steric clash of the phenyl group of **8.6** with the Leu residue that replaces Val690 in AMPA receptors (*91*). As predicted, compound **8.6** exhibited improved potency and selectivity for native and cloned human GluK1 receptors versus native AMPA and cloned human GluA2 receptors (*91, 98, 100*). It was shown that **8.6** at a concentration of 100 nM completely blocked GluK1-containing dorsal root C-fiber responses to KA (10 μM), with and in the absence of ConA (*101*). As for **8.4**, **8.5** and **8.6** showed excellent selectivity for human recombinant GluK1 over GluK2 subtypes and no effect on GluK2/GluK5 heteromers up to a concentration of 100 μM (Table 8).

The most potent GluK1 antagonists **8.4** (UBP-304), **8.5** (UBP310), and **8.6** (UBP316 or ACET) were tested on native AMPA receptors expressed on spinal motor neurons giving K_D values of 71.4, 83.4, and 108 μM, respectively, for blocking (S)-5-fluorowillardiine (5-F-will)-induced depolarization, suggesting that these antagonists were highly selective for the GluK1 subtype over AMPA receptors (*91, 92, 95*). Pharmacological characterization of **8.2** (50 μM), **8.4** (50 μM), **8.5** (10 μM), and **8.6** (1 μM) at NMDA and group I metabotropic glutamate receptors expressed in neonatal rat motor neurons demonstrated that these compounds displayed no activity at these receptors (*91*−*93*). Thus, these willardiine derivatives, and in particular **8.6**, are useful pharmacological tools for studying the roles of GluK1 in CNS function (*91, 93, 99*), though care should

Table 10. Functional Characterization (K_B) of Antagonists **9.1** and **9.2** in a TEVC Oocyte Assay at Cloned Non-Desensitizing Homomeric Subtypes GluK1-3[a]

cmpd	GluA1/2	GluK1	GluK2	GluK3
9.1[c]	—[f]	11.4	> 300	273
9.2 ((*S*)-ATPO)[d]	3.6[b]	24[b]	> 300[e]	—[f]

[a] All values in [μM]. [b] Inhibition of KA-induced responses in *Xenopus* oocytes (K_i). [c] From ref *102*. [d] From ref *104* [e] From ref *107*. [f] Not tested.

be taken in interpreting the data for compounds **8.5** and **8.6** given the GLUK3 activity of these compounds.

Other Chemical Classes

Based on structure-based design, the compound (2*S*,3*R*)-*m*-carboxyphenylproline **9.1** was recently synthesized as a novel class of selective iGluR antagonists (*102*). In binding studies, **9.1** exhibited medium range micromolar affinity for native AMPA, and KA receptors and low micromolar affinity for native NMDA receptors. At cloned homomeric GluK1 and GluK3 receptors, **9.1** exhibited low micromolar affinity (3 and 8.1 μM respectively) whereas it was without affinity for GluK3 (Table 9). At the mGluRs, **9.1** has been shown to be without activity (rat mGluR1, $K_i >$ 10 000 μM) (*103*). Functional characterization at non-desensitizing mutants of homomeric GluK1−3 receptors revealed that the compound is an antagonist of GluK1,3 (Table 10) (*102*).

A phosphonic acid analogue of AMPA, compound **9.2** ((*S*)-ATPO), was shown to have antagonistic activity at both recombinant GluA1−4 (K_i values of 2.0, 3.6, 3.6, and 6.7 μM, respectively) and GluK1 subtypes ($K_B = $ 24 μM), but no activity at the GluK2 subtype (Tables 9 and 10) (*104*). X-ray crystal structures of (*S*)-ATPO in complex with GluA2 (*46*) and GluK1 (*105*), respectively, show that (*S*)-ATPO stabilizes the open form of the LBD by binding to residues in domains D1 and D2 otherwise engaged in agonist binding.

A recent SAR study on a number of 3-substituted phenylalanine compounds focused on exploring compounds based on an alanine-substituted biphenyl core (*106*). The study concluded that a 3-carboxylic acid functionality was essential for GluK1 affinity as in compounds **9.3−9.5** (Table 9). Furthermore, the two aromatic rings had to be directly attached, that is, not connected by a methyl linker (structures not shown). The GluK1 affinity observed for **9.3** was found to reside in the *S*-isomer (**9.4**) which was to be expected from earlier reported α-amino acid-based KA receptor antagonists (Tables 7−9). The GluK1 affinity could be further increased (3-fold) by the addition of two chlorine atoms, as in **9.5**. None of the mentioned compounds showed affinity for homomeric GluK2 or GluK3 receptors
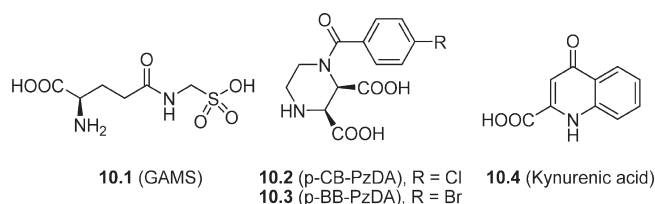
Figure 3. Chemical structures of compounds **10.1**−**10.4**.

10.1 (GAMS)   10.2 (p-CB-PzDA), R = Cl   10.3 (p-BB-PzDA), R = Br   10.4 (Kynurenic acid)

or even native AMPA, KA, or NMDA receptors. Compounds **9.3** and **9.5** were docked into the LBD of GluK1 complexed with **7.3** (UBP302), and, as expected, the compounds adopted a binding mode similar to that for **7.3**. In this *in silico* study, the chlorine atom in the 5-position of **9.5** filled a partly hydrophobic cavity, which may explain the improved affinity compared to **9.3**. On the other hand, the chlorine atom in the 4-position filled a hydrophilic cavity.

Miscellaneous

Early SAR studies suggested that the D-glutamine analogue **10.1** (GAMS) (*108*) and the benzaldehyde-substituted piperazines **10.2** (p-BB-PzDA) and **10.3** (p-CB-PzDA) (*109*) (Figure 3) were selective antagonists for AMPA/KA receptors over NMDA receptors. However, in these studies, quisqualate was used as the AMPA/KA receptor agonist and it is now known that this compound also activates group I metabotropic glutamate receptors (*22*, *110*, *111*). Compound **10.1** has been reported to be a weak antagonist of KA receptors on DRG neurons (*49*) and to preferentially antagonize KA receptors (*112*). In assays on recombinant human KA receptors, weak antagonist activity was observed at GluK1, GluK1/GluK2, and GluK1/GluK5, but not GluK2 or GluK2/GluK5 (IC$_{50}$ values in the millimolar range at each subtype) (*50*). While compounds **10.2** and **10.3** blocked KA-induced responses, they blocked NMDA-induced responses with greater potency on CA1 hippocampal neurons (*113*). Both of these compounds have been shown to display moderate antagonist activity at GluK1-containing receptors on dorsal root C-fibres (*114*), but they have not been tested across recombinant AMPA/KA receptor subtypes.

The tryptophan metabolite **10.4** (kynurenic acid or KYNA) (Figure 3) has often been referred to as a broad-spectrum Glu receptor antagonist (*115*), although at low levels the compound is an antagonist of only the strychnine-insensitive glycine site of the NMDA receptor (IC$_{50}$ of 8−15 μmol/L) (*116*). However, the compound also antagonizes the glutamate recognition site of the NMDA receptor. At higher concentrations (IC$_{50}$ in the millimolar range), **10.4** is a competitive antagonist at AMPA and KA receptors (*117*) and exhibits IC$_{50}$ values in the 500 μM range for blocking homomeric and

heteromeric GluK1 and homomeric GluK2 receptors, but does not block GluK2/GluK5 receptors at concentrations up to 3 mM (*101*). The metabolite **10.4** is thought to act as a tonic modulatory agent in the healthy CNS and has been shown to be involved in pathological states such as schizophrenia and cognitive deficits (*118*).

Conclusion and Perspectives

This review provides the reader with an overview of the field medicinal chemistry of competitive KA receptor antagonists. From this overview, it is evident that despite extensive efforts only selective antagonists for the GluK1 subunit have been developed. The discovery of selective antagonists for subunits GluK2−5 is yet to be accomplished and may call for an alternative strategy such as development of negative allosteric modulators. In perspective, selective KA receptor antagonists which discriminate between heteromeric receptor compositions may be of high value. Such pharmacological tools will allow for the investigation of the function and distribution of heteromeric KA receptors in the CNS.

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