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*Perspective*

# Ligands for Glutamate Receptors: Design and Therapeutic Prospects

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## Introduction

(*S*)-Glutamic acid (Glu), which is the main excitatory neurotransmitter in the central nervous system (CNS), and other excitatory amino acids (EAAs) operate through four different classes of receptors. In addition to the three heterogeneous classes of ionotropic EAA receptors (iGluRs), named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainic acid (KA) receptors,[1,2] a heterogeneous class of G-protein coupled EAA receptors (mGluRs) has been shown to have important functions in neuronal signaling processes.[3,4] It is now generally agreed that iGluRs as well as mGluRs play important roles in the healthy as well as the diseased CNS, and that all subtypes of these receptors are potential targets for therapeutic intervention in a number of diseases.

The cloning of the different subunits of the iGluRs and of the eight subtypes of mGluRs represents a major breakthrough. Whereas at present six NMDA receptor subunits (NR1, NR2A-2D, and NR3A) have been cloned and characterized in regards to primary structure, four AMPA receptor subunits (iGluR1−4) have similarly been characterized, and so far five subunit building blocks for KA-preferring receptors (iGluR5−7, KA1, and KA2) have been identified. Most if not all physiological iGluRs have heterotetra- or -pentameric structures, but the number of functional NMDA, AMPA, and KA receptors in the CNS is not known. At present, eight subtypes of the seven transmembrane (7TM) mGluRs have been characterized, but there is evidence to suggest that further subtypes of mGluRs may be identified.

These achievements form the basis of the almost explosive increase of research activities in the EAA receptor field. A major goal of these widely ramified molecular biological, physiological, structure/function, and medicinal chemistry approaches is to identify subtype-selective EAA receptor ligands. Such compounds capable of activating, blocking, or modulating iGluRs or mGluRs are essential pharmacological tools, which may be further developed into therapeutically useful drugs. Meanwhile, the search for further subunits of iGluRs and subtypes of mGluRs continues. While new receptor proteins will probably be minor in terms of density and distribution in the CNS, they may constitute EAA receptors involved in the regulation of distinct physiological mechanisms of particular interest in certain disease states.

## Toward Design of EAA Receptor Ligands on a Rational Basis

A large number of enzymes have been characterized structurally, kinetically, and in terms of mechanism(s) of action, and these studies have paved the way for rational design of various types of enzyme inhibitors, notably mechanism-based irreversible inhibitors and transition state analogues as high affinity reversible enzyme inhibitors.[5−7] In contrast to the active sites of enzymes, the recognition sites of receptors do not catalyze chemical reactions. This means that it is

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meaningless to discuss design of mechanism-based ligands or transition state analogues as approaches to the development of novel types of receptor blocking agents. Furthermore, projects aiming at structure-based design of new receptor ligands have been severely hampered by the, so far, unsuccessful attempts to crystallize entire iGluRs or mGluRs alone or with ligands bound to the receptor recognition or allosteric sites. These experimental difficulties undoubtedly reflect that receptor proteins are membrane bound, making it difficult for these proteins, which contain alternating hydrophobic and hydrophilic regions, to stabilize themselves outside the membrane matrix.

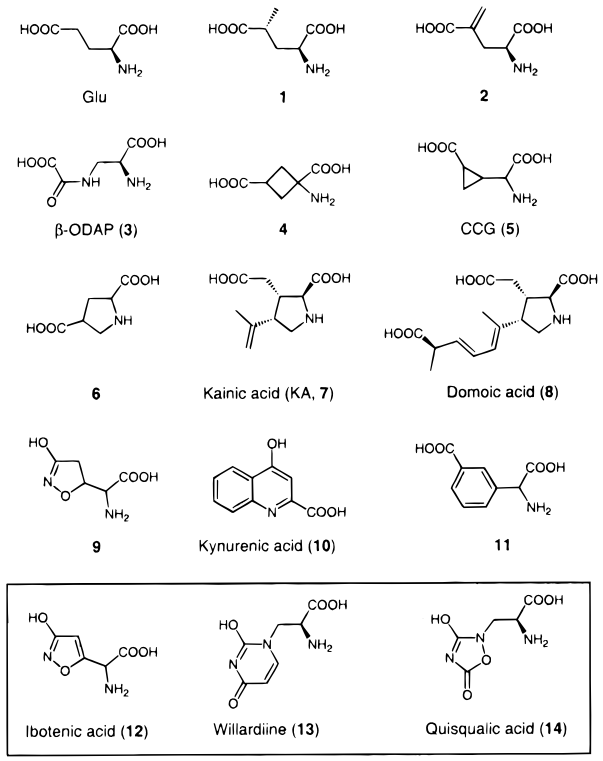
Nevertheless, attempts to crystallize receptor/ligand complexes with the aim of performing X-ray structure analyses continue. One branch of this research field, which focuses on the identification, biotechnological production, and structure determination of binding domains of the receptor proteins, is in a state of fruitful development. Such approaches are based on the systematic mapping of the regions of receptor proteins, which contain the ligand recognition site(s), using point mutation and chimeric receptor construction strategies. In this field of receptor research, the publications of a three-dimensional model of the AMPA receptor ligand-binding site verified by site-directed mutagenesis[8] and the crystal structure of the ligand-binding domain of an AMPA receptor in a complex with the naturally occurring KA/AMPA receptor agonist KA (**7**)[9] represent pioneer work. These achievements have greatly accelerated research initiatives in this field, and detailed analyses and mapping of the points of interaction between receptor recognition site and ligand molecule will pave the way for attempts to design novel receptor ligands on a rational structural basis.

A large number of plants ranging from microorganisms to flowering plants biosynthesize amino acids structurally related to Glu.[10] Several of these naturally occurring Glu analogues are recognized by iGluRs and/or mGluRs as agonists or antagonists, and some of these compounds acting as iGluR agonists show potent excitotoxic effects in vitro and in vivo.[1,2] A few of these compounds have been extensively used as neurotoxins in experimental neurobiology, although the lack of receptor specificity of these naturally occurring Glu analogues limits their use as tools. Some of these compounds have, however, been very valuable leads for the design of specific or highly selective ligands for subtypes of EAA receptors, based on systematic and semirational re-design approaches.

Members of this very large group of Glu analogues of natural origin[10] are depicted in Chart 1, but the isolation, structure determination, and synthesis of these compounds will not be described here. The possible physiological roles of these amino acids in the host plants are unknown, but the recent discovery of putative iGluRs in plants suggest that these amino acids could regulate signal transduction in plants.[11] Furthermore it may be imagined that some of these excitotoxic compounds may play a role as defense tools.

With the exception of kynurenic acid (**10**), which is a metabolite of tryptophan and an endogenous NMDA receptor antagonist of potential physiological relevance,[12] it is possible to identify a Glu structure

**Chart 1.** Structures of Some Naturally Occurring Amino Acids Showing Effects on Glu Receptors




element in these compounds, although (*S*)-2-amino-3-(*N*-oxalylamino)propionic acid (*β*-ODAP, **3**) and 3-carboxyphenylglycine (**11**) actually are homologues of Glu. With the exception of the acyclic Glu analogues, **1** and **2**, and the monocyclic analogues, **4** and CCG (**5**), all of the Glu analogues depicted are heterocyclic compounds, of which **6**, KA (**7**), and domoic acid (**8**) contain amino groups incorporated into pyrrolidine rings.

Tricholomic acid (**9**), ibotenic acid (**12**), willardiine (**13**), and quisqualic acid (**14**) are all analogues of Glu, where the distal carboxyl group has been replaced by the acidic heterocyclic 3-hydroxy-2-isoxazoline, 3-hydroxyisoxazole, pyrimidine-2,4-dione, and 1,2,4-oxadiazole-3,5-dione units, respectively. All of these compounds are very potent, though not selective, agonists at EAA receptors, indicating that these biosynthesized heterocyclic units are effectively recognized and bound by the agonist conformations of different EAA receptors. Lack of selectivity is a limiting factor for the pharmacological utility of these heterocyclic amino acids as exemplified by compounds **12** and **14** which activate both iGluR and mGluR subtypes.[3,13] On the other hand, these natural products, in particular compounds **12**−**14**, have been versatile leads for the development of selective iGluR or mGluR agonists[13] as exemplified in Figure 1. Whereas **12** interacts more or less effectively with all subtypes of EAA receptors, the *R*-form of the aspartic acid analogue of **12**, (*R*)-AMAA (**15**), is a selective NMDA receptor agonist,[14] (*S*)-AMPA (**16**) is a highly selective AMPA receptor agonist,[15,16] and the *tert*-butyl analogue of **16**, (*S*)-ATPA (**17**), has been shown to be a very potent and selective agonist at the KA-preferring iGluR5.[17,18] (*S*)-Homo-AMPA (**18**) does not show detectable affinity for any of the iGluRs, but **18** is a specific but weak agonist at mGluR6.[19,20] Whereas **13** is a relatively nonselective AMPA receptor agonist, (*S*)-

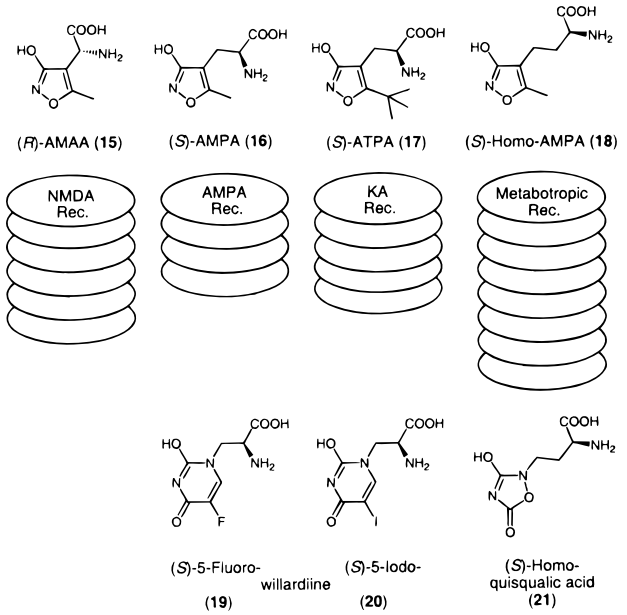



**Figure 1.** Schematic illustration of the multiplicity of EAA receptors and the structures of some key ligands.

5-fluorowillardiine (**19**) is a selective and very potent agonist at AMPA receptors,[21,22] and the 5-iodo (**20**) analogue a potent agonist at iGluR5, more potent but less selective than (*S*)-ATPA.[23,24] In analogy with the conversion of **16** into **18**, homologation of quisqualic acid (**14**) to give **21** leads to complete loss of effect at iGluRs, but whereas **18** is a subtype-selective mGluR agonist, **21** shows a complex mGluR receptor profile[25] (see later section).

## Ionotropic Glutamate Receptor Subunits

The first Glu receptor subunit was cloned by an expression cloning approach using *Xenopus* oocytes.[26] Subsequent application of molecular biological screening techniques led to the identification of 17 genes encoding proteins which, based on sequence comparisons, belong to the Glu receptor family.[27]

On the basis of sequence identities, the Glu receptor subunits can be divided into six groups. One group, containing the iGluR1−iGluR4 (also called GluRA−GluRD) subunits, generates the AMPA receptors. The low affinity KA receptor subunits, iGluR5−iGluR7, and the high affinity KA receptor subunits, KA1 and KA2, form the KA receptors, whereas the NMDA receptor subunits fall into three groups—the NR1, NR2A−NR2D, and NR3A (Figure 2). A seventh group consists of two orphan subunits, $\delta$1 and $\delta$2, but the subunits do not form channels activated by Glu, nor do they assemble with any of the other Glu receptor subunits.[28] However, several observations suggest that at least $\delta$2 forms functional receptors. In mice with genetic knock-out of $\delta$2, the long-term depression at the parallel fiber-Purkinje cell synapse was impaired[29] and, in addition, the neurodegenerative mutant Lurcher mouse turned out to have a mutation in $\delta$2, which makes the receptor constitutively active.[30]

Shorter homologous KA binding proteins have also been cloned from goldfish, *Xenopus*, and chicken. These proteins are homologous to the C-terminal 500 amino acids in the non-NMDA receptors, but lack the N-terminal part. They exhibit binding properties similar to the Glu receptors and do form functional channels.[27]

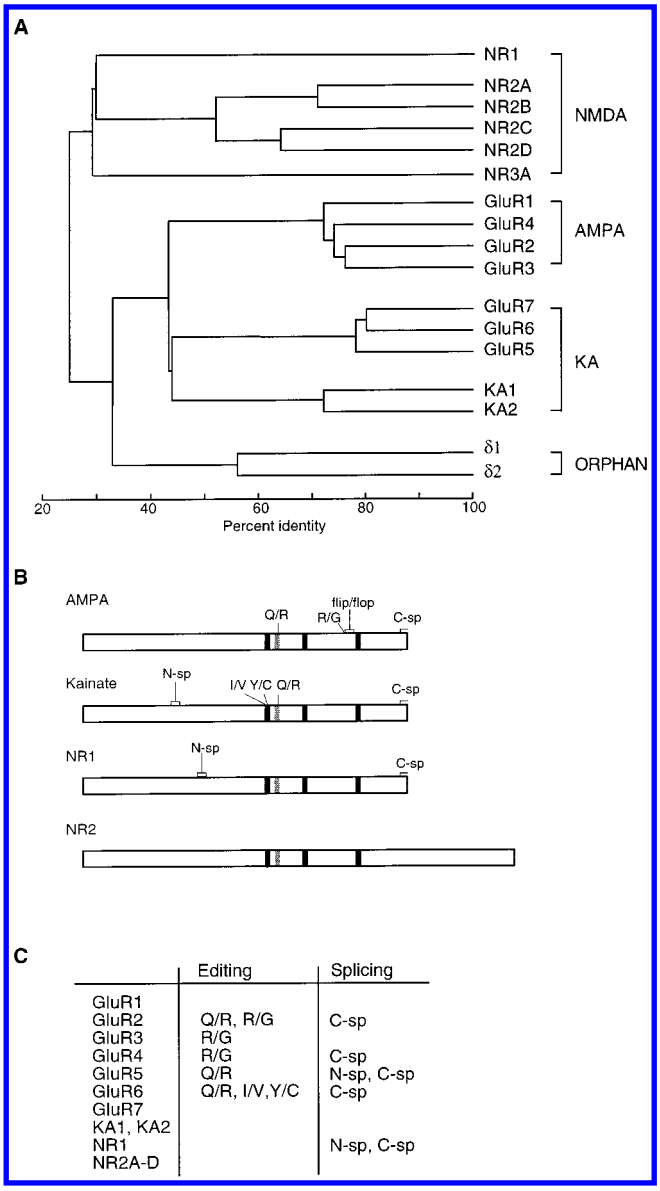



**Figure 2.** (A) Phylogenetic comparison of the glutamate receptor subunits. Only the N-terminal 920 amino acids of NR2A−D are included. (B) Schematic representation of the receptor proteins; NR2A−D are between 1218 and 1445 amino acids while the others are from 875 to 965 amino acids long. Black boxes indicate transmembrane regions; gray box indicates the re-entry loop; flip/flop, N-sp, and C-sp indicate alternative spliced areas; Q/R, R/G, I/V, and Y/C indicate edited residues. (C) Table indicating the different types of post-transcriptional modifications at the different subunits.

## Synaptic Localization of Ionotropic Glutamate Receptors

Evidence derived from different lines of studies is consistent with a predominantly postsynaptic localization of NMDA and AMPA receptors.[31] They are coexpressed in most excitatory terminals, but some terminals only express NMDA receptors and would require membrane depolarization to allow activation.[32] Early lesion studies combined with [$^3$H]KA binding suggested that a substantial number of KA receptors were located at presynaptic terminals.[24] Activation of presynaptic KA receptors seems to reduce Glu release. On hippocampal

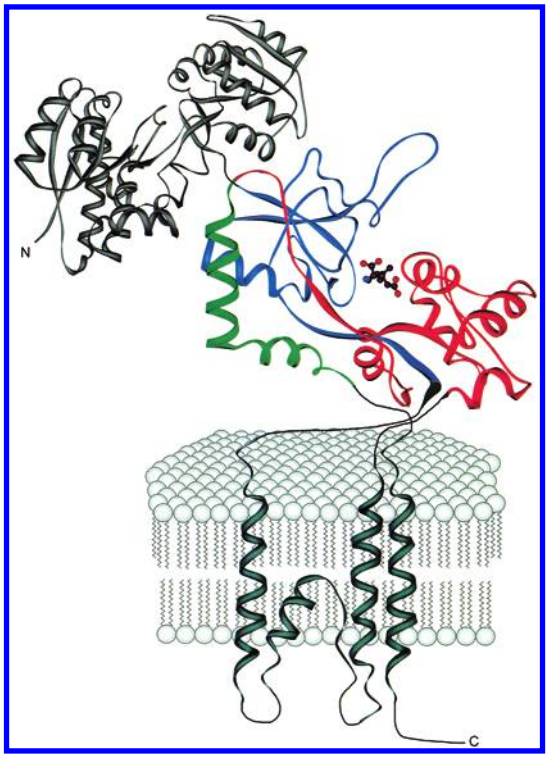



**Figure 3.** Illustration of a single glutamate receptor subunit. The N-terminal domain (gray) is folded as a periplasmatic binding protein. The binding domain is depicted as the crystal structure of the soluble GluR2 binding domain; segment 1 is blue, the part of segment 2 that forms domain 2 is red, and the flip/flop domain is green.

synaptosomes, activation of KA receptors decreases the release of [$^3$H]Glu,[33] and similar reduction in Glu release was observed in excitatory synapses in the CA3 region when iGluR5 containing KA receptors were activated.[34] Employing the iGluR5 partial selective antagonist LY294486 (**90**) (see Chart 11), it was demonstrated that iGluR5 containing receptors also are involved in the reduction of evoked GABA release from hippocampal inhibitory interneurons.[35] The effect can be abolished by pertussis toxin, which suggests that the presynaptic inhibition might involve G-protein coupled mechanisms. It is currently debated if the G-protein activation is a direct or an indirect effect of KA receptor activation.[35−37] KA receptors are also located at postsynaptic terminals revealed by high frequency electric stimulation.[38] Application of **90** or genetic knock-out of iGluR6 in mice inhibits the high frequency stimulated current,[39] suggesting that postsynaptic KA receptors might contain both iGluR5 and iGluR6 subunits.

## Ionotropic Glutamate Receptor Topology

The topology of the Glu receptors has been the topic of many controversies since the first proposed model suggested a subunit topology with four transmembrane segments similar to the nicotinic acetylcholine/$GABA_A$ superfamily of receptors. However, defining the extracellular surface of the subunits by mapping endogenous phosphorylation as well as glycosylation sites, and introduction of new glycosylation sites revealed that the receptor subunits have an extracellular N-terminus and three transmembrane regions[40,41] (Figure 3). The proposed second transmembrane segment, M2, does not pass the membrane but forms a re-entry loop from the cytoplasmic side. This model was confirmed by additional studies where protease sensitivity of truncated receptor subunits was examined,[42] and also the accessibility to sulfhydryl reagents of genetically introduced cysteine residues in the NMDA receptor supported the three-transmembrane model.[43] Since the re-entry loop, M2, forms part of the pore, the Glu receptor channel appears to structurally resemble the voltage gated channels rather than the nicotinic acetylcholine/$GABA_A$ superfamily.

Whether there are four or five subunits in the receptor complex is still debated. The problem has been addressed by various biochemical approaches including chemical cross-linking[44] and purification of solubilized receptor complex followed by sedimentation analysis[45,46] or gel filtration.[47] Neither careful electrophysiological analysis of neither wild type nor mutant subunits have provided conclusive evidence (for review, see ref 48). Single-channel recordings from a receptor formed from iGluR6−iGluR3 chimeric subunits suggested that the conductance level of the channel depends on the number of agonists bound to the receptor. Binding of agonists to two of the subunits (with antagonists bound at the remaining subunits) resulted in a low conductance channel activation, while further replacement of the antagonists resulted in increased conductance.[49] A stepwise increase in the conductance depending on the number of bound agonists certainly will complicate the interpretation of dose−response curves.

## Agonist Binding Sites of Ionotropic Glutamate Receptors

The amino acid sequences of the iGluRs have revealed a weak similarity between bacterial periplasmic amino acid binding proteins and two iGluR regions, S1 preceding M1 and S2 between M3 and M4.[50,51] Chimeric receptor subunit approaches taking advantage of the distinct pharmacology of the AMPA and KA receptors revealed that the agonist binding site indeed is formed by a 130 amino acid segment, S1, preceding M1 and the segment, S2, between M3 and M4.[52] Based on the homology to the periplasmic binding proteins, several models of the agonist binding site have been developed and confirmed by site-directed mutagenesis.[8,52] The two binding domains could be expressed in a soluble form when the two transmembrane regions, M1 and M3, and the re-entry loop were replaced by a hydrophilic linker and the protein was truncated before M4. The soluble protein exhibited a pharmacological profile similar to the receptors, suggesting that the agonist binding site was correctly folded even in the absence of the transmembrane regions.[53] Further truncations and optimization of the linker resulted in a soluble form of iGluR2 that retained the binding properties and was suitable for cocrystallization with KA (**7**).[9,54]

The crystal structure showed that domain 1 was formed by segment S1 and the 33 amino acids in the C-terminal end of S2 including the alternative spliced flip/flop region, which is located on the back of the domain relative to the binding site (Figure 4). Domain 2 is formed by the N-terminal 134 amino acids of S2. KA (**7**) was bound between the two domains with the Glu-like backbone bridging between the two lobes and most likely holding them together or stabilizing a twist

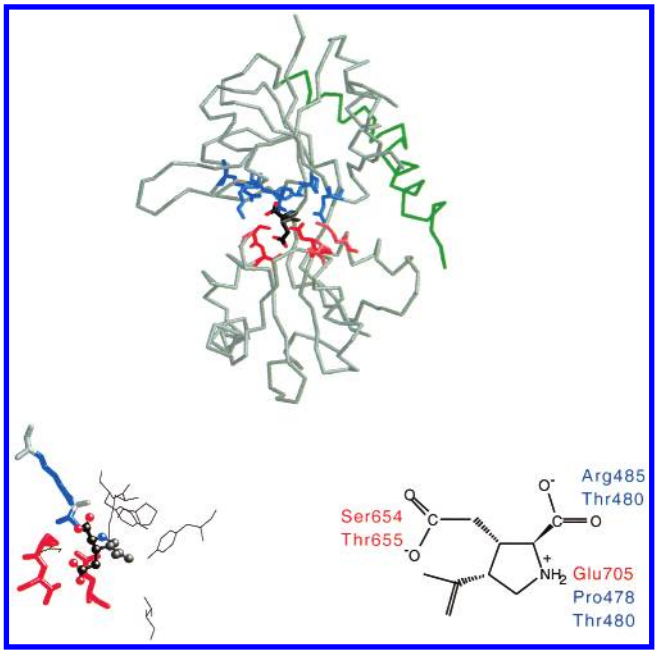



**Figure 4.** Top: Crystal structure of the soluble binding domain from iGluR2. Residues interacting with the glutamate backbone (black) of kainate are indicated for domain 1 (blue) and domain 2 (red). Residues involved in additional interactions with selective compounds are also indicated in light blue (domain 1) and light red (domain 2) (see Table 2). The flip/flop region is shown in green. Bottom left: The glutamate backbone of kainate forms a bridge between domain 1 and domain 2 (colors as in A). Bottom right: Kainate structure with the interacting amino acids.

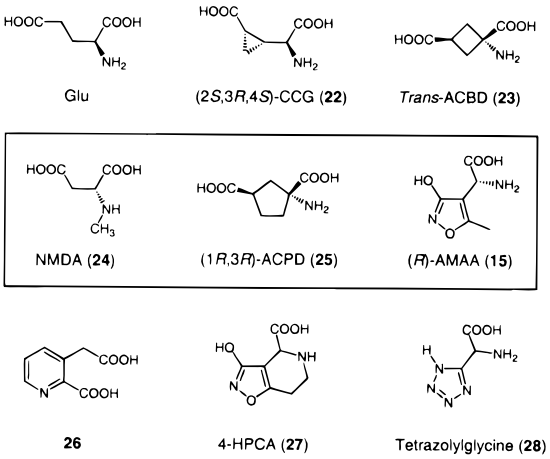
between the domains. Five amino acids were identified in these interactions which most likely are involved in all agonist interactions. The α-carboxyl group interacts with domain 1 through an ionic interaction with the guanidinium group of Arg485 (iGluR2 numbering) and the peptidyl NH group of Thr480. Thr480 also interacts by the hydroxyl group with the protonated amino group of KA. The other interactions involve domain 2, where the positively charged amino group of KA also interacts with Glu705 and the ω-carboxyl group interacts with the backbone NH groups of Ser654 and Thr655, and the hydroxyl group of Thr655. Mutations in these five residues, at equivalent positions in other Glu receptors, abolish or greatly reduce agonist affinity or potency (see ref 48 for review). The proposed agonist mediated closure of the domains is further stabilized by interdomain interaction which does not participate in the KA interaction such as Glu402-Thr686 and Lys449 interaction with Asp651 and Ser652.[9] However these residues might interact with other ligands since the position equivalent to Thr686 in iGluR6 (Asn721) prevents binding of AMPA (**16**) to iGluR6.[55]

A number of additional residues are proposed to interact with specific ligands.[9] One example is Tyr450, which forms a wedge between the pyrrolidine ring and the isopropenyl group of KA and domain 1 preventing further closure of the domains. It has been proposed that the gating might relate to the degree of domain closure in a model where an intermediate closure will correspond to the gating state while further closure will induce the desensitized state. Thus, the steric clashes between KA and Tyr450 would result in a partially closed form with low level of (or no) desensitization and a low binding affinity, while AMPA and Glu easily can accommodate Tyr450 and allow further closure resulting in desensitization and high binding affinity.

## NMDA Receptors

**Molecular Structure of NMDA Agonists.** Until recently, the NMDA receptors received most attention. The synthetic amino acid NMDA (**24**) (Chart 2), which

**Chart 2.** Structures of *N*-methyl-D-aspartic Acid (NMDA, **24**) and Some NMDA Receptor Agonists



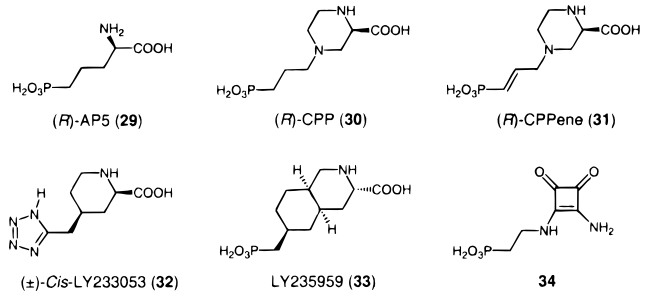

is a very selective agonist at NMDA receptors, has been available for more than 30 years. Equally important, a large number of very potent and selective NMDA antagonists have been developed during the past two decades, and the availability of these compounds has greatly facilitated studies of the physiological and pathophysiological roles of NMDA receptors.[56,57]

From a structure−activity point of view, NMDA, which has (*R*)-configuration, is quite unique. It is the sole *N*-methylated acidic amino acid showing an affinity for the NMDA receptors similar to that of its unmethylated form. *N*-Methyl-(*S*)-Glu and, in particular, *N*-methyl-(*R*)-Glu interact much less potently with NMDA receptor sites than does (*S*)-Glu.[56] Although (*R*)-Glu is much weaker than (*S*)-Glu as an NMDA agonist, NMDA is more potent than its (*S*)-form. Despite its high potency, NMDA binds to NMDA receptor sites with very low affinity, and attempts to use radioactive NMDA as a ligand for binding studies have been unsuccessful.[56,58] These properties of NMDA are shared by another potent NMDA agonist, AMAA (**15**)[14] (Chart 2).

Three carbocyclic acidic amino acids, in which different parts of the molecule of Glu have been conformationally restricted, have been shown to be very potent NMDA agonists, namely the (2*S*,3*R*,4*S*)-enantiomer of CCG (**22**),[59] (1*R*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid [(1*R*,3*R*)-ACPD] (**25**),[60] and *trans*-1-aminocyclobutane-1,3-dicarboxylic acid (*trans*-ACBD) (**23**).[61] *trans*-ACBD (**23**) is achiral, but it is interesting to note that whereas the glycine moiety of **22** has (*S*)-configuration, the same structure element of **25** has (*R*)-configuration. These and a number of other structure−activity aspects of NMDA agonists, including the bicyclic AMAA analogue, 4-HPCA (**27**)[62] and compound **28**,[63] have been analyzed using computational methods.[58]

**Molecular Structure of Competitive NMDA Antagonists.** Using (*R*)-2-aminoadipic acid and, in particular, (*R*)-AP5 (**29**) (Chart 3) as lead structures, a very large number of potent and selective competitive NMDA antagonists have been developed. Most of these NMDA antagonists are phosphono amino acids, in which the carboxyl and phosphono groups typically are separated by four or six atoms.[64−67] Not all of these compounds have been resolved, but for those that are available in stereochemically pure forms, the NMDA antagonist effects reside in the (*R*)-enantiomer, but a few exceptions from this rule have been described.[67] The phosphono amino acids (2*R*)-4-(3-phosphonoprop-1-yl)piperazine-2-carboxylic acid [(*R*)-CPP, **30**],[64] (2*R*)-4-(3-phosphonoprop-2-(*E*)-en-1-yl)piperazine-2-carboxylic acid [(*R*)-CPPene, **31**], and LY235959 (**33**)[65] are among the most potent competitive NMDA antagonists described, and these compounds have been extensively studied pharmacologically.[12] Substitution of a tetrazole group for a phosphono group afforded the NMDA antagonist, *cis*-4-(1*H*-tetrazol-5-ylmethyl)piperidine-2-carboxylic acid [(±)-*cis*-LY 233053, **32**] with only limited loss of activity.[68]

**Chart 3.** Structures of Some Competitive NMDA Receptor Antagonists

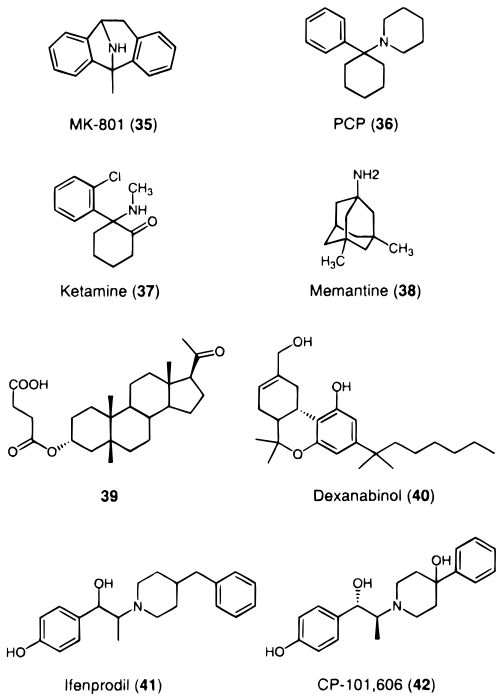



Compound **34**, {2-[(2-amino-3,4-dioxo-1-cyclobuten-1-yl)amino]ethyl}phosphonic acid, represents a unique structural class of NMDA antagonists.[69] In this compound, the α-amino acid group, normally present in competitive NMDA antagonists, has been bioisosterically replaced by the nonionized and achiral 3,4-diamino-3-cyclobutene-1,2-dione unit. The physicochemical properties of this class of compounds probably are more favorable than those of phosphono amino acids, making **34** or analogues thereof pharmacologically interesting.

**Molecular Structure of Noncompetitive NMDA Antagonists.** The polycyclic amine (+)-5,10-epimino-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene (MK-801, dizocilpine, **35**) is a very potent noncompetitive NMDA antagonist, which acts as an open channel blocker.[70] The specific binding of [$^3$H]MK-801 is competitively inhibited by a large number of compounds including the dissociative anesthetics PCP (**36**) and ketamine (**37**) (Chart 4). It has been shown that the antagonist effect of **35**−**37** is agonist-dependent, supporting the view that these compounds bind to a site within the ion channel of the NMDA receptor complex.[70,71]

MK-801 has been extensively studied in a number of animal models of epilepsy and ischemia and is very effective in most of these models.[12] In animal studies, adverse effects of MK-801 and PCP have been observed, and such effects may be predictive of side effects in the human clinic (see later sections).[72]

**Chart 4.** Structures of Some Noncompetitive NMDA Receptor Antagonists




A very large number of analogues of MK-801 and PCP have been synthesized, and it has been possible to develop a pharmacophore of the MK-801 binding site, which accounts for the binding of all of the known ligands.[73]

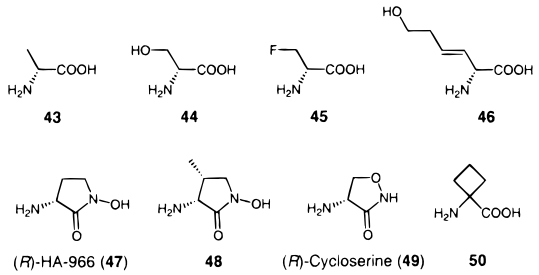
The sterol hemisuccinate **39** has been shown to act as a noncompetitive NMDA antagonist and to be neuroprotective against NMDA induced seizures in mice.[74] Similarly, the cannabinoid dexanabinol (**40**)[75] shows noncompetitive NMDA antagonism and significant neuroprotective properties. The mechanism of action of **39** and **40** is unclear, but it seems unlikely that these compounds interact with the same site(s) as those recognizing **35**−**38**, all of which contain protonizable amino groups as key structure elements.[73] Ifenprodil (**41**) and CP-101,606 (**42**) (Chart 4) represent a unique structural class of noncompetitive NMDA antagonists of major pharmacological and therapeutic interest[76] (see later sections).

**Molecular Structure of Glycine$_B$ Agonists and Antagonists.** Glycine is acting as an inhibitory neurotransmitter, primarily in the spinal cord of the mammalian CNS, and this effect of glycine is mediated by strychnine sensitive ionotropic receptors named glycine$_A$ receptors.[77] More recently, glycine has been shown also to act as a co-transmitter at the NMDA receptor, and these receptors operated by glycine, which are insensitive to strychnine, have been named glycine$_B$ receptors.[78] The precise mechanism of action of glycine at glycine$_B$ receptors of the NMDA receptor complex is as yet unclear.[78] Although supramaximal amounts of glycine normally are present in the extracellular milieu, the NMDA receptors may not in general be maximally activated by glycine under physiological conditions. It remains likely that the NMDA receptor is principally

an EAA receptor at which Glu is the transmitter, whereas glycine is a co-transmitter, which modulates the Glu receptor site and perhaps plays a role in the desensitization mechanism of the NMDA receptor.[78] Recent evidence derived from studies on the enzyme serine racemase suggests that (*R*)-serine may be an endogenous ligand for the glycine$_B$ receptor.[79]

A number of small neutral α-amino acids (Chart 5)

**Chart 5.** Structures of Some Compounds Showing Agonist or Partial Agonist Effects at the Co-Transmitter Glycine Site (Glycine$_B$ Receptor) of the NMDA Receptor Complex



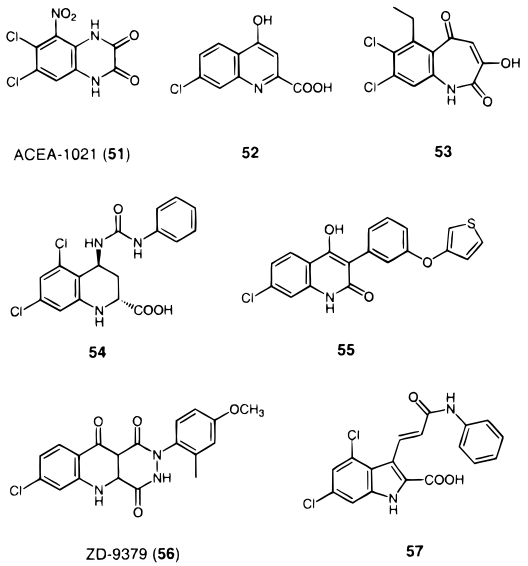

such as (*R*)-alanine (**43**), (*R*)-serine (**44**),[79] and (*S*)-fluoroalanine (**45**) enantioselectively bind to and activate the glycine co-transmitter site.[80] The glycine analogue, (*E*)-2-amino-6-hydroxy-3-hexenoic acid, containing a rather bulky substituent is also a glycine agonist, the (*R*)-enantiomer (**46**) being approximately equipotent with the (*S*)-enantiomer.[81]

The cyclobutane analogue (**50**) is a partial agonist at the glycine site.[82] Similarly, the cyclic amino hydroxamic acids, (*R*)-*N*-hydroxy-3-amino-2-pyrrolidone [(*R*)-HA-966, **47**],[83] the methylated analogue, (3*R*,4*R*)-*N*-hydroxy-3-amino-4-methyl-2-pyrrolidone (**48**),[84] as well as (*R*)-cycloserine (**49**)[85] are partial glycine agonists, (*R*)-cycloserine showing the highest efficacy.[86] These three pyrrolidone analogues, which penetrate the blood−brain barrier (BBB) after systemic administration, have become very useful tools for studies of the in vivo roles of the glycine site.[87,88]

During the past few years a large number of structurally different antagonists at the glycine site of the NMDA receptor have been developed. It is interesting to note the structural similarity of the glycine antagonist 5,6-dichloro-4-nitroquinoxaline-2,3-dione (ACEA-1021, **51**)[89] (Chart 6) and the AMPA/KA antagonist CNQX (**69**)[90] (Chart 8). The 4-amido-2-carboxytetrahydroquinoline derivative **54**,[91] and the 3-aryl-4-hydroxyquinolin-2(1*H*)-one **55**[92] are among the most potent and selective glycine antagonists so far described. Compounds containing the 3-aryl-4-hydroxyquinolin-2(1*H*)-one acidic functionality were the first examples of glycine antagonists showing oral bioavailability.[92] More recently, compounds **56** and **57**[93] have been described as potent glycine$_B$ antagonists of potential therapeutic interest.[94]

**Molecular Pharmacology.** Formation of functional NMDA receptors in heterologous expression systems requires coexpression of NR1 and at least one of the NR2 subunits. Reports of activity of homomeric NR1 receptors in *Xenopus* oocytes were most likely due to NR1 assembly with an endogenous NR2-like subunit.[95] NR1 receptor subunits have three alternative spliced exons, one in the N-terminal domain and two generating

**Chart 6.** Structures of Some Compounds Showing Antagonist Effects at the Co-Transmitter Glycine Site (Glycine$_B$ Receptor) of the NMDA Receptor Complex




four different C-termini (Figure 2). Splice variants with or without the N-terminal exon included is referred to as NR1b or NR1a, respectively. The N-terminal splice variants result in differential sensitivity to numerous modulatory endogenous ligands, and a common mechanism appears to be distortion of the pH sensitivity (see below).

Activation of NMDA receptors requires glycine as a co-agonist. Increasing concentrations of glycine potentiate the response to agonists by reduction of the desensitization, resulting in an increased steady state current after prolonged NMDA exposure.[96] Endogenously, both glycine and/or (*R*)-serine[79] can act as a ligand. However, it is debated whether the glycine or (*R*)-serine concentration in the synaptic cleft will saturate the glycine site (for review, see ref 97).

The potency of glycine is independent of subunit composition when tested on the mouse clones,[98] while the potency on rat NR1/NR2D is almost 10-fold higher than for NR1/NR2A receptors.[99,100] Detailed mutagenesis studies show that mutations in the NR1 subunit greatly affect the glycine interaction. In particular, the mutants Gln387 and Phe466 (equivalent to 402 and 450 in iGluR2) in S1 reduce the potency for glycine more than 2000-fold, but also mutants Ser669 (653 in iGluR2) in S2 affect the glycine interaction.[101] Interestingly, only the mutations in S1 affect the ability of 7-chlorokynurenic acid (**52**) (Chart 6) to inhibit the glycine potentiation, suggesting that **52** exerts its effect via selective binding to one of the binding lobes.[101] Mutations in NR2B greatly alter the Glu potency but impose no significant changes in the glycine potency. The antagonists affinities are affected differently by the mutations: inhibition of (*R*)-CPP (**30**) is only affected by mutants in domain S1, while (*R*)-AP5 (**29**) is sensitive to mutations in the S1 as well as the S2 binding domain.[102] The current model of the NMDA receptor complex suggests that the glycine site is formed by the NR1 subunit and the Glu site is formed by the NR2 subunit. Thus, the model suggests that subtype specific competitive glycine antagonists should not be expected.

**Table 1.** $EC_{50}$ Values for Selected NMDA Receptor Agonists and $K_i$ Values for Two Antagonists[a]

| | NR1/NR2A | NR1/NR2B | NR1/NR2C | NR1/NR2D |
|---|---|---|---|---|
| glycine | 0.84 (2.1) | 0.19 (0.3) | 0.15 (0.2) | 0.096 (0.09) |
| (*R*)-serine (**44**) | 0.32 | 0.26 | 0.21 | 0.17 |
| NMDA (**24**) | 36 | 20 | 22 | 9 |
| (*S*)-Glu | 1.7 | 0.8 | 0.7 | 0.4 |
| (*R*)-AP5 (**29**) | 0.28 | 0.46 | 1.64 | 3.71 |
| (*R*)-CPPene (**31**) | 0.11 | 0.14 | 1.46 | 1.84 |

[a] All Values are in $\mu$M.

**Table 2.** Key Residues in the Agonist-Binding Cavity[a]

| | iGluR1−R4 | iGluR5/6/7 | KA1−2 | NR1 | NR2A−D |
|---|---|---|---|---|---|
| 402 | Glu | Glu | Glu | Gln | Glu |
| 449 | Lys | Lys | Val/Leu | Lys | Lys |
| 450 | Tyr | Tyr | Tyr | Phe | His |
| 478 | Pro | Pro | Gly/Ala | Pro | Ser |
| **480** | **Thr** | **Thr/Ala/Thr** | **Thr** | **Thr** | **Thr** |
| **485** | **Arg** | **Arg** | **Arg** | **Arg** | **Arg** |
| 653 | Gly | Gly | Gly | Ser | Gly |
| **654** | **Ser** | **Ser/Ala/Ser** | **Ser** | **Ser** | **Ser** |
| **655** | **Thr** | **Thr** | **Ser/Thr** | **Val** | **Thr** |
| 686 | Thr | Ser/N/N | Thr | Ala | Val |
| **705** | **Glu** | **Glu** | **Glu** | **Asp** | **Asp** |
| 708 | Met | Ser/Thr/Thr | Met | Val | Val |

[a] Residues in bold interact with the Glu backbone of kainate. Other residues have been involved in selective binding of other agonists and competitive antagonists.

A number of highly potent and selective antagonists have been developed for the glycine site such as **51** and **53** (Chart 6), with binding affinities in the low nanomolar range, and **56** with an $IC_{50}$ value of 3.3 nM. Indeed, no subtype specificity was observed for these $glycine_B$ antagonists.

The molecular diversity of the Glu binding site might be a potential target for competitive subtype specific drugs. However, examining the key residues in the binding pocket (Table 2), determined from comparisons with the iGluR2 crystal structure, reveals a highly conserved binding site between the NR2 subtypes. The differences in potency of NMDA and Glu between the subtypes is also less than 4-fold with the order of potency NR1/NR2D > NR1/NR2B = NR1/NR2C > NR1/NR2A (Table 1). Other agonists such as homoquinolinic acid (**26**) (Chart 2) exhibit a different relative subunit selectivity, with the rank order NR1/NR2A > NR1/NR2B > NR1/NR2C > NR1/NR2D.[103] Interestingly, antagonists exhibit some subunit selectivity, as (*R*)-AP5 (**29**) and (*R*)-CPPene (**31**) inhibit NR1/NR2A with more than 10-fold higher potency than NR1/NR2D receptors. Mutagenesis data show that (*R*)-AP5 inhibition is affected by mutations, which do not affect Glu interaction, suggesting that (*R*)-AP5 binding site exceeds the Glu binding pocket.[102] In the analysis of these data, it should, however, be taken into consideration that whereas the NR2A subunit is widely distributed in brain tissue, the other NR2 subunits have been identified in restricted areas of the brain.[48,76]

The noncompetitive antagonists ifenprodil (**41**) and its analogues, e.g., CP-101,606 (**42**) (Chart 4), have recently received much attention because of the subtype selectivity and the mode of action, which is an activity-dependent blockade of NR2B containing NMDA receptors.[76,104] Ifenprodil inhibits NR1/NR2B receptors ($IC_{50}$ 0.3 $\mu$M) and is at least 150-fold weaker against NR1/NR2C or NR1/NR2D receptors.[105] The potency is strongly pH-dependent in a physiological range, and changes of the ionization of the drug cannot explain the effect. Strong evidence supports the view that the inhibitory effect is due to a drug induced increase in the sensitivity to protons. The molecular determinants for the pH sensor are not well defined, but the action of a number of other modulators seems to converge to the pH sensor as a common structural element. Thus, the presence of exon 5 (e.g., NR1b) or spermine abolishes the pH inhibition, while ifenprodil (**41**) and CP-101,606 (**42**) (at NR1a/NR2B receptors) or nM $Zn^{2+}$ (in NR1a/NR2A receptors) enhances the pH inhibition.[104,106,107] Zinc inhibits NMDA receptors in a voltage-dependent and -independent manner. The $IC_{50}$ for the voltage independent $Zn^{2+}$ inhibition of the recombinant NR1/NR2A receptor is in the nanomolar range compared to micromolar for the NR1/NR2B.[108,109] Recent studies have identified two histidines, His42 and His44, in the N-terminal part of NR2A as involved in the high affinity $Zn^{2+}$ binding related to the voltage independent inhibition.[106]

The endogenous proton inhibition might function as a "rescue" mechanism to reduce NMDA receptor mediated currents under ischemic insults where the pH might be as low as 6.5. Thus, novel strategies which are targeted against the endogenous modulatory pathways might be considered for the development of subunit selective drugs or perhaps drugs that interfere with the $Zn^{2+}$ modulation.

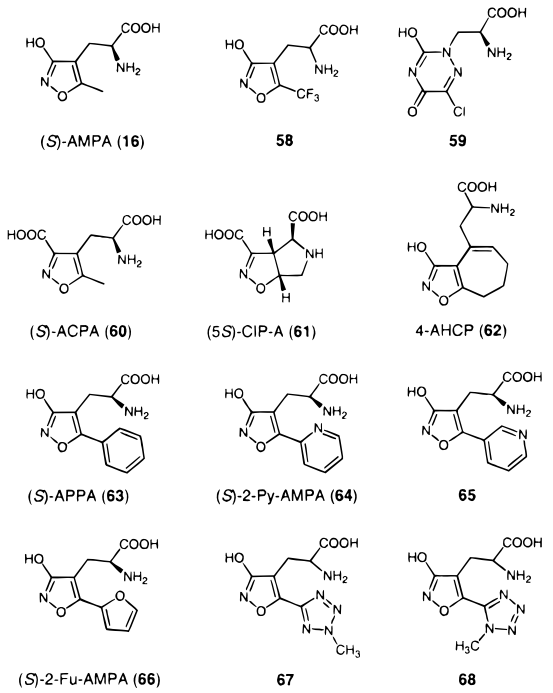
The inhibition of the NMDA channel by compounds such as MK-801 (**35**) and PCP (**36**) (Chart 4) depends on channel activation. Both MK-801 and PCP are trapped in the channel after removal of the agonist, and the inhibition is difficult to reverse. The open-channel blocker memantine (**38**) also binds in the channel, but a fraction of the channels apparently releases the blocker.[110] The molecular mechanism for the partial trapping and the difference between the reversability of blockers are not understood but might reflect how deep the compounds bind within the channel or a poor fit of **38** into the binding site.

## AMPA Receptors

**Molecular Structure of AMPA Agonists and Functional Partial Agonists.** The introduction of [$^3$H]-AMPA as a radioligand[111,112] greatly facilitated the pharmacological characterization of AMPA receptors and the development of new AMPA receptor ligands, including the very potent AMPA agonists trifluoro-AMPA (**58**),[113] (*S*)-5-chloro-6-azawillardiine (**59**),[114] and (*S*)-ACPA (**60**)[115] (Chart 7). Like **60**, 4-AHCP (**62**)[116] actually is a homologue of Glu, and both these compounds are highly potent agonists at AMPA receptors. (5*S*)-CIP-A (**61**), which is a semirigid analogue of (*S*)-Glu showing potent agonist effect at AMPA receptors,[117] is a useful tool for the development of AMPA agonist pharmacophore(s) (T. Liljefors, unpublished).

In addition to [$^3$H]-(*S*)-AMPA[22] and [$^3$H]-(*S*)-5-fluorowillardiine,[21,22] [$^3$H]ACPA[118] has more recently been introduced as an AMPA agonist radioligand. Although these three AMPA agonists show very similar pharmacology on native receptors, they seem to bind to and activate AMPA receptors in a nonidentical fashion, with dissimilar binding distributions in rat brain (Figure

**Chart 7.** Structures of Some AMPA Receptor Agonists




5).[118] One explanation of these observations may be a difference in receptor subtype selectivity, but it should also been taken into consideration that all of these AMPA agonists show weak but different inhibitory effects on [$^3$H]KA binding. The three structurally quite different compounds may be useful tools for studies of AMPA receptor mechanisms and for the development of AMPA agonist pharmacophores.

Analogues of AMPA having different substituents in the 5-position of the isoxazole ring have been synthesized and used in structure−activity studies on the AMPA receptors.[119−121] The results of these quite extensive studies have given rise to a hypothesis concerning the topography of AMPA receptors, which seems to contain a cavity which can accommodate lipophilic substituents of a certain size.[120] An important compound in this respect is phenyl-AMPA (APPA).[122] The racemic form of APPA was originally described as a weak partial agonist at AMPA receptors with an intrinsic activity of approximately 60% relative to that of AMPA. Subsequently, APPA was resolved into optically pure (*R*)- and (*S*)-APPA (**63**),[123] and these two enantiomers were tested in the rat cortical wedge preparation.[123,124] These electrophysiological experiments showed **63** to be a full agonist at AMPA receptors, whereas the (*R*)-form was devoid of excitatory activity. (*R*)-APPA was shown to be an antagonist at AMPA receptors, and rightward parallel shifts of both the AMPA and the **63** dose−response curves were obtained by coadministration with (*R*)-APPA, indicating competitive antagonism. This means that the observed partial agonist profile of racemic APPA is due to the combined effects of the full agonist, (*S*)-APPA (**63**), and the competitive antagonist, (*R*)-APPA.

This prompted further experiments using different relative concentrations of these enantiomers. Different fixed concentration ratios of (*S*)-APPA and (*R*)-APPA (1:1, 1:2, and 1:3), gave dose−response curves with decreasing levels of relative efficacy, and these curves

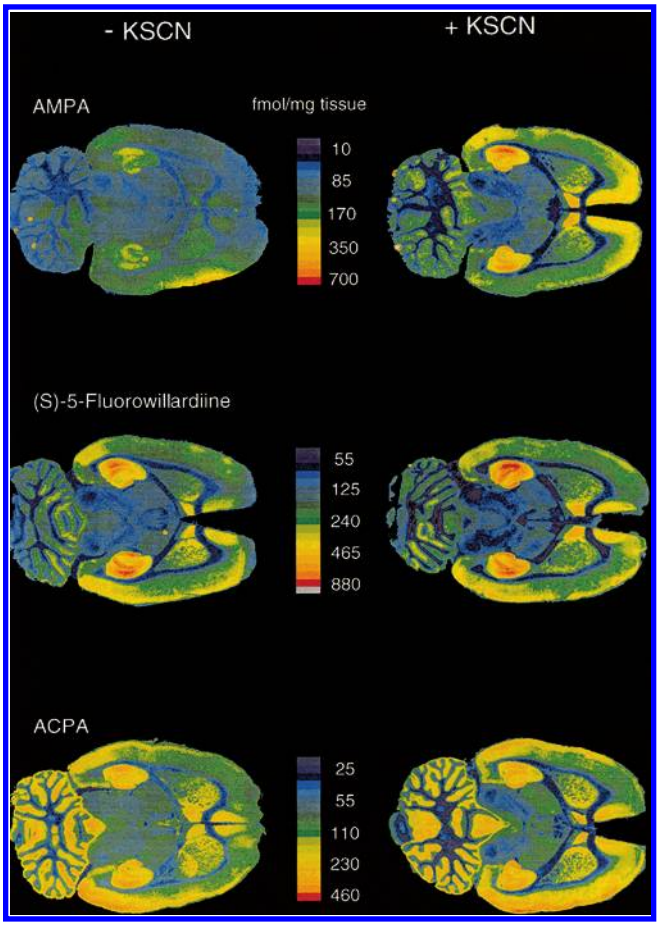



**Figure 5.** Autoradiographic representation of the binding of [$^3$H]AMPA (**16**) (top), [$^3$H]-(*S*)-5-fluorowillardiine (**19**) (middle), and [$^3$H]ACPA (**60**) (bottom) in the presence or absence of 100 nM KSCN (for details, see ref 118). (Reproduced with permission.)

illustrate the principle of functional partial agonism,[124] which can be obtained by using a fixed ratio of an agonist and a competitive antagonist.

This principle opens up the possibility of producing partial agonist activity at any level, meaning that any desired intrinsic activity between 0 and 100% can be achieved by coadministration of the appropriate concentration ratio of a full agonist and a competitive antagonist.[124] In principle, establishment of "tunable" functional partial agonism in a brain region of particular relevance to iGluR-stimulating therapeutic intervention should be possible. Thus, by proper choice of efficacy level, the establishment of nontoxic and nondesensitizing EAA receptor stimulation therapies may, in principle, be possible.

A prerequisite for the development of practically useful therapies along these lines obviously is the availability of agonists and competitive antagonists capable of penetrating the BBB.[124] In this regard, (*S*)-APPA (**63**) and (*R*)-APPA have been studied in a drug discrimination model, showing (*R*)-APPA to have antagonistic effects toward the central stimulus properties of ATPA, whereas no agonist (substituting) effect was observed with **63**.[125] ATPA was subsequently shown to be a very potent KA receptor (iGluR5) agonist (see later section), and since (*R*)-APPA does not significantly affect [$^3$H]AMPA or [$^3$H]KA binding,[123] the mechanism(s) underlying this in vivo effect is enigmatic.

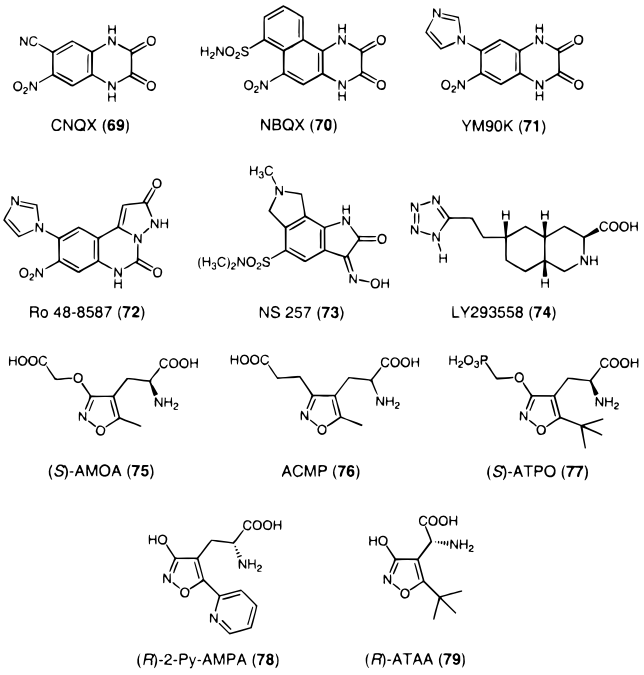
This principle is, of course, not restricted to AMPA receptor agonists and competitive antagonists. It should be applicable to competitive ligands at any receptor, and since it is potency-dependent, the dose ratios required for a desired level of efficacy is a function not only of the relative potencies of the agonist and competitive antagonist but also of the pharmacokinetic and pharmacodistribution characteristics of the two compounds.

These observations for (*S*)-APPA (**63**) and (*R*)-APPA prompted the synthesis of 5-heteroaryl substituted AMPA analogues. Whereas the (*S*)-form of the 2-pyridyl analogue, **64**, is much more potent than **63** as an AMPA agonist,[126] the 3-pyridyl analogue, **65**, is inactive.[127] On the other hand, (*R*)-2-Py-AMPA (**78**) is a weak AMPA antagonist equipotent with (*R*)-APPA, and **64** and **78**, administered together, also produce functional partial agonism.[126] The 2-furyl analogue of **64**, (*S*)-2-Fu-AMPA (**66**), is even more potent than **64** as an AMPA agonist, whereas (*R*)-2-Fu-AMPA, quite surprisingly, does not show significant AMPA antagonist effect.[127] Although **66** is highly photosensitive, attempts to use **66** as a photolabel for the AMPA receptor recognition site(s) have been unsuccessful.[127] Within this group of AMPA analogues, the 2-methyltetrazolyl analogue **67** is the most potent AMPA agonist whereas the 1-methyltetrazolyl isomer **68** is essentially inactive.[128]

The mode of binding of these heteroaryl analogues of AMPA to the AMPA receptors is unclear, but evidently electrostatic as well as steric effects play a key role in the interaction of the heteroaryl substituent with the proposed cavity at the AMPA recognition site(s). Recent molecular pharmacological data indicate that subtype-selective AMPA agonists will be found within this family of compounds. A number of the very potent AMPA agonists shown in Chart 7, notably **16**, **60**, **66**, and **67**, have been cocrystallized with the recombinant S1−S2 binding domain[9,54] of an AMPA receptor. X-ray crystallographic analyses in progress undoubtedly will shed light on the mode of interaction of these compounds with the agonist conformation of the AMPA receptor and will form the basis for the development of AMPA agonist pharmacophore model(s).

**Molecular Structure of Competitive AMPA Antagonists.** Different series of competitive AMPA receptor antagonists have been developed. Early pharmacological studies on AMPA and KA receptors were hampered by the lack of selective and potent antagonists. CNQX (**69**) (Chart 8) and related compounds offered a breakthrough in this respect, being quite potent antagonists, though nonselective.[129,130] Subsequently, NBQX (**70**) was shown to have improved AMPA receptor selectivity compared to **69**.[131] A large number of compounds have been developed with the quinoxalinedione structure, not only as AMPA receptor antagonists, but also as compounds showing effects at other Glu receptor sites, notably glycine$_B$ antagonists. YM90K (**71**) shows high potency compared to **70** as an AMPA receptor antagonist and is systemically active.[132] Within this family of compounds the highly selective AMPA antagonist, Ro 48-8587 (**72**), has been radiolabeled and shown to be a very useful tool for studies of AMPA receptors.[133] A number of isatin oximes, such as NS 257 (**73**), have shown antagonist activity, and these compounds, like **71** and **72**, show higher water solubility than **70**, and they have been shown to be systemically active anticonvulsants.[134]

**Chart 8.** Structures of Some Competitive AMPA Receptor Antagonists

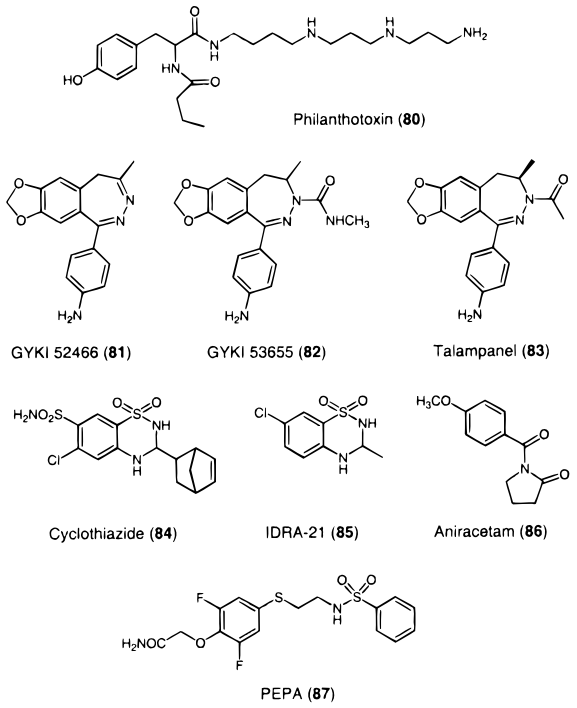



One group of AMPA receptor antagonists contain the acidic amino acid structure generally found in Glu receptor agonists, whereas another group of antagonists are acidic amino acids containing a longer backbone. One member of the latter group is the tricyclic compound LY293558 (**74**), which shows significant antagonist effects at KA receptors in addition to the potent AMPA receptor blocking effects.[135] The AMPA receptor antagonists (*S*)-AMOA (**75**)[136,137] and (*S*)-ATPO (**77**),[138] which have been developed using AMPA as a lead structure, also have carbon backbones longer than that normally found in AMPA agonist molecules. For **74**, **75,** and **77**, the (*S*)-form has been shown to be the more potent enantiomer.[135,138] Recently, the carbon analogue of **75**, ACMP (**76**), has shown improved AMPA antagonist potency compared to **75**.[139]

Within the group of AMPA antagonists having "agonist chain lengths", the (*R*)-form is generally the active enantiomer, as exemplified by (*R*)-APPA,[123] (*R*)-2-Py-AMPA (**78**),[126] and (*R*)-ATAA (**79**).[140] These antagonists are remarkable because, despite their "agonist-like" structure and AMPA antagonist activity, they show very low receptor affinity in the [$^3$H]AMPA binding assay.

**Molecular Structure of Noncompetitive AMPA Antagonists and Modulatory Agents.** Like the NMDA receptors, the AMPA receptors appear to be biomolecular complexes comprising a number of binding sites in addition to the Glu recognition sites.[141] Whereas philanthotoxins, as exemplified by **80**, normally block iGluRs with a low degree of selectivity (se later section), a number of 2,3-benzodiazepines such as GYKI 52466 (**81**)[142] and GYKI 53655 (**82**)[143,144] (Chart 9) have been characterized as potent and selective noncompetitive AMPA antagonists. The noncompetitive nature of these antagonists, assumed to interact with an allosteric receptor site, suggests that they may be capable of

**Chart 9.** Structures of Some Noncompetitive AMPA Receptor Antagonists (**80**−**83**) and Some AMPA Receptor Modulatory Agents ("Ampakines") (**84**−**87**)




inhibiting AMPA receptor functions in the presence of high levels of Glu.[145] Within this group of compounds, Talampanel (**83**) is under clinical evaluation.[145]

The rapid agonist induced desensitization of AMPA receptors can be markedly inhibited by a number of structurally dissimilar compounds including cyclothiazide (**84**),[146] the related sulfonamide IDRA-21 (**85**),[141] and aniracetam (**86**), a group of compounds named "Ampakines".[147] These compounds essentially block AMPA receptor desensitization and, consequently, enhance excitatory activity several-fold, depending on the initial level of desensitization observed for the individual agonist. The recognition sites for cyclothiazide (**84**), aniracetam (**86**), and the 2,3-benzodiazepine noncompetitive antagonists are different and are distinct from the agonist recognition site of the AMPA receptor.[148,149]

Potassium thiocyanate is used to enhance agonist affinity in the [$^3$H]AMPA binding assay.[112] It has been shown that this chaotropic agent blocks AMPA receptors by converting the receptors into the desensitized state showing higher affinity for AMPA.[150] It has been suggested that the sites of interaction of **84** and the thiocyanate ion are identical and that these compounds have "opposite" effects on the AMPA receptor desensitization mechanism(s).[151] There is evidence to suggest that this binding site is located at the iGluR2 subunit.[152] Interestingly, the binding of [$^3$H]ACPA (**60**) (Figure 5), an AMPA receptor agonist showing weak desensitization, is not affected by thiocyanate ions.[118]

**Molecular Pharmacology.** The AMPA receptor subunits iGluR1−iGluR4 are expressed in different variants due to splicing and RNA editing. All subunits occur in two alternative spliced forms where the C-terminal part of the binding segment, S2, can be in either a flip (i) or a flop (o) form. The flip forms are predominantly expressed before birth and desensitize slowly and less strongly to Glu when compared to the flop forms. The flop forms are generally expressed at the same level as the flip forms in adults.

Expression of the various forms of the AMPA receptor subunits in heterologous expression systems show that the most significant pharmacological and physiological differences depend on the presence of iGluR2 in the complex or the type of splice variant in the individual subunits. The iGluR2 pre-mRNA is edited by a process that changes an adenosine to an inosine, resulting in incorporation of an arginine rather than a glutamine at a critical position in the pore (Q/R site).[153] The presence of an arginine, i.e., an iGluR2 subunit in the receptor complex, at that position in the pore abolishes $Ca^{2+}$ permeability and changes the rectifying properties. AMPA receptors without iGluR2 subunits are $Ca^{2+}$ permeable and exhibit a strong inward rectification due to block by intracellular polyamines (see refs 27 and 48 for review). Different channel blockers, such as philanthotoxin (**80**), selectively inhibit AMPA receptors without the iGluR2 subunit most likely because the positively charged arginine in iGluR2 repels the positively charged blockers.[154,155]

The allosteric modulator cyclothiazide (**84**) potentiates the Glu activated current measured in oocytes from 67-fold (on iGluR3$_i$) to 170-fold (on iGluR4$_i$) by completely abolishing the desensitization.[156] The $EC_{50}$ is about 5 $\mu$M for the flip forms.[157] Compound **84** also affects the flop forms but only by reducing the rate of transition into the desensitized state and at lower potency ($EC_{50}$ > 70 $\mu$M). The major structural determinant for the difference between flip and flop is the amino acid at position 750 (in iGluR1 or 754 in iGluR2).[158] A mutation of Ser750 in iGluR1$_i$ to an asparagine (as in flop) reduces the **84** sensitivity to the flop level, and mutation to a glutamine, as in the insensitive iGluR6, completely abolishes the action of **84**.[158] Aniracetam (**86**) potentiation is also affected by mutations at the 750 position. The **86** potentiation can be explained as a slower channel closure while the mechanisms underlying the **84** potentiation are not clearly understood.[159] The sulfonylamino compound PEPA (**87**) exhibits almost the opposite profile by preferentially potentiating the flop forms. Homomeric iGluR1$_o$ expressed in oocytes is potentiated 8-fold, while iGluR3$_o$ and iGluR4$_o$ are potentiated more than 50-fold with an $EC_{50}$ of 50 $\mu$M for iGluR3$_o$.[160] The effect of **87** is also affected by mutations at the 750 (iGluR1) position. Examination of the iGluR2 crystal structure shows that the residue 750 (754 in iGluR2) is located adjacent to a remarkably hydrophobic solvent exposed surface, suggesting that the region might be involved in subunit−subunit interaction in the assembled receptor.[9] Other mutagenesis studies have shown that aromatic substitutions of Leu497 in the S1 domain in iGluR1 completely relieve desensitization,[161] suggesting that either these regions might interact or desensitization might result from allosteric transitions involving different parts of the receptor subunits.

The currently most selective AMPA antagonists are the allosteric modulating 2,3-benzodiazepines **81** and **82** which, as mentioned previously, interact at a site different from that recognizing **84**. However, the reduction in the potencies of the 2,3-benzodiazepines by **84**

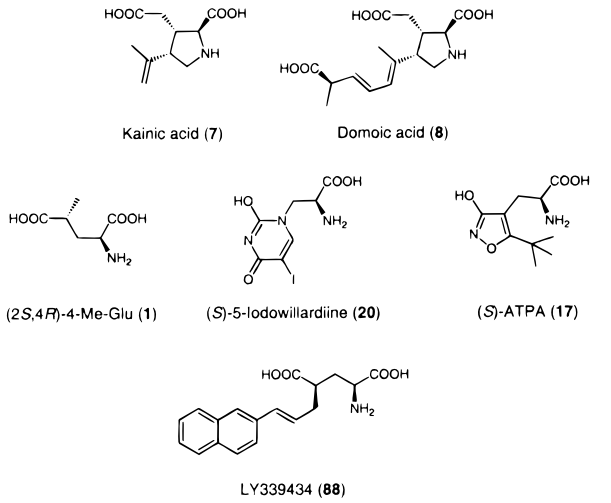
are directed through an allosteric mechanism. Most of the characterized agonists or competitive antagonists exhibit less than 5-fold selectivity in potency or affinity between the AMPA receptor subtypes, a reflection of the high conservation of the residues covering the surface of the ligand-binding pocket (Table 2). However, the maximal steady state currents evoked by the agonists often exhibit subtype selectivity. As an example, the $EC_{50}$ for (*S*)-ATPA (**17**) (Figure 1) is 7.9 $\mu$M and 7.6 $\mu$M on $iGluR3_o$ and $iGluR4_o$, respectively, while the steady state current is 5 and 30%, respectively, of the KA (**7**) induced currents.[18] Thus **17** might in more complex neuronal based assays act, due to the low steady state current, as a functional antagonist on iGluR3 receptors but as an agonist on iGluR4 receptors.

## Kainate Receptors

**Molecular Structure of Kainate Agonists.** Pharmacological characterization of KA receptors have for many years been hampered by the lack of selective ligands, both agonists and in particular antagonists. KA has been the standard agonist despite its nonselective action, and [$^3$H]KA is the ligand of choice for studies of high and low affinity KA binding sites.[162,163] KA shows relatively potent interaction with AMPA receptors as well, and it is frequently used as the agonist for studies on AMPA receptors, because KA, in contrast to AMPA itself, does not desensitize AMPA receptors.

Other naturally occurring kainoids also interact potently with KA receptors, e.g., domoic acid (**8**) (Chart 10) has been identified.[164−166] Domoic acid is a highly

**Chart 10.** Structures of Some Kainate Receptor Agonists



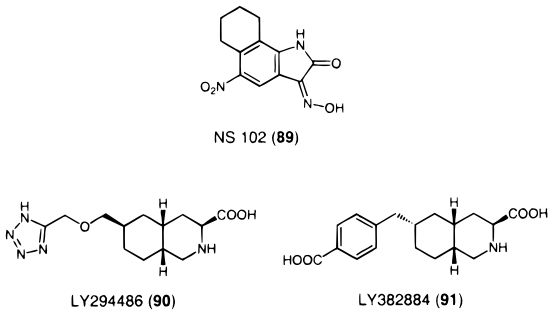

potent KA receptor agonist, more potent than KA, and also a very effective neurotoxin.

(2*S*,4*R*)-4-Me-Glu (**1**) has been reported to have selective affinity for [$^3$H]KA binding sites,[167] and potent agonist activity at recombinant homomeric iGluR5 and iGluR6 receptors.[168,169] Significant potency of compound **1** has, however, been reported at mGluRs as well.[170] Potent and selective iGluR5 agonist activity has also been shown for (*S*)-5-iodowillardiine (**20**), whereas very weak or no activity was observed at iGluR6 or iGluR7.[171,172] Potent iGluR5 agonist activity has been observed for (*S*)-ATPA (**17**), which shows high selectivity toward iGluR5 relative to iGluR6 and AMPA receptors, both in binding experiments and in electrophysiological studies.[17,18]

Activity at KA receptors can be modulated by lectins, such as Concanavalin A (Con A), which attenuates the desensitization of KA receptors and thus enhances the excitatory activity.[173−175]

**Molecular Structure of Kainate Antagonists.** So far, only very few KA receptor antagonists have been reported. NS 102 (**89**) (Chart 11) does, however, show

**Chart 11.** Structures of Some Competitive KA Receptor Antagonists




selective affinity for the low affinity [$^3$H]KA site.[176] NS 102 also shows some affinity for AMPA receptors and antagonist effect at homomeric iGluR6.[177] Low water solubility may limit the pharmacological utility of **89**. LY294486 (**90**) has been shown to have competitive antagonist activity with some selectivity toward iGluR5, relative to AMPA-preferring subunits, and without activity at other KA-preferring subunits.[17,34,178]

Future studies on receptors of known subunit combination will be of importance for the development of new KA receptor ligands. The AMPA receptor antagonist LY293558 (**74**) also does show some antagonist activity at iGluR5 receptors, but no antagonism of iGluR6 receptors.[178]

It must be emphasized that most of the Glu receptor ligands mentioned in this and previous sections have only been tested at a limited number of Glu receptor subtypes. As exemplified by (*S*)-ATPA,[17,18] compounds previously described as family- or subtype-selective receptor ligands may possess more potent, but not yet disclosed, pharmacological effects at other Glu receptor subtypes.

**Molecular Pharmacology.** The KA receptor subunits iGluR5−iGluR7 are often referred to as the low affinity KA receptors due to a binding affinity for KA (**7**) in the range of 15−100 nM, while the KA1−KA2 receptors form the high affinity KA receptors with affinities in the low nanomolar range.[179,180] The activity of the KA receptors can be distinguished from the AMPA receptors by desensitization to KA and the very slow recovery from the desensitized state.

Homomeric iGluR6 was the first recombinant KA receptor to be characterized functionally.[174] KA evoked a desensitizing current while AMPA (**16**) did not activate the receptor. Furthermore, Con A treatment abolished the desensitization. Interestingly, AMPA evoked a nondesensitizing current on heteromeric complexes between iGluR6 and KA2, despite neither iGluR6 nor KA2 can bind AMPA.[181] This led to the suggestion that the binding site might be located at the interface

between the two subunits, and an allosteric effect of AMPA cannot, at present, be excluded.

Homomeric iGluR5 and iGluR6 exhibit similar properties with respect to KA activated current and Con A treatment. Con A potentiates the steady state current 100−200-fold on iGluR5 and iGluR6 receptors expressed in oocytes, but less than 10-fold on the AMPA receptors.[156] The Con A effects on iGluR6−KA2 complexes are not clear, but at least there is no potentiation of the AMPA induced current. Con A potentiation requires interaction with the carbohydrate moieties, thereby probably slowing the structural transition in the activated receptor.[182] In contrast to iGluR6, iGluR5 receptors are activated by AMPA with an $EC_{50}$ of ∼1 mM.[183] The difference is due to the sequence heterogeneity at the position equivalent to 686 in iGluR2 (Asn721 in iGluR6).[55] More careful analysis of AMPA derivatives revealed that hydrophobic residues at the 5-position in the isoxazole ring, as the *tert*-butyl substituent in ATPA (**17**), greatly increased the potency at iGluR5 receptor ($EC_{50}$ 0.6 $\mu$M).[17,18] The high potency for ATPA on iGluR5 is difficult to explain structurally, since there is no obvious hydrophobic pocket that can accommodate the *tert*-butyl group and facilitate the binding. However, the ATPA preference for iGluR5 relative to the AMPA receptors seems to some extent to depend on the amino acid at position 708 (iGluR2 numbers) (unpublished data). The 5-substituted willardiines (Figure 1) show a gradual change in KA vs AMPA receptor preference depending on the size of the halogen at the 5-position.[184] Fluorowillardiine (**19**) preferentially activates the AMPA receptors while Iodowillardiine (**20**) has a high binding affinity to the iGluR5 receptor but shows no affinity for iGluR6 receptors.[171] The selectivity between iGluR5 and iGluR6 also depends on the amino acid at position 721 in iGluR6 (686 in iGluR2).[172]

Screening of decahydroisoquinoline derivatives on recombinant Glu receptors led to the identification of LY293558 (**74**),[185] LY294486 (**90**),[17] and LY382884 (**91**)[186] as iGluR5 antagonists (Chart 11). Both **74** and **90** also inhibit AMPA receptors although the latter exhibit more than 10-fold selectivity for iGluR5. LY382884 (**91**) binds to homomeric iGluR5 with a $K_i$ of 4.0 $\mu$M and >100 $\mu$M for the other KA and homomeric AMPA receptors. Interestingly, heteromeric iGluR5/iGluR6 receptors are also inhibited by LY382884 (**91**).

Other KA vs AMPA receptor specific agonists are the Glu analogues LY339434 (**88**) (Chart 10) and **1** (Chart 1).[187,188] The former is selective for iGluR5 while the latter does not discriminate between iGluR5 and iGluR6 homomeric receptors.[168,169]

Homomeric iGluR7 receptors have recently been shown to form functional receptors, with properties distinct from the other low affinity receptors. Domoic acid (**8**) acts as a high affinity agonist on iGluR5 and iGluR6 (Table 2) but as an antagonist on iGluR7. The potency for KA (**7**) is >5 mM for iGluR7, and pretreatment with Con A does not affect iGluR7.[189]

The properties of heteromeric KA receptors have distinct physiological and pharmacological properties compared to the homomeric receptors,[190] complicating the understanding of the molecular mechanisms underlying the responses observed in native KA receptors.

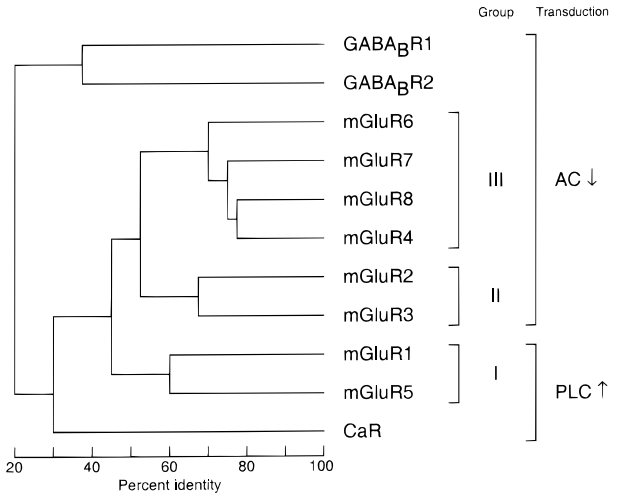



**Figure 6.** Phylogenetic tree showing the sequence identity between cloned mammalian family C G-protein coupled receptors. The signal transduction pathway and the division of mGluRs into three groups are also shown.

## Structure and Function of Metabotropic Glutamate Receptors

**Receptor Genes.** The mGluRs belong to the superfamily of G-protein coupled receptors (GPCRs) as judged by their signal transduction pathways and hydropathy plots revealing seven transmembrane (7TM) segments. In 1991 when mGluR1 was cloned,[191,192] two major subfamilies of GPCRs had been discovered: the rhodopsin-like (family A) and the secretin-like (family B). The eight presently cloned mGluRs do not show homology to any members of these two subfamilies and, accordingly, a new family has been established (family C, mGluR-like).[191−194] As shown in Figure 6, three additional family C members, the calcium-sensing receptor (CaR)[195] and two $\gamma$-aminobutyric acid type B receptors (GABA$_B$R1-2),[196] which show 20−30% sequence identity with the mGluRs, have also been cloned. Furthermore, several putative pheromone family C receptors have been cloned from the vomeronasal organ of mammals.[197] The overall topology of all the family C receptors is distinctly different from those of family A and B. Most notable is the unusually long amino-terminal domain (ATD) consisting of some 500 amino acids connected to the 7TM domain by a cysteine rich region (Figure 7).

Analysis of the eight mGluR protein sequences has revealed that the subtypes can be divided into three groups. As shown in Figure 6, receptors within a group show more than 60% sequence identity, whereas there is 40−50% sequence identity between the groups. This grouping coincides with the signal transduction pathways used by the receptors. For example, the Group I receptors mGluR1 and mGluR5 stimulate phospholipase C (PLC), causing an increase in intracellular inositol phosphates (IPs) and $Ca^{2+}$ levels. Group II consists of mGluR2 and mGluR3 while Group III includes mGluR4, mGluR6, mGluR7, and mGluR8, all of which inhibit adenylate cyclase (AC) causing a decrease in intracellular cAMP levels.[193,194] Until recently, selectivity of known agonists and antagonists did not extend beyond the group level; however, as will be discussed in detail in later paragraphs, the discovery of a number of ligands with selectivity for individual mGluRs have now been reported.

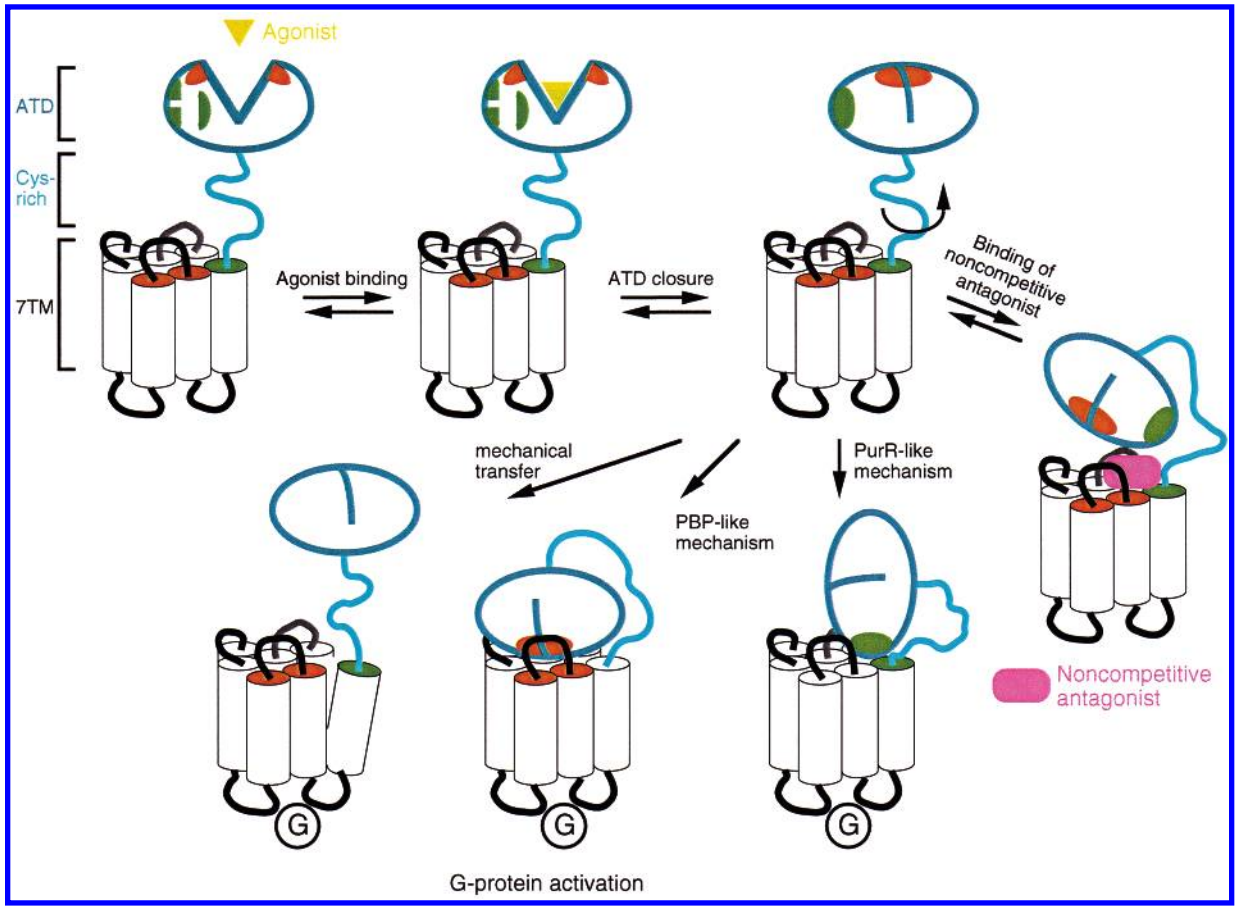



**Figure 7.** As shown on the top left figure, mGluRs are generally believed to consist of an extracellular amino-terminal domain (ATD) connected to the 7 transmembrane (7TM) domain by a cysteine-rich hinge. Agonists bind to the open form of the ATD which then close and surround the agonist. Three models of the activation of the 7TM by the closed ATD are shown. (1) "Mechanical transfer", the structural changes in the ATD upon closure are relayed to the cysteine-rich region and on to the 7TM region. (2) "PBP-like mechanism", like the periplasmic binding proteins (PBP) a motif is generated from both lips when these are brought together upon ATD closure. The motif then recognizes and activates the 7TM region. (3) "PurR-like mechanism", like the purine repressor (PurR) agonist binding closes the cleft and induces a structural rearrangement of one of the globular domains whereby a motif is generated. The motif then recognizes and activates the 7TM region. Finally it is shown how 7TM binding noncompetitive antagonists are thought to hinder the intramolecular signaling mechanism.

**Agonist Binding.** In 1993 it was shown that the ATD of mGluR1 was homologous to the leucine/isoleucine/valine binding protein (LIVBP) which is a member of the family of bacterial periplasmic binding proteins (PBP).[51,198] All PBPs fold in a similar overall three-dimensional (3-D) structure consisting of two distinct globular domains, arranged as a central $\beta$-sheet flanked on both sides by α-helices, connected by a hinge region and separated by a cleft. LIVBP has been crystallized in the open form in the absence and presence of leucine.[199] Using this information, a 3-D model of the ATD of mGluR1 in the open form has been built, and it was predicted that the glycine moiety of Glu forms hydrogen bonds to Ser165 and Thr188. Accordingly, it was shown that mutations of these residues severely impaired Glu binding.[51] As can be seen in the 3-D model shown in Figure 8, Ser165 and Thr188 are located in the cleft of mGluR1. Since leucine does not contain a distal acidic function like Glu, it has not been possible to predict the amino acid(s) binding this part of the ligand from the LIVBP structure. However, it has been shown that mutations of the conserved Arg78 in mGluR1 (Figure 8) and mGluR4 severely impair binding of [$^3$H]-quisqualic acid and tritiated 2-amino-4-phosphonobutyric acid ([$^3$H]AP4), respectively. These data suggest that the distal acidic group of the agonists forms a salt bridge with Arg78.[200]

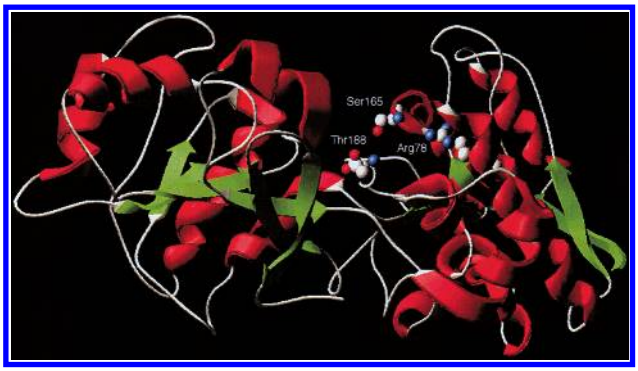



**Figure 8.** Three-dimensional model of the amino-terminal domain (ATD) of mGluR1 generated as described previously.[51] Helices and $\beta$-sheets are shown in red and green, respectively. Like the periplasmic binding proteins, the ATD consists of two distinct globular domains, arranged as a central $\beta$-sheet flanked on both sides by α-helices, connected by a hinge region and separated by a cleft. Amino acids which have been suggested to bind Glu in the cleft are also shown. Ser165 and Thr188 are believed to bind the glycine moiety by hydrogen bonds, and Arg78 presumably binds the distal carboxylic acid of Glu by a salt bridge. Coordinates for the model were kindly provided by Paul Sheppard and Patrick O'Hara.

**Receptor Activation.** It is presently not known how agonist binding in the ATD leads to activation of the 7TM domain. However, based on the known activation mechanism of PBPs and the structurally related purine repressor (PurR), several models can be suggested.

In PBPs, ligands bind to amino acids on one side of the open cleft. Driven by the favorable entropy of dehydration of the ligand and expulsion of water from the cleft, the PBP then closes around the ligand by bending and twisting of the hinge.[198] In analogy with this, it seems plausible that Glu binds to the globular domain containing Arg78, Ser165, and Thr188, followed by closure of the ATD. After closure, the soluble PBPs interact with proteins in the bacterial membrane. Since mutations on both "lips" of the globular domains affect this protein−protein interaction, it has been suggested that the closing of the PBP brings a motif on the lips together which is then recognized by the membrane protein.[201] In analogy with this chain of events, one can advance a hypothesis that a similar mechanism is responsible for the activation of mGluRs. Thus, as shown in Figure 7, closure of the ATD forms a motif from the two "lips" (shown in red) which then recognize and activate the 7TM region.

A second plausible mechanism can be deduced from the known structure and activation mechanism of PurR.[202,203] PurR consists of a corepressor binding domain (CBD) which is connected with a hinge to the DNA-binding domain. The CBD shares the overall 3-D structure of the PBPs, but like the mGluRs the ligand-binding domain is covalently connected to the effector region. In analogy with the PBPs, the repressor compounds bind in the cleft and cause closure of the CBD around the ligand by hinge-bending (but not hinge-twisting). However, in contrast to the PBPs, closure of the CBD cause structural rearrangements in the globular domain adjacent to the DNA-binding domain, which allow these two domains to interact in a fashion that ultimately leads to DNA binding.[202,203] Thus it can be hypothesized that closure of the ATD in mGluRs induces a structural rearrangement in the ATD which creates a motif (shown in green in Figure 7) that interacts with the 7TM region.

Other models of activation can also be envisaged such as delivery of Glu from the ATD to the 7TM region (like PBPs delivering ligands to the membrane proteins in active transport). However, we and others have shown that the ATD and 7TM regions of mGluR1 and CaR can be interchanged without loss of receptor activity,[204,205] which is not compatible with this hypothesis. Finally, one could envisage activation of the 7TM region as a mechanically transferred structural rearrangement brought from the ATD through the cysteine rich region to TM segment 1 (Figure 7).

Recently it has been shown that the noncompetitive antagonists ethyl 7-(hydroxyimino)-cyclopropan[*b*]-chromen-1a-carboxylate (CPCCOEt, **135**) and 2-methyl-6-(phenylethynyl)-pyridine (MPEP, **140**) (Chart 16) bind to the 7TM domain of mGluR1 and mGluR5, respectively.[206−208] Both compounds bind to the top of TM segment 7, and it has thus been suggested that they act by blocking intramolecular interaction of the ATD with the 7TM (Figure 7).[206] This hypothesis is in agreement with the suggested PBP- or PurR-like mechanisms.

Important information regarding the activation mechanism has also been obtained by construction of chimeric receptors in which ATDs and 7TM domains have been interchanged between family C receptors. Fully functional receptors have been obtained with mGluR2/1,[209] mGluR3/1,[210] mGluR4/1,[211] mGluR5/1,[206] mGluR1/CaR,[205] and CaR/mGluR1[204,205,207] chimeric receptors. Thus, the 7TM domain of mGluR1 can be equally well activated by the ATD of mGluR1,2,3,4,5 and CaR, which shows that the mechanism of signal transfer between the ATD and 7TM region has been conserved within these receptors. This should provide important information in the search for, e.g., the motif activating the 7TM domain.

Finally, it has been reported that truncated soluble forms of the ATD of mGluR1,[212,213] mGluR4,[214] and mGluR5[213] bind agonists with affinities close to those from the full receptor. The ATDs from both mGluR1 and mGluR5 form crystals,[213] and it is thus anticipated that the 3-D structure of the ATD will be determined in a not too distant future. As has been the case for the PBPs, PurR, and iGluR2, this should provide detailed information regarding ligand binding and the activation mechanism of the mGluRs.

## Molecular Structure of Metabotropic Glutamate Receptor Agonists

The cloning of the mGluRs and the following evolving evidence of their potential use as drug targets in a variety of neurological disorders have encouraged medicinal chemists to design ligands targeted for the mGluRs. During the first decade of the cloned mGluRs, our knowledge of the structure−activity relationship of these receptors has increased dramatically and has led to the discovery of new potent and selective ligands. The vast majority of these ligands are Glu analogues consisting of the glycine moiety and a distal acidic function, but recently noncompetitive antagonists with no structural similarity to Glu have also been discovered. In the following we have outlined some of the principles of drug design that have been employed by medicinal chemists in order to design ligands with increased receptor selectivity and potency (Table 3). We would also like to point the reader to a recent review by Schoepp et al. which describes mGluR pharmacology based on the three groups of receptors.[194]

**Conformational Constraints.** The first agonist shown to be selective for mGluRs compared to iGluRs was (1*SR*,3*RS*)-1-aminocyclopentane-1,3-dicarboxylic acid [(1*SR*,3*RS*)-ACPD also named *trans*-ACPD[215,216]], (1*S*,3*R*)-ACPD (**92**) (Chart 12) being the active enantiomer.[217] When tested on the eight cloned mGluRs it acts rather nonselectively,[218−224] but because of the compound's lack of activity at iGluRs, it has been used intensively as a template for design of new mGluR ligands. Within these series of compounds, it is very interesting to note the profound changes in receptor selectivity, potency, and efficacy caused by relatively small structural changes.

Introduction of a nitrogen atom in the C4-position of **92** afforded (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxy-

**Table 3.** Potencies of the Compounds on Cloned mGlu Receptors[a]

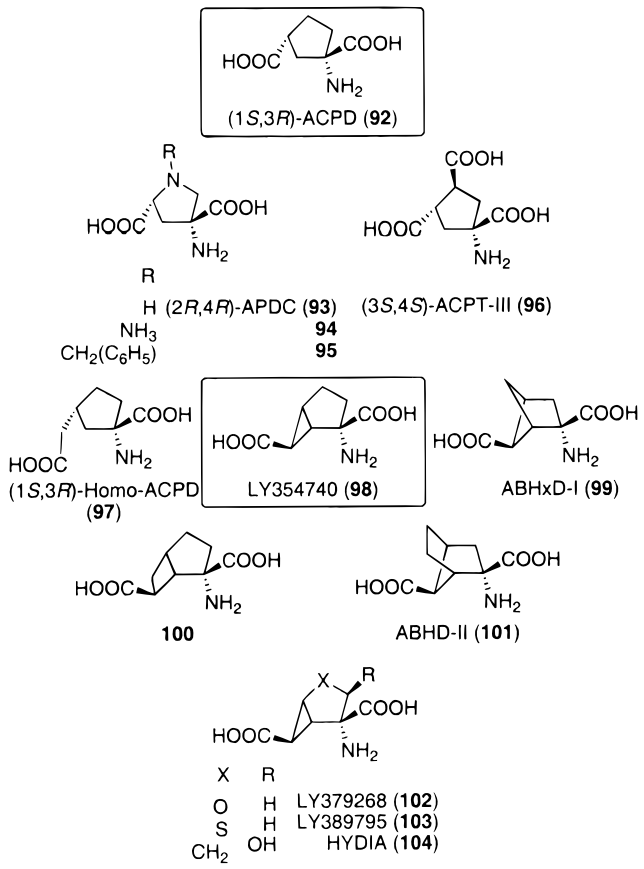
| compound | mGluR1 | mGluR2 | mGluR3 | mGluR4 | mGluR5 | mGluR6 | mGluR7 | mGluR8 | refs |
|---|---|---|---|---|---|---|---|---|---|
| Glu | 1.1−10 | 0.29−12 | 1.9−9.0 | 9.8−17 | 3.1−11.4 | 4.9−38 | 2300−5400 | 9.5 | 20, 235, 242 |
| (1*S*,3*R*)-ACPD (**92**) | 42 | 5 | | 98 | 15 | 60 | >1000 | 45 | 227, 276 |
| (2*R*,4*R*)-APDC (**93**) | >100 | 0.30−0.45 | 0.41 | >300 | >100 | 110 | >300 | >100 | 226, 227, 276 |
| 1-amino-APDC (**94**) | >1000 | 4.8 | 52 | >1000 | >1000 | >1000 | | | 229 |
| 1-benzyl-APDC (**95**) | >1000 | **200** | | | **600** | 20 | | | 227 |
| (3*S*,4*S*)-ACPT-III (**96**) | >1000 | >1000 | | 8.8 | | | | | 230 |
| (1*S*,3*R*)-homo-ACPD (**97**) | >1000 | 122[pa] | | >1000 | | | | | 231 |
| ABHxD-I (**99**) | 1.6 | 0.33 | 2.2 | 23 | 0.72 | 5.3 | | | 235 |
| ABHD-II (**101**) | weak pa | weak agonist | | | | | | | 240 |
| LY354740 (**98**) | >100 | 0.011 | 0.038 | >100 | >100 | 3.0 | >100 | 12 | 234 |
| LY379268 (**102**) | >100 | 0.0027 | 0.0046 | 21 | >100 | 0.40 | >100 | 1.7 | 234 |
| LY389795 (**103**) | >100 | 0.0039 | 0.0076 | >100 | >100 | 2.4 | >100 | 7.3 | 234 |
| **100** | >1000 | >1000 | | >1000 | >1000 | >1000 | | | 239 |
| (2*S*,3*S*,4*S*)-CCG (**127**) | 1.9−50 | 0.30−0.49 | 0.44 | 3.7−50 | 3.1 | 5.8 | 47−230 | 0.35−3.4 | 241, 242, 276 |
| (*S*)-2-aminoadipic acid (**105**) | >1000 | 35 | | >3000 | >1000 | 140 | | | 19, 245 |
| (*S*)-homo-AMPA (**18**) | >1000 | >1000 | >1000 | >1000 | >1000 | 58 | >5000 | | 20 |
| ibotenic acid (**12**) | 43 | 110 | | >1000 | 17 | | | | 245 |
| quisqualic acid (**14**) | 1.1 | >1000 | 40 | ∼1000 | 0.055 | ∼1000 | | | 25, 220, 222 |
| (*S*)-homoquisqualic acid (**21**) | **184** | 23 | | >1000 | 36 | | | | 25 |
| (*S*)-AP4 (**106**) | >1000 | >1000 | >1000 | 0.32−0.91 | >1000 | 0.90 | 175 | 0.061 | 25, 220, 222, 276 |
| (*S*)-AP5 (**107**) | >1000 | **205** | | ∼1000 | >1000 | | | | 25 |
| (*RS*)-PPG (**113**) | >500 | >300 | >200 | 5.2 | >500 | 4.7 | 185 | 0.21 | 266 |
| (*S*)-HPG (**110**) | 68−1000 | >1000 | | >1000 | 14 | | | | 253−255 |
| (*S*)-DHPG (**111**) | 6.6 | >1000 | | >1000 | 1.9 | | >1000 | >1000 | 255, 270, 276 |
| (*S*)-CHPG (**112**) | 750 | | | | >10000 | | | | 256 |
| (*S*)-Me-Glu (**132**) | 10 | 2.0 | | 470 | | | | | 170 |
| **2** | 0.15 | 0.23 | | >1000 | | | | | 170 |
| HYDIA (**104**) | >**100** | **0.11** | **0.10** | **22** | >**100** | | | 15 | 243 |
| (*S*)-HIBO (**108**) | **250** | >**1000** | | >**1000** | **490** | | | | 245 |
| (*S*)-Bu-HIBO (**109**) | **110** | >**1000** | | >**1000** | **97** | | | | 245, 247 |
| (*S*)-4CPG (**116**) | **18−84** | 500−577 | | >**1000** | >**2000** | | | | 245, 253−255 |
| (*S*)-4C3HPG (**117**) | **30** | 20 | | >**1000** | | | | | 253 |
| (*S*)-M4CPG (**120**) | **70−540** | **51**, 340 | | >**1000** | >**2000** | | | | 253−255 |
| (+)-4C2MPG (**118**) | **8.8** | | | | >**100** | | | | 258 |
| (*RS*)-4C3H2MPG (**119**) | **6.0** | | | | >**100** | | | | 258 |
| (*RS*)-AIDA (**123**) | **214** | >**1000** | | >**1000** | >**1000** | | | | 259, 260 |
| (*S*)-CBPG (**124**) | **25** | >**300** | | >**300** | 103[pa] | | | | 264 |
| (*S*)-ACUDA (**125**) | **232** | 55[pa] | | >**1000** | >**1000** | | | | 265 |
| (*RS*)-MPPG (**114**) | >**1000** | **11** | | | >**1000** | **480** | | | 268 |
| (*RS*)-APICA (**115**) | >**1000** | **30** | | | >**1000** | >**1000** | | | 268 |
| MCCGI (**128**) | | **84** | | >**1000** | | | | | 270 |
| MAP4 (**126**) | | **447** | | **88** | | | | | 270 |
| (*S*)-MetQUIS (**131**) | ∼**500** | **40** | | >**1000** | ∼**1000** | >**1000** | | | 272 |
| **133** | >**300** | **18** | **6.1** | >**300** | >**300** | | >**300** | >**300** | 274 |
| **134** | >**300** | **50** | **30** | >**300** | >**300** | | >**300** | >**300** | 274 |
| LY341495 (**129**) | **6.8** | **0.021** | **0.014** | **22** | **8.2** | | **0.99** | **0.17** | 277 |
| XE-CCG-I (**130**) | | **0.075** | **0.20** | | | | | | 278 |
| LY393675 (**122**) | **0.35** | | | | **0.47** | | | | 279 |
| LY367366 (**121**) | **5.7** | | | | **3.4** | | | | 262 |
| CPCCOEt (**135**) | **9.9** | >**100** | | >**100** | >**100** | | >**100** | >**100** | 206 |
| **136** | >**100** | **1** | | >**100** | | | | | 283 |
| Ro 64-5229 (**137**) | >**100** | **0.11** | >**100** | >**100** | >**100** | | | | 284, 285 |
| SIB-1757 (**138**) | >**100** | >**100** | >**100** | >**100** | **0.37** | >**100** | >**100** | >**100** | 286 |
| SIB-1893 (**139**) | >**100** | >**100** | >**100** | 26 | **0.29** | >**100** | >**100** | >**100** | 286 |
| MPEP (**140**) | >**30** | >**100** | >**100** | >**100** | **0.036** | >**10** | >**100** | >**100** | 287 |

[a] Plain and bold text refer to agonist and antagonist potencies in $\mu$M, respectively. pa = partial agonist.

late [(2*R*,4*R*)-APDC, **93**], which displayed an increased potency for the group II receptors as compared to the parent compound while loosing affinity for the group I and III receptors.[225−227] N1-Substitutions of **93** have been reported to cause significantly reduced potency at the group II receptors.[227−229] However, interestingly 1-amino-APDC (**94**) acts as a partial group II agonist[229] and 1-benzyl-APDC (**95**) shifts selectivity from mGluR2 and mGluR3 toward mGluR6.[227] Similarly, an addition of a carboxylic acid moiety at the corresponding C4-position of **92** afforded (3*S*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid [(3*S*,4*S*)-ACPT-III, **96**], which displayed agonist potency at mGluR4 comparable to Glu and weak antagonist effects at mGluR1 and mGluR2.[230]

In 1997, (1*S*,3*R*)-homo-ACPD (**97**), the homologue of **92**, was shown to have increased selectivity for

**Chart 12.** Structures of Some Amino Acids Showing Agonist Effects at Metabotropic Glu Receptors (mGluRs)




mGluR2 compared to mGluR1 and mGluR4, albeit with lower potency and efficacy than the parent compound.[231] At the same time, a dramatic 10 000-fold increase in potency for the group II receptors was reported by further restraining the conformation of **97** by introduction of a single bond between the side chain and the 5-position in the cyclopropane ring, affording (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740, **98**).[232−234] Compound **98** displays low nanomolar agonist potency for mGluR2 and mGluR3, low micromolar agonist potency for mGluR6 and mGluR8, while showing no activity for the remaining mGlu receptors.

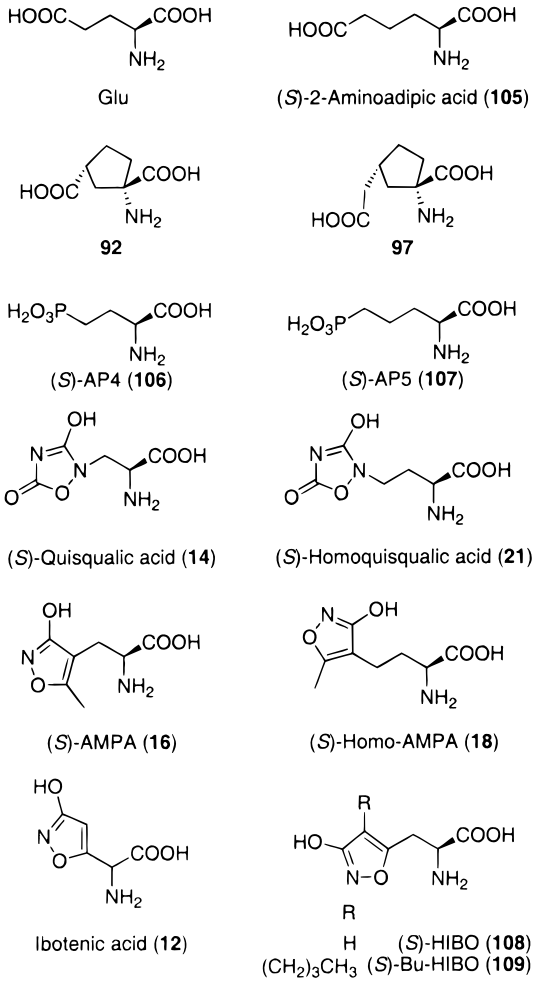
Recently, the synthesis and pharmacology of (1*S*,2*S*,4*S*,5*S*)-2-aminobicyclo[2.1.1]hexane-2,5-dicarboxylic acid (ABHxD-I, **99**) was reported by Kozikowski et al.[235] Interestingly, this compound displayed agonist potency comparable to Glu on mGluR1 to mGluR6, thus the compound is less selective than the parent compound **92**. In the context of molecular modeling, this observation has proven to be of vital importance. Compound **99** is a quite rigid molecule which adopts a conformation corresponding to the extended (anti-anti, aa) conformation of Glu. The observation that the compound is a potent agonist on all three mGluR groups led the authors to suggest that Glu adopts the same extended conformation on all three receptor groups, and that group selectivity is thus not a consequence of different conformations but rather a consequence of other factors such as steric hindrance.[235] This hypothesis has later been confirmed by several more extensive pharmacophore models.[236−238] One example in this context is the comparison of the highly selective group II agonist **98** with the nonselective agonist **99**. Both compounds prefer conformations corresponding to the extended aa conformation of Glu.[227,235] However, overlay of the compounds with Glu (all in the aa conformation) shows that **98** does occupy an extra volume compared to **99**.[235] Accordingly, it has been suggested that the group II receptors can accept this extra volume whereas group I and III receptors cannot.[235] The unique pharmacological profile of **98** is further stressed by other close analogues such as 2-aminobicyclo[3.2.0]heptane-2,5-dicarboxylic acid (**100**) and ABHD-II (**101**), which are inactive and weak group I/II agonists, respectively.[239,240]

The 2-(carboxycyclopropyl)glycines (CCGs, **5**) are also conformationally restricted analogues of Glu, albeit with more flexibility than the bicyclic compounds described above. In agreement with the pharmacophores, it is also the isomers [in particular, (2*S*,3*S*,4*S*)-CCG-I (**127**)] with the extended conformation that are agonists at the mGluRs.[241] Also in agreement with the findings from the pharmacophores showing that it is the fully extended conformation of Glu that recognizes mGluR1, mGluR2, and mGluR4, **127** (Chart 15) has been shown to display potent agonist activity on members from all three mGluR groups, albeit with some preference for the group II receptors.[241,242]

The high potency and group II selectivity of **98** has inspired the synthesis of analogues thereof. The heterobicyclic analogues (−)-2-oxa-4-aminobicyclo-[3.1.0]hexane-4,6-dicarboxylate (LY379268, **102**) and (−)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY389795, **103**) are 3- to 8-fold more potent at mGluR2 and mGluR3 than the parent compound **98**. However, the activities at mGluR6 and mGluR8 are also increased by a similar factor.[234] Thus, the overall selectivity of these analogues does not increase compared to the parent compound. Very recently, the 3-hydroxy analogue of **98**, (1*S*,2*R*,3*R*,5*R*,6*S*)-3-hydroxy-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (HYDIA, **104**), was reported.[243] It is very interesting to note that such a modest structural change converts the compound from an agonist into a potent group II antagonist.

**Extension of Backbone Chain Length.** On the basis of the observation that (*S*)-2-aminoadipic acid (**105**) (Chart 13), the homologue of Glu, displays increased mGluR selectivity compared to Glu, the effects of this backbone extension have been studied intensively. Compound **105** selectively activates mGluR2 and mGluR6, whereas it has no effect on mGluR1, mGluR4, or mGluR5.[19,20,244,245] In the same reports it was discovered that (*S*)-2-amino-4-(3-hydroxy-5-methyl-4-isoxazolyl)butyric acid [(*S*)-Homo-AMPA, **18**] was a specific agonist at mGluR6 with no activity at mGluR1−5 or mGluR7.[19,20] Since mGluR4, mGluR6, and mGluR7 all belong to group III, it is highly surprising that **105** and **18** only activated mGluR6 within this group. It is also interesting to note the dramatic pharmacological difference between (*S*)-AMPA (**16**) and the homologue **18** on the AMPA receptors. As described previously, the former is a highly selective agonist on the AMPA receptor whereas the latter is completely inactive at this receptor.[20] As well as their previously described effects

**Chart 13.** Structures of Some Glu Analogues Showing Effects at Ionotropic Glu Receptors (iGluRs) and/or Metabotropic Glu Receptors (mGluRs) (left column) and the Corresponding Homologues Interacting Preferentially with mGluRs (right column)




as AMPA receptor agonists, ibotenic acid (**12**) and quisqualic acid (**14**) were, in the mid 1980s, also shown to stimulate inositol phosphate accumulation, indicative of metabotropic effects.[246] The cloning of the mGluRs confirmed these results by showing that both compounds activate the group I receptors.[193,194,221] Whereas **14** is more potent than Glu on the group I mGluRs, **12** is less potent. Furthermore, the latter compound also shows activity at the group II receptors.[245] Recently, the pharmacological effects of the homologues of **12** and **14** were reported. Interestingly, (*S*)-homoibotenic acid [(*S*)-HIBO, **108**] was shown to be a selective (albeit weak) antagonist at mGluR1 and mGluR5.[245] Introduction of lipophilic/bulky side chains such as a butyl group in the 4-position, to give (*S*)-Bu-HIBO (**109**), increases the activity of the compounds as group I antagonists. However, like the parent compound **12**, compound **108** and the analogues also activate AMPA receptors.[245,247] (*S*)-Homoquisqualic acid (**21**) also produced effects quite different from the parent compound **14**. On mGluR5, **21** was also an agonist albeit with 500-fold lower activity than **14**, whereas the homologue surprisingly was an antagonist at mGluR1.[25] Furthermore, **21**, in contrast to the parent compound, was an agonist at mGluR2.[25]

(*S*)-2-Amino-4-phosphonobutyric acid [(*S*)-AP4, **106**] is also a classical mGluR agonist, which in the early 1980s was shown to reduce evoked release of Glu via a presynaptic mechanism.[248] The cloning of the group III receptors has shown that **106** is an agonist at these receptors, some 10-fold more potent than Glu, and that many of the effects of the ligand in the CNS can be ascribed to activation of group III receptors.[220,222,223,249] Again, the homologue, (*S*)-2-amino-5-phosphonopentanoic acid [(*S*)-AP5, **107**], showed a rather different pharmacological profile than the parent compound. Compound **107** also activates group III receptors, but with a markedly lower potency;[25,250] but in contrast to the parent compound, **107** also displays antagonist activity at mGluR2.[25,251] It should be kept in mind that (*R*)-AP5 (**29**) (Chart 3), which does not interact detectively with mGluRs, is a potent and selective competitive NMDA antagonist.

Taken together, the results from the homologous compounds clearly demonstrate that the distance between the glycine moiety and the distal acidic group is of vital importance for the pharmacological activity. As pointed out, the selectivity profile of the homologues are often quite different from the parent compound. Furthermore, in several cases agonists have been converted into antagonists when the backbone chain length is increased. Although the effects are often unpredictable, the results show that chain length is a parameter of utmost importance when analogues of newly identified mGluR leads are being designed.

**Bioisosteres.** We have already described a number of compounds in which the distal carboxylic acid of Glu has been replaced by an acidic bioisosteric group. Compounds such as **14** (bioisosteric group: 1,2,4-oxadiazol-3,5-dione), **12**, and **18** (bioisosteric group: 3-hydroxyisoxazole) and **106** (bioisosteric group: phosphonic acid) show that all three receptor groups are capable of accepting bioisosteric groups as a replacement for the distal carboxyl group. The increased receptor selectivity of these compounds compared to Glu also demonstrates that this region of the receptor, which interacts with the distal carboxyl group, differs among the receptor subtypes. Thus further research into new bioisosteres may be a fruitful path to new subtype-selective mGluR ligands. This argument is further substantiated by group I-selective agonists based on phenol as the distal acidic group. Thus, both (*S*)-3-hydroxyphenylglycine [(*S*)-HPG, **110**] and (*S*)-3,5-dihydroxyphenylglycine [(*S*)-3,5-DHPG, **111**] (Chart 14) are selective agonists at mGluR1 and mGluR5,[252−255] whereas (*RS*)-2-chloro-5-hydroxyphenylglycine [(*RS*)-CHPG, **112**] selectively activates mGluR5 with no activity at mGluR1.[256] Compound **111** is equipotent with Glu on both group I subtypes, whereas both **110** and **112** are significantly less potent than the endogenous agonist.

## Molecular Structure of Competitive Metabotropic Glutamate Receptor Antagonists

**Phenylglycines.** One of the first potent mGluR antagonists to be reported was (*S*)-4-carboxyphenylglycine [(*S*)-4CPG, **116**] which antagonized **92** induced IP formation in cerebral cortical slices.[257] In agreement with these results, **116** has been shown to antagonize IP formation by mGluR1, and furthermore the compound has been shown to be an agonist[253] or antago-

**Chart 14.** Structures of Some Amino Acids Showing Competitive Antagonist Effects at Metabotropic Glu Receptors

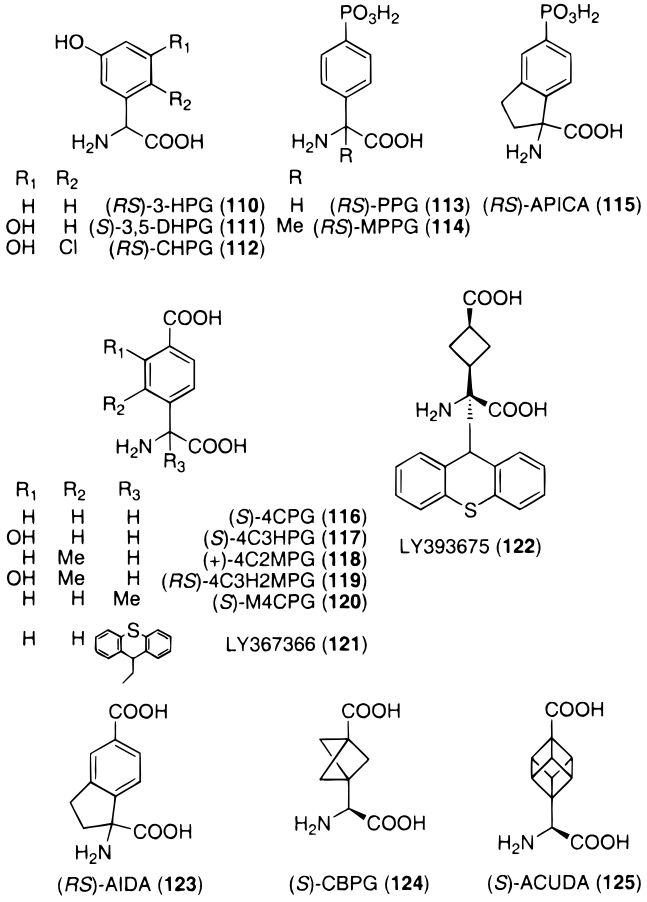
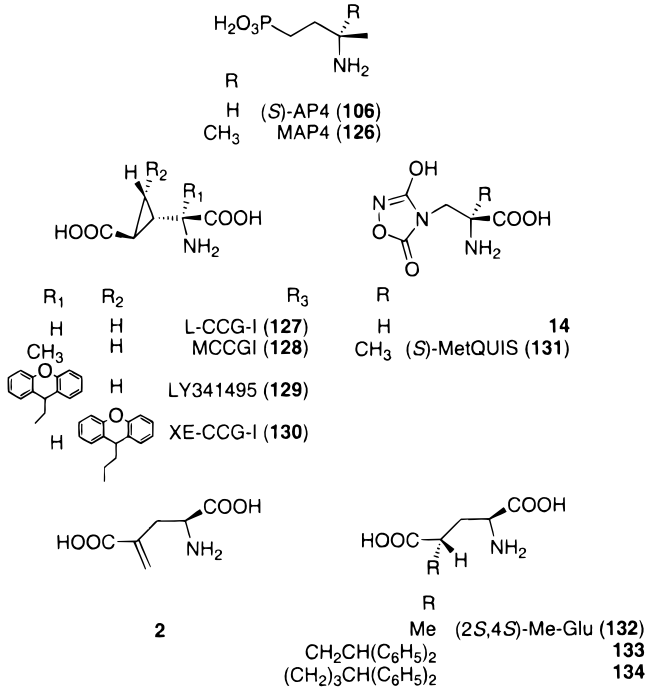



**Chart 15.** Structures of Some Amino Acids Showing Agonist or Competitive Antagonist Effects at Metabotropic Glu Receptors




nist[254] on mGluR2 with no effect on mGluR4.[253,254] Compound **116** has been used intensively as a template for design of more potent and selective antagonists at mGluR1. Whereas it has been debated whether **116** is an agonist or antagonist at mGluR2,[253,254] it is generally agreed that (*S*)-4-carboxy-3-hydroxyphenylglycine [(*S*)-4C3HPG, **117**] is an mGluR1 antagonist/mGluR2 agonist and that the α-methylated analogue, (*RS*)-α-methyl-4-carboxyphenylglycine [(*RS*)-M4CPG, **120**], is an antagonist at both subtypes.[253,254] More recently, it has been shown that the antagonist potency is increased by methylation of the 2-position of the phenyl ring. Thus, (+)-4-carboxy-2-methylphenylglycine [(+)-4C2MPG, **118**] and (*RS*)-4-carboxy-3-hydroxy-2-methylphenylglycine [(*RS*)-4C3H2MPG, **119**] are both approximately 5-fold more potent than the nonmethylated parent compounds.[258] (*RS*)-1-Aminoindan-1,4-dicarboxylic acid [(*RS*)-AIDA, **123**], a compound which can be viewed as a cyclized analogue of both **120** and **118**, has also been shown to possess mGluR1 antagonist activity, albeit with lower potency than the noncyclic compounds.[259,260] The antagonist potency of the 4-carboxyphenylglycines discussed above shows mGluR1 antagonist potencies in the 5−200 $\mu$M range. It is interesting to note that the potencies are somewhat affected by the agonist used to determine the functional potency.[255] Furthermore, it is notable that the 4-carboxyphenylglycines discussed above show selectivity for the mGluR1 subtype with no or weak activities at the closely related mGluR5 subtype.[245,255,260,262] One exception from this rule is the α-thioxanthylmethyl analogue LY367366 (**121**), which has been shown to be equipotent at mGluR1 and mGluR5 with low micromolar potency (Table 3).[262]

On the basis of modeling studies it has been suggested that the antagonist action of the 4-carboxyphenylglycines is not caused by the phenyl ring in this series of compounds, but rather by the coplanar arrangement of the glycine moiety and the distal acidic group due to the flat nonflexible phenylring.[263] To test this hypothesis, Pellicciari and co-workers synthesized (*S*)-2-(3′-carboxy[1.1.1]bicyclopentyl)glycine [(*S*)-CBPG, **124**] and (*S*)-2-(4′-carboxycubyl)glycine [(*S*)-ACUDA, **125**].[264,265] Both of these compounds retain the coplanar arrangement of the glycine moiety and the distal acidic group, and in agreement with their hypothesis both compounds have been shown to be moderately potent mGluR1 antagonists with no or weak activity at mGluR2, mGluR4, and mGluR5.[264,265]

The phosphonic acid bioisostere of **116**, [(*RS*)-PPG, **113**], is a potent group III agonist.[266] On the basis of the results from the 4-carboxyphenylglycines, the phosphonic acid bioisosteres of M4CPG (**120**), MPPG (**114**),[267] and **123**, (*RS*)-APICA (**115**)[268] has been designed. In analogy with the results showing that α-methylation of **116** converts the compound from being an agonist to an antagonist at mGluR2,[253] **114** is an antagonist on group III mGluRs.[268−270] Compound **114** is slightly more potent as an antagonist on mGluR2, which is rather surprising given that neither **106** nor **113** has any appreciable effect at this receptor subtype.[268−270] Compound **115** showed a pharmacological profile quite similar to **114**, being slightly less potent as an mGluR2 antagonist than the parent compound.[268]

**α-Substitutions.** As eluted to above, α-methylation has become a widely used tool in the design of antagonists derived from agonists. Some of these analogues are illustrated in Charts 14 and 15. In agreement with

their agonist selectivity profile, MCCGI (**128**) and 2-amino-2-methyl-4-phosphonobutanoic acid (MAP4, **126**) antagonize mGluR2 and mGluR4, respectively, albeit with significantly reduced antagonist potency compared to the parent agonist compounds.[270,271] We previously explained how α-methylation of the potent and selective group III agonist **113** resulted in the mixed group II/III antagonist **114**.[268−270] Another example of the "group switching" and loss of potency obtained by α-methylation is (*S*)-α-methylquisqualic acid [(*S*)-MetQUIS, **131**], which is a moderately potent antagonist at mGluR2 and a very weak antagonist at mGluR1 and mGluR5.[272] Taken together, the results from α-methylation show that, albeit some important compounds have been obtained, it does not appear to be a viable strategy to design potent antagonists.

**"Fly-Swatter" Substitutions.** As another approach, potent antagonists have been obtained from agonists by substitution with bulky and lipophilic side chains. As with several other receptor and transport systems, this strategy has proven very successful. Some of the pioneering compounds in this respect are 4-substituted analogues of Glu such as (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)pentane-1,5-dioic acid (**134**) and (2*S*,4*S*)-2-amino-4-(2,2-diphenylethyl)pentane-1,5-dioic acid (**133**) (Chart 15), described by the Eli Lilly group. Both compounds are selective antagonists for mGluR2 and mGluR3 with potencies in the low micromolar range.[273,274] Interestingly, small substituents in the same position, such as methyl, [(2*S*,4*S*)-Me-Glu, **132**], or methylene, (*S*)-4-methylene-Glu (**2**), are more potent agonists at mGluR2 than Glu, with some activity at mGluR1 but with no appreciable activity at mGluR4.[170] Thus, by increasing the bulk and lipophilicity at the 4-position to these "fly-swatter" substituents, the selectivity for group II is retained (and even increased) but the compounds are converted from agonists to antagonists. Furthermore, in contrast to the results from α-methylation, the potency of the antagonists was retained as compared to the parent agonist compound.

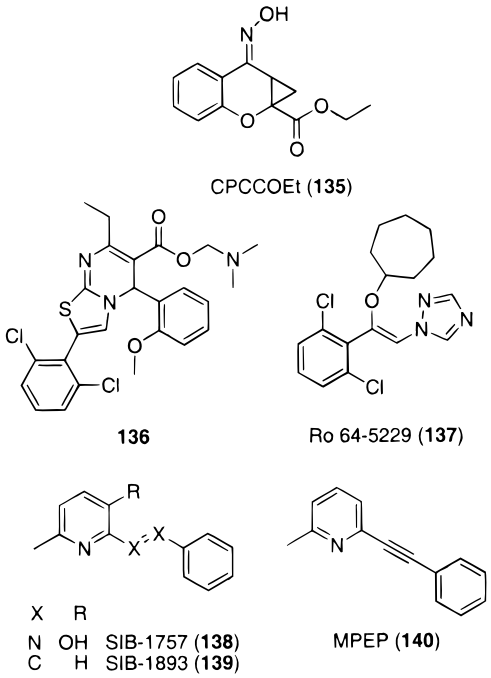
These results have inspired the design of a number of new potent antagonists with "fly-swatter" side chains. Compound **127** was first substituted with "fly- swatters" in the 2-position by the Eli Lilly group. It was found that the most potent compound was obtained with xanthylmethyl as the substituent, LY341495 (**129**),[275,276] which displays antagonist activity at the group II receptors in the low nanomolar range. However, like the parent compound **127**, **129** also shows affinity for other subtypes, especially mGluR8.[277] The group of Pellicciari recently reported the synthesis and pharmacology of **127** analogues with "fly-swatter" substituents in the 3′-position. In this series, the compound with the xanthylethyl substituent, (2*S*,1′*S*,2′*S*,3′*R*)-2-[2′-carboxy-3′-(9*H*-xanthen-9-ylethyl)cyclopropyl]glycine (XE-CCG-I, **130**), was found to be the most potent antagonist with potencies at mGluR2 and mGluR3 comparable to **129**.[278] Finally, the "fly-swatter" principle has also been successfully applied to 3-carboxycyclobutylglycine, providing, for example, LY393675 (**122**),[279,280] and to 4CPG (**116**) to give LY367366 (**121**).[262] Compounds **121** and **122** show submicromolar and low micromolar potency, respectively, on both group I subtypes, which for the latter is quite remarkable given that most other phenylglycine analogues have shown preference for the mGluR1 subtype.[245,255,260−262]

It can be concluded that receptors from all three mGluR groups can accommodate quite large and lipophilic side chains in a variety of positions. In general, the large "fly-swatter" side chain substituents confer potent antagonist properties, thus providing a more attractive strategy for preparing potent subtype specific antagonists than simple α-methyl substitution.

## Molecular Structure of Noncompetitive Metabotropic Glutamate Receptor Antagonists

In 1996, Annoura et al. reported CPCCOEt (**135**) (Chart 16), a non-amino acid compound with no structural similarity with Glu, as a group I-selective antagonist.[281] Since then, the compound has been shown to be a selective mGluR1 noncompetitive antagonist with no mGluR5 activity, acting at the 7TM region rather than the agonist-binding ATD.[206,207,282] Recently, a number of other non-amino acid mGluR antagonists have been discovered. Thus, 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives, such as **136**, selectively antagonize mGluR2 in the low micromolar range with no affinity for mGluR1 or mGluR4, but with weak activity at the NMDA and AMPA receptors.[283] It has yet to be determined whether this class of ligands is selective within group II and whether the antagonists act in a competitive or noncompetitive manner. The enol ether Ro 64-5229 (**137**) show submicromolar antagonist potency for mGluR2 with no activity at mGluR1, mGluR3, mGluR4, mGluR5, NMDA, or AMPA receptors.[284,285] Thus, like **135**, this compound discriminates between receptors in a group, and also like **135**, compound **137** acts in a noncompetitive manner.[284,285] Recently, 6-methyl-2-(phenylazo)-3-pyridinol (SIB-1757, **138**), (*E*)-2-methyl-6-(2-phenylethenyl)pyridine (SIB-1893, **139**), and 2-methyl-6-(phenylethynyl)pyridine (MPEP, **140**) have been reported.[286,287]

**Chart 16.** Structures of Some Compounds Showing Noncompetitive Antagonist Effects at Metabotropic Glu Receptors



All three compounds are selective noncompetitive antagonists with nanomolar potency at mGluR5, **140** being the most potent compound, with no activity at the remaining seven mGluRs or the iGluRs.[286,287] Like **135**, the compound has been shown to act on the 7TM region rather than the agonist-binding ATD.[208]

Most of the noncompetitive antagonists described in the preceding paragraph have been discovered by high throughput screening of compound libraries. It is interesting to note the diverse structures that have come out of these screens and that the compounds are highly selective even within groups I and II mGluRs. As has been demonstrated for **135** and **140**, most if not all of the noncompetitive antagonists act outside the agonist-binding pocket in the ATD, which can very well be the explanation for the high degree of subtype selectivity. The overall sequence identity between mGluR1 and mGluR5 and between mGluR2 and mGluR3 is 61% and 70%, respectively.[219,221] However, the amino acids in the mGluRs aligning with the LIVBP ATD-binding pocket are even more conserved. Thus the sequence identity between mGluR1 & mGluR5 and mGluR2 & mGluR3 in the suggested agonist-binding pockets are 88% and 82%, respectively.[51] Given that the binding of the endogenous agonist Glu is of immense importance for prober receptor function, it is not surprising that this part has been conserved to a higher degree during evolution than the remaining part of the receptor. Likewise, it is not surprising that ligands acting outside the agonist-binding pocket show a higher degree of receptor selectivity than ligands with structures similar to Glu acting in the pocket. With this in mind, high throughput screening using functional assay systems seems like an attractive strategy for discovery of new subtype specific ligands, in addition to structure-based ligand design.

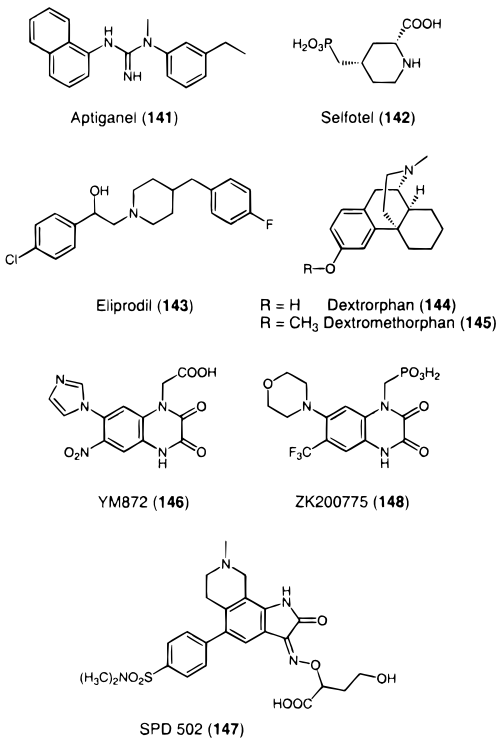
## Therapeutic Prospects

It is well known that Glu can act as a neurotoxin, especially when energy supply is compromised.[288] This has given rise to the proposal that injury to neurons in many neurologic disorders may be caused, at least in part, by overstimulation of Glu receptors, which, at least to some extent, may involve reversal of Glu transporters. These neurologic conditions range from acute insults such as stroke, trauma, and epilepsy to chronic neurodegenerative states such as Huntington's disease, Parkinson's disease, AIDS, dementia, and amyotrophic lateral sclerosis. There is also growing support for the proposal that chronic pain can be relieved by Glu antagonists. Finally, there are suggestions that slowing AMPA receptor desensitization may have a cognitive enhancing effect.

**Stroke and Brain Injury.** Stroke was the first clinical indication considered for Glu receptor antagonists, because the evidence for an etiological role for excitotoxic mechanisms is most convincing and, in consequence, the hopes for a therapy have been the highest.[289,290]

Hundreds of studies have been performed with EAA receptor antagonists in experimentally produced ischemic stroke in laboratory animals, and the majority of these show that the extent of the lesions can be attenuated by such agents. An extensive amount of data has been generated over more than 10 years, showing quite clearly that NMDA receptor antagonists of all classes demonstrate robust neuroprotection in animal models of focal ischemia. This has been demonstrated using a number of different experimental paradigms in a variety of species. However, in general, the therapeutic time window of effectiveness for NMDA receptor antagonists in animal models of focal ischemia is narrow.[291] As far as studies in global ischemia models is concerned, the situation is less clear. It seems that when body temperature is controlled, the neuroprotective effect of MK-801 (**35**) (Chart 4) is diminished, but in a gerbil model of global ischemia, MK-801 has been shown to be a neuroprotectant independent of its hypothermic effects.[292−294] However, the criticisms that have been aimed at channel blockers do not seem to hold for glycine site antagonists, where clear neuroprotection is seen in global ischemia models. In focal ischemia models, the therapeutic time window is significantly longer than for MK-801 (**35**).[295] Similarly, ifenprodil (**41**) (Chart 4) and eliprodil (**143**) (Chart 17) show similar neuroprotective properties in both focal and global ischemia models.[296,297]

**Chart 17.** Structures of Some Ionotropic Glu Receptor Antagonists of Clinical Interest




A number of NMDA receptor antagonists have been subjected to early clinical trials for stroke, over the last 10−12 years. These include the noncompetitive channel blocker aptiganel (**141**), the competitive Glu antagonist selfotel (**142**) (Chart 17), the competitive glycine site antagonist ACEA-1021 (**51**) (Chart 6), and the ifenprodil analogue eliprodil (**143**). The results of these studies have been disappointing. Although blockade of Glu receptors protects against excitotoxicity, it can also have serious unwanted effects, such as induction of psychotomimetic effects, respiratory depression, or cardiovascular dysregulation. The selfotel (**142**) trial was termi-

nated early due to lack of efficacy at tolerable doses,[298] as was the eliprodil (**143**) trial,[299] although eliprodil (**143**) as well as CP-101,606 (**42**) (Chart 4) have shown significantly improved side effect profiles relative to the nonsubtype-selective NMDA antagonists which preceded them.[76] These studies did, however, reveal some CNS-related side effects such as agitation, hallucinations, confusion, and dizziness, indicating that only compounds with robust effects without adverse side effects may be effective. Clinical studies of MK-801 (**35**) and dextrorphan (**144**) (Chart 17) were stopped for safety reasons before efficacy studies were even initiated.

Glycine antagonists may be associated with fewer side effects, but preclinical studies suggest that brain penetration may be low. However, glycine site antagonists with improved brain penetration are under development.[94,300] Clinical trials with ACEA-1021 (**51**) (Chart 6) have been complicated by problems of renal toxicity.

Treatment with the competitive AMPA/KA receptor antagonist NBQX (**70**) and YM90K (**71**) (Chart 8) have been demonstrated to reduce infarct size in rodent models of global and focal ischemia.[131,301,302] Similarly, the noncompetitive AMPA receptor antagonist GYKI 52466 (**81**) (Chart 9) is active in both types of ischemia models.[303,304] Unlike treatment with NMDA receptor antagonists, however, treatment with AMPA/KA antagonists is efficacious, even when administered several hours after the ischemic insult.[131,305,306] In terms of safety, AMPA/KA receptor antagonists, so far, appear to be relatively well tolerated and devoid of the psychostimulant effects observed with NMDA receptor antagonists.[307]

Early stroke trials with the AMPA antagonist NBQX (**70**) were discontinued due to insolubility of the drug, which precipitated in kidneys and, thus, caused necrosis. Second-generation AMPA receptor antagonists that are much more water soluble are under development in several pharmaceutical companies. For example, the novel water soluble AMPA antagonists, YM872 (**146**) and SPD 502 (**147**) (Chart 17), are neuroprotective in animal models of focal and global ischemia.[308−310] Introduction of a phosphonomethyl group into the quinoxalinedione skeleton results in a water soluble AMPA receptor antagonist, ZK200775 (**148**), which exhibits a remarkably long therapeutic time window of >4 h for neuroprotection following permanent occlusion of the middle cerebral artery (MCA) in rats.[311] The crucial clinical trials for stroke in man with AMPA/KA antagonists are ongoing, and it is still unknown whether the window of opportunity, the degree of neuroprotection, and side effect profile will allow efficacy to be convincingly demonstrated with these compounds.

Little is known about the role of mGluRs in ischemia. As mentioned earlier, group I mGluRs often mediate excitation or increase excitability of neurons while group II/III mGluRs generally mediate depression of synaptic transmission. On the supposition that reduction of excitation or excitability is a useful therapeutic strategy for treatment of neurodegeneration following ischemia, antagonists for group I mGluRs and/or agonists for group II/III mGluRs might be neuroprotective.[312] A recent report postulates a key role for mGluR1 receptors in the pathological mechanisms responsible for the postischemic neuronal cell death. Indeed, the selective mGluR1 antagonists AIDA (**123**) and (*S*)-CBPG (**124**) (Chart 14) significantly reduce neuronal cell death in vivo after global ischemia.[313]

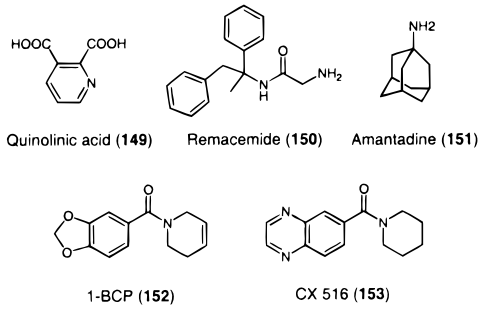
Furthermore, there is growing evidence that activation of group II and group III mGluRs results in neuroprotection. Neuroprotective effects of group II agonists have been seen in vivo, although with some discrepancies. Thus, LY354740 (**98**) (Chart 12) significantly reduced hippocampal damage after global ischemia in gerbils,[314] whereas the compound was without neuroprotective effect in the MCA model of focal ischemia in rats.[315] Similar data have been obtained with LY379268 (**102**) (Chart 12), a more potent derivative of LY354740 (**98**), in models of global and focal ischemia, suggesting that group II mGluR agonists may have more utility in global than in focal cerebral ischemia.[316]

The primary lesions in traumatic head and spinal injury are physical; however, it is possible that Glu may contribute to the appearance of delayed secondary symptoms. Several of the NMDA antagonists that have been evaluated in the clinic in stroke have also been tested in traumatic brain injury. Although aptiganel (**141**) (Chart 17) was reported to be well tolerated in traumatic brain injury patients, the clinical trial has been suspended due to lack of effect and to safety concerns.[317] Similarly, the development of selfotel (**142**) and (*R*)-CCPene (**31**) (Chart 3) have been discontinued. Although the clinical trial results to date are not encouraging, traumatic brain injury remains a significant therapeutic target for Glu receptor antagonists.

**Huntington's Disease.** Huntington's disease (HD) is a fatal hereditary progressive neurodegenerative disease, with onset in middle-age, and is characterized by dyskinesias with associated cognitive and psychiatric impairment. Histopathologically, atrophy of the caudate putamen is observed.

Intrastriatal lesions with the NMDA agonist quinolinic acid (**149**) (Chart 18) mimic some of the neuro-

**Chart 18.** Structures of Some Ionotropic Glu Receptor Ligands or Modulatory Agents of Interest as Experimental Tools or Therapeutic Agents




chemical and neuropathological characteristics in the brain of patients with HD; this has led to the hypothesis that an endogenous excitotoxin, possibly **149**, may be involved in this disease.[318,319] Unfortunately, it is not realistic to expect that NMDA antagonists should find use in the symptomatological therapy of Huntington's disease since no symptomatological improvement has been obtained in clinical trials with remacemide (**150**) (Chart 18) or ketamine (**37**) (Chart 4) in HD pa-

tients.[320,321] However, the favorable side effect profile seen for remacemide (**150**) and ketamine (**37**) are encouraging for the use of these agents as neuroprotective agents in delaying disease progression. A long term study is now underway to evaluate potential effects of **150** on disease progression.

An alternative mechanism of neuroprotection may be obtained by inhibition of Glu release via presynaptic group III mGluRs. Recently, a potent and selective group III mGluR agonist, PPG (**113**) (Chart 14), has been demonstrated to have neuroprotective effect in NMDA and quinolinic acid induced striatal lesions in rats after intrastriatal infusion.[266] These data provide novel in vivo evidence for group III mGluRs as attractive targets for neuroprotective therapy and encourage the search for systemically active group III mGluR agonists as promising drugs for the treatment of neurological disorders, such as HD.

**Parkinson's Disease.** Parkinson's disease (PD), one of the most common neurodegenerative disorders, is characterized by a massive and selective loss of dopaminergic neurons in the nigrostriatal pathway. This causes imbalance in the motor circuits in the basal ganglia, which leads to a net increase in the inhibitory output from the basal ganglia to the thalamus. There are several lines of preclinical evidence that neurodegeneration of dopaminergic pathways of the substantia nigra pars compacta in PD involves excitotoxicity.[322] Glu antagonists might intervene at two different levels: as neuroprotective agents, by counteracting the neurotoxic effects of Glu, and in the symptomatic treatment of the disease, by correcting the changes in basal ganglia glutamatergic neurotransmission. So far, no Glu receptor antagonist has been shown to influence the progression of PD. However, it is now widely accepted that NMDA receptor antagonists manifest their antiparkinson effects by attenuating the imbalance between dopaminergic and glutamatergic pathways within the basal ganglia network.[323] Of the NMDA receptor antagonists, only NMDA channel blockers seem to show convincing efficacy. However, few clinical studies have been undertaken, principally because there is a shortage of Glu antagonists which are considered safe for human use, but drugs such as memantine (**38**) (Chart 4) and amantadine (**151**) (Chart 18) are in clinical use for PD in order to provide symptomatic treatment of the disease.[324,325] However, the antiparkinson activity of these compounds alone is rather weak, but the clinical response to coadministration of (*S*)-3,4-dihydroxyphenylalanine (L-dopa) and remacemide (**150**), has been shown to be more effective than L-dopa alone.[326] There are reports on antiparkinson effects of NR2B-selective NMDA antagonists related to ifenprodil (**41**) (Chart 4).[76]

Also, coadministration of the AMPA antagonist NBQX (**70**), or the competitive NMDA antagonist, CPP (**30**), and threshold doses of L-dopa ameliorates parkinson symptoms in animal models of PD.[327] Noncompetitive antagonists, like memantine (**38**) and amantadine (**151**), have furthermore been reported to diminish L-dopa-associated motor "on−off" fluctuations after chronic L-dopa treatment, which is a serious therapeutic problem.[324,328]

L-Dopa induced dyskinesias in monkeys and humans can be diminished significantly by the NMDA antagonists LY235959 (**33**) (Chart 3), dextromethorphan (**145**), and amantadine (**151**).[329,330] It has recently been reported that LY354740 (**98**) (Chart 12), a group II mGluR agonist, dose-dependently diminished parkinson-like muscle rigidity induced by pretreatment with haloperidol.[331]

Although L-dopa still remains the most effective drug in PD therapy, iGluR antagonists and/or mGluR group II agonists might enrich the therapeutic arsenal, particularly if used in combination with L-dopa. In fact, coadministration of iGluR antagonists enhances the efficacy of L-dopa, thus enabling a reduction of the dose of L-dopa and, hopefully, will delay or suppress the adverse effects related to long term treatment with this drug.

**Alzheimer's Disease.** Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cell loss, pathological changes in neuronal transmission, and the presence of senile plaques, and neurofibrillary tangles. The prominent symptoms of AD patients are cognitive impairment and memory deficiencies. Several studies have investigated the relationship between excitotoxicity and $\beta$-amyloid toxicity in vitro and demonstrated that $\beta$-amyloid peptides appear to sensitize cultured neurons to excitotoxic cell death induced by Glu or NMDA (**24**), but not AMPA (**16**).[332−334] This may imply that the formation of extracellular $\beta$-amyloid renders neighboring healthy neurons vulnerable to excitotoxicity, and it may provide a mechanism where excitotoxic damage aggravates neurodegeneration. Hence, there are reasons to believe that NMDA receptor antagonists may provide neuroprotection and possibly delay the progression of AD. There has been one report of the clinical evaluation of chronic memantine (**38**) in AD patients, which showed no worsening of symptoms over a 12 month period.[335] However, this finding requires confirmation in a large placebo-controlled trial. It would be surprising if the recognized deleterious effect of NMDA receptor antagonists on cognitive function was not to cause an aggravation of symptoms in AD, although the balance between desired and undesired effects will be a function of the doses administered.

Little work has been performed on glutamatergic anomalies in AD, with the exception of receptor binding studies using post-mortem tissue,[336] the interpretation of which is confounded by the marked neuronal loss in this disease, and the subsequent difficulty in attributing observed changes in binding to *cause* or *consequence* of neuronal death. However, there is a number of indications that Glu might be involved in the pathophysiology of AD,[13,94] but this issue is still controversial. Studies in the past years have shown that both NMDA and AMPA receptors play critical roles in learning and some forms of associated memory in animals.[337,338] Could drugs that facilitate Glu receptor transmission enhance certain forms of cognition in humans? Several attempts have been initiated to develop positive modulators of Glu receptors to treat the cognitive deficits in AD. The first of these was (*R*)-cycloserine (**49**) (Chart 5), a partial agonist at the glycine site of NMDA receptors, which despite promising animal data and initial acute studies in healthy volunteers failed to demonstrate any support for beneficial cognitive effects in AD patients.[339] How-

ever, in a later study in AD patients, a 10-week regimen study of (*R*)-cycloserine (**49**) appeared to improve recall in an implicit memory test in Alzheimer's patients.[340]

Recently, drugs that positively modulate AMPA receptors by reducing AMPA receptor desensitization and/or slowing AMPA receptor deactivation, such as cyclothiazide (**84**) and IDRA-21 (**85**) (Chart 9), and other "Ampakines" such as 1-BCP (**152**) and CX 516 (**153**) (Chart 18), have been reported.

CX 516 (**153**), which produces a mild relief from desensitization in AMPA receptors,[341] has been reported to improve memory scores in a small sample of young individuals.[342] In a study of elderly individuals, CX 516 (**153**) produced a dose-dependent improvement in the ability to recall nonsense syllables.[343]

CX 614, a close analogue of **153** and which completely blocks desensitization of AMPA receptors,[344] has also been reported to improve learning in rats in the radial maze.[345] These findings are encouraging and suggest a future for positive AMPA receptor modulators in ameliorating memory deficits in AD patients.[346] Although overt toxic effects, such as seizures, tremor, and hyperactivity, have not been reported after administration of 1-BCP (**152**), IDRA-21 (**85**), or CX 516 (**153**) to rodents or after oral administration to humans,[342,347,348] one has to be aware that AMPA receptor mediated excitotoxicity may occur with excessive AMPA receptor activation.

**Amyotrophic Lateral Sclerosis.** Although the cause of amyotrophic lateral sclerosis (ALS) is unknown, this is one of the neurodegenerative diseases for which there is most evidence that excitotoxicity may contribute to the pathology of the disease.[349−351] The disease is characterized by progressive muscular weakness, leading to paralysis and eventually death, usually due to respiratory failure.[352] Death usually occurs between 2 and 5 years after diagnosis, making it one of the most lethal of all neurological diseases. Histopathologically, ALS is characterized by a selective loss of both upper and lower motorneurons.

A number of studies have demonstrated that ALS patients have significant elevations in plasma and cerebrospinal fluid (CSF) levels of Glu.[353−355] In cultured motorneurons, excitotoxicity due to an increase in Glu concentration is blocked by NBQX (**70**) (Chart 8) and GYKI 52466 (**81**) (Chart 9), but not by NMDA receptor antagonists.[356,357] Rothstein and co-workers have come up with a more precise idea of the nature of the proposed glutamatergic defect in ALS by demonstrating a reduction in glial Glu uptake by the Glu transporter subtype GLT-1.[358,359] Another factor affecting the sensitivity of motorneurons to Glu may be related to the receptor subunit composition of Glu receptors that they bear. The iGluR2 receptor has been shown to be absent from human spinal motorneurons.[360] Absence of the iGluR2 receptor subunit would make AMPA/KA receptors permeable to calcium, and this could account for the pharmacology of the excitotoxic response.

The preclinical evidence suggests that AMPA/KA receptor antagonists would be the most promising drugs for the treatment of ALS. It is presently not clear whether both AMPA and KA receptors are involved, but with new selective compounds, it should be possible to resolve this. Potent AMPA and/or KA receptor antagonists with minimal side effects are needed before appropriate clinical trials can be performed.

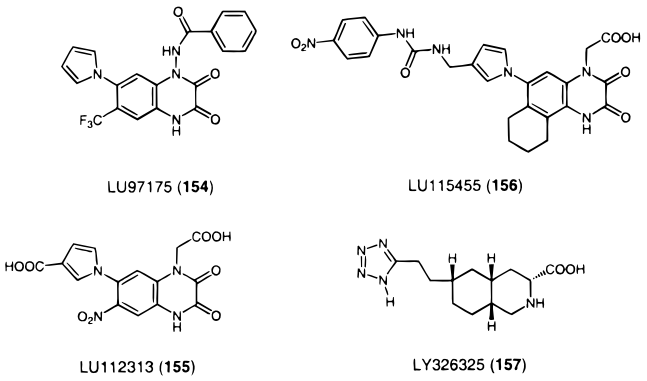
**AIDS.** AIDS dementia is a devastating complication of HIV-infection characterized by a triad of disturbances in cognition, motor performance, and behavior in adults.[361] In children, developmental delay, cognitive impairment, poor brain growth, and other neurological symptoms are seen.[362] The exact pathophysiological mechanisms of AIDS dementia are not known. However, there are indications that Glu might be involved in some aspects of AIDS-related cognitive neurological deficits.[363] The neurotoxic effects by the HIV envelope glycoprotein (gp120) in cell cultures can be blocked by a variety of NMDA receptor antagonists, including (*R*)-AP5 (**29**) (Chart 3), memantine (**38**), and MK-801 (**35**) (Chart 4), but not by the AMPA receptor antagonist CNQX (**69**) (Chart 8), suggesting a role for NMDA receptor stimulation in AIDS dementia.[364−367] The gp120 protein also provokes NMDA receptor mediated cell death in human neurons.[368−370] These data suggest that the use of NMDA receptor antagonists, like memantine (**38**), may ameliorate certain of the neurological complications of HIV infection.[371] In fact, **38** is currently in clinical trials for inhibition of neurological deficits in AIDS patients.

**Epilepsy.** Epilepsy has been considered a potential therapeutic target for iGluR antagonists since the demonstration that NMDA receptor antagonists block seizures in rodent models of epilepsy more than 15 years ago.[372] Subsequently, both competitive and noncompetitive antagonists of AMPA receptors have been shown to be anticonvulsive.[373,374] To date, however, no selective NMDA receptor antagonist has been reported to produce therapeutic benefit in clinical trials in epilepsy.[375]

KA is a potent excitant of CA3 neurons in the hippocampus, and recent pharmacological studies suggest involvement of KA receptors of the iGluR5 subtype in regulation of excitatory synaptic transmission in both the CA1 and CA3 regions of the hippocampus.[34,376] It has thus been suggested that KA receptors may have a role in the pathogenesis of epileptiform activity.[377] Furthermore, recent studies in iGluR6 deficient mutant mice[39] have also indicated a role of the iGluR6 subtype of KA receptors in epileptiogenic effects of systemic administration of KA.

To our knowledge, there are no ongoing clinical trials of Glu receptor antagonists in epilepsy. The way ahead for iGluR antagonists in epilepsy is likely to involve identification of antagonists with selectivity for receptors with particular subunit compositions and matching these to specific types of epilepsy.[378,379] A new pyrrolylquinoxalinedione series of non-NMDA antagonists with marked differences in their AMPA and KA receptor affinities have recently been evaluated in the kindling model of temporal lobe epilepsy, the most common type of epilepsy in humans. Thus, LU97175 (**154**), showing high affinity for iGluR5-7, and LU115455 (**156**) (Chart 19), which potently binds to both AMPA receptors and low affinity KA receptor subunits, were more potent anticonvulsants in the kindling model than the AMPA receptor-selective compound LU112313 (**155**). Furthermore, the three compounds exerted potent anticonvulsant effect without inducing motor impairment. These data suggest that non-NMDA receptor antagonists

**Chart 19.** Some Ionotropic Glu Receptor Antagonists of Therapeutic Interest




acting at both AMPA and KA receptors are more effective in this model than AMPA-selective drugs.[380]

Modulation of epileptic seizures by group III mGluRs has been reported,[266,381−383] indicating that group III mGluRs may be critically involved in maintaining the delicate balance between neuronal inhibition and excitation, thus encouraging the search for systemically active group III mGluR agonists.

**Schizophrenia.** Schizophrenia is a mental illness characterized by hallucinations, delusions, thought disorder, affective blunting, and asociality over a lifelong course. Although the behavioral and cognitive manifestations of the illness are characteristically unmistakable, no neural pathology has yet been identified, and neither has any pathophysiological explanation been confirmed. Post-mortem tissue examination has failed to provide strong evidence for a neurodegenerative etiology of schizophrenia. However, several lines of evidence suggest that glutamatergic mechanisms may contribute to the pathophysiology of schizophrenia.[384] PCP (**36**) (Chart 4) and other antagonists of NMDA receptors have, as earlier mentioned, psychotomimetic properties in healthy individuals[385,386] and exacerbate preexisting symptoms of schizophrenia.[387,388] These observations provide some evidence that schizophrenia may be associated with decreased production and/or release of Glu in critical brain areas. Therefore, treatments with NMDA agonists would be expected to provide antipsychotic effects. The most difficult aspect of the pharmacology of the NMDA receptor complex, therapeutically speaking, is that direct acting NMDA agonists are all excitatory and potentially excitotoxic. Indirect acting NMDA agonists, such as glycine agonists or spermidine ligands, may potentiate the natural actions of endogenous NMDA ligands and be effective therapeutically, but this has yet to be demonstrated in humans.[384]

Recently, PCP (**36**) and other psychotomimetic NMDA antagonists have been shown to increase Glu efflux[389,390] in prefrontal cortex, suggesting that these types of drugs may produce their cognitive and locomotor effects, at least in part, by potentiating glutamatergic neurotransmission at non-NMDA receptors. Hence, reduction of presynaptic glutamatergic activity by targeting group II mGluRs may present an approach for reversing those behavioral effects of PCP (**36**) associated with increased glutamatergic activity. Recently, an agonist of this group of mGluRs, LY354740 (**98**) (Chart 12), has been shown to block schizophrenia-like symptoms in a rat model of schizophrenia, the PCP model, at a dose not producing apparent side effects.[391] Thus, targeting this group of receptors may present a new nondopaminergic therapeutic strategy for treatment of schizophrenia. New drugs are badly needed as current schizophrenia medications are far from ideal.

Drugs that target mGluRs may, in contrast to drugs acting at iGluRs, be considered therapeutically more attractive from a side effect perspective because this subtype of GluRs does not seem to mediate fast synaptic transmission.

**Pain.** Currently, the only available agents for treatment of, e.g., neuropathic pain are opioids, with their known limitations of tolerance, addiction, and CNS depressive effects. There remains an unmet need for effective therapeutic agents that can be utilized on a regular basis without side effects.

Glu and its ionotropic receptors in the spinal cord play a key role in pain transmission and also appear to be involved in the development of neuronal plasticity which accompanies sensitization to pain.[392,393] The involvement of NMDA receptors in chronic pain is well established as NMDA antagonists such as (*R*)-CPP (**30**), ketamine (**37**), amantadine (**151**), and dextromethorphan (**145**) have antinociceptive activity in a variety of chronic neurogenic pain states in humans and in animal models.[394−397] Unfortunately, the effects of the compounds are often seen at doses causing side effects typical for NMDA receptor antagonists. The future development of NMDA receptor antagonists for hyperalgesia will be dependent on identifying antagonists that lack cognitive and motor side effects, and in this respect, much interest is focused on NR2B-selective NMDA antagonists.[76]

The role of AMPA/KA receptors in spinal pain transmission and injury induced central sensitization is not well characterized due to lack of specific AMPA and KA antagonists. However, peripheral applications of KA (**7**) or domoic acid (**8**) (Chart 1) produce nociceptive reflexes comparable to those elicited by capsaicin and bradykinin, which is consistent with KA receptor expression by nociceptive neurons.[398] Recently, spinal KA receptors have been reported to contribute to transmission of somatosensory inputs from the periphery to the brain.[399] A recent study has investigated the role of AMPA and KA receptors in the processing of persistent nociceptive information, and compounds with varying activities at these receptors were examined for effects on the formalin induced paw-licking behavior in rats.[400] In this study, mixed AMPA/KA antagonists, like LY293558 (**74**) (Chart 8), induced ataxia at antinociceptive doses, whereas a selective AMPA antagonist did not cause antinociception at doses that did not produce ataxia. However, a selective iGluR5 antagonist, LY382884 (**91**) (Chart 11), was without ataxic effect at antinociceptive doses. LY382884 (**91**) has been reported to be without effect on acute physiological nociceptive responses.[401] These data strongly suggest an involvement of iGluR5 in the processing of sensory information in the spinal cord related to chronic pain.

In studies in humans with the mixed AMPA/KA receptor antagonist, LY293558 (**74**) (Chart 8), reduction of capsaicin-evoked hyperalgesia and allodynia with no

effect on hyperalgesia was reported.[402] Only a low incidence of side effects at effective doses of LY293558 (**74**) was seen, suggesting that this class of drugs may prove to be useful in clinical pain states.

The KA-selective ligand SYM-2081 (**1**) (Chart 1) inhibited hyperalgesia and allodynia following chronic sciatic nerve ligation in rats.[403] This effect was probably due to functional antagonism at KA receptors in the continuous presence of SYM-2081 (**1**), as this agonist causes profound desensitization of KA receptors.[168,188,404]

Electrophysiological experiments have revealed an involvement of mGluRs in transmission of noxious signals in the spinal cord.[405] Group I mGluRs are expressed in the rat dorsal root ganglion and spinal dorsal horn.[406] On this basis, it is tempting to speculate that mGluR5 may be involved in pain transmission and drugs acting at this mGluR subtype might be used in the treatment of chronic pain. Recently, a structurally novel, potent, and systemically active selective noncompetitive mGluR5 receptor antagonist MPEP (**140**) (Chart 16) has been identified, and the compound inhibits mechanical hyperalgesia in an animal model of inflammatory pain.[407]

**Anxiety.** The initial observation that NMDA receptor antagonists are anxiolytic[408] has been supported by several more recent studies.[409−411] However, even modest cognitive side effects are unlikely to be acceptable for this clinical application. Recently, the AMPA receptor antagonist LY326325 (**157**) (Chart 19) has been shown to produce anxiolytic-like effects without altering locomotor activity in rats,[412] suggesting a future for non-NMDA receptor antagonists as anxiolytics.

A potent agonist for group II mGluRs, LY354740 (**98**) (Chart 12), has shown anxiolytic activity in various animal models of anxiety after oral administration.[232,413] Furthermore, acute administration of LY354740 (**98**) did not produce side effects known for currently used anxiolytics, for example, diazepam, such as sedation and deficits in neuromuscular coordination. These data may indicate a functional role for group II mGluRs in fear/anxiety and suggest that mGluR2/mGluR3 agonists may be beneficial in the treatment of anxiety-related disorders in humans without the side effects seen with currently used medications.

## Future Directions

The Glu receptor field has been, and continues to be, in a state of almost explosive development. A major driving force in this research area of increasing industrial as well as academic interest is the observation of implications of the Glu neurotransmitter system in a steadily increasing number of chronic and acute diseases and disease conditions.

During the past three decades, a variety of Glu receptor ligands have been designed and developed using semirational approaches. A large number of such compounds have been developed by re-design of naturally occurring amino acids showing effects at Glu receptors. These natural products normally interact nonselectively with different subtypes of Glu receptors, but as the result of systematic structural modifications, a number of family- or subtype-specific Glu receptor ligands have been made available as experimental tools and compounds of clinical interest.

Naturally occurring compounds will continue to be a rich source of leads for the design of specific Glu receptor ligands and of tools for the identification of novel types of recognition sites at Glu-operated synapses. Animal and plant toxins of peptide or non-peptide structure undoubtedly will play an increasingly important role in the disclosure of such recognition sites as potential drug targets.

In addition to this line of research, new Glu receptor ligands will be designed on the basis of the mapping of Glu receptor recognition sites by computational methods in combination with X-ray crystallographic analyses of cocrystallized complexes of receptor ligands and receptor binding domains. This line of research is now in a state of rapid development, particularly in the AMPA receptor field, and within the next few years, a number of such X-ray structure analyses on iGluR as well as mGluR binding domains will be reported.

Simultaneously, the identification of novel types of compounds showing effects at Glu receptor subtypes based on high throughput screening in functional assays will accelerate. Such compounds will be used as leads for the design of novel types of Glu receptor agonists, antagonists, or modulatory agents. A number of Glu receptor ligands discovered using this kind of technology will interact with the receptors in a noncompetitive manner as already shown in a large number of cases. Since such compounds are likely to bind to sites different from the Glu recognition sites, this may be a fruitful approach to the development of truely specific receptor ligands.

So far, the Glu receptors have been the pharmacological targets of primary interest, and since the cloned subtypes of all, so far, known Glu receptors are now available for pharmacological studies, these receptors will remain in focus for drug design and development projects in this field. However, the multiple forms of Glu transporters are now of rapidly growing interest as pharmacological targets, and this research area is already in a state of fruitful development.

The multiple enzymes associated with the Glu neurotransmission process have, so far, only played a minor role as pharmacological targets. Although a number of details of the complex biochemical pathways at Glu-operated synapses remain to be elucidated, various enzymes in this system are likely to be interesting pharmacological targets. The recent reports on serine racemase[79] and on the enzymes involved in the biosyntheses of the endogenous NMDA agonist quinolinic acid and NMDA antagonist kynurenic acid[414] are likely to stimulate the design of different types of enzyme inhibitors as potential modulators of Glu neurotransmission.

The Glu receptor field evidently is an increasingly interesting and challenging research area, not least for medicinal chemists specialized in different fields of drug design. Collaborative academic and industrial research projects involving medicinal chemists and pharmacologists undoubtedly will form the basis of the marketing of a range of therapeutic agents in diseases, which, so far, have escaped effective treatment.

**Acknowledgment.** The secretarial assistance of Mrs. Anne Nordly is gratefully acknowledged.

## Biographies

**Hans Bräuner-Osborne** received his Ph.D. in molecular pharmacology from the Royal Danish School of Pharmacy in 1993 where he has continued to work as a postdoctoral fellow and assistant professor at the Department of Medicinal Chemistry. He has carried out extensive structure−activity studies on muscarinic acetylcholine receptor agonists and partial agonists and on ligands for metabotropic glutamate receptors, in both cases using cloned receptors. In recent years, his research has focused on cloning, structure−function analysis, and pharmacological characterization of family C G-protein coupled receptors. Within this family of receptors, he has carried out structure−function studies on the calcium sensing receptor, and he has recently cloned a group of novel human orphan receptors.

**Jan Egebjerg** finished his Ph.D. in biostructural chemistry from University of Aarhus in 1990. He joined Molecular Neurobiology Laboratory at The Salk Institute for Biological Studies in 1990 as a postdoctoral fellow. He was engaged by Novo-Nordisk A/S as a neuroscientist in 1990 and as head of Molecular and Cellular Biology from 1991. He returned to University of Aarhus as associate professor in 1996, and from 1999 he has also been employed at H. Lundbeck A/S as head of Molecular Genetics. His main research focus is molecular mechanisms involved in ionotropic glutamate receptor function.

**Elsebet Ø. Nielsen** received her Ph.D. in medicinal chemistry from the Royal Danish School of Pharmacy in 1984, followed by postdoctoral training with Peter Roberts at the University of Southampton, U.K., and Povl Krogsgaard-Larsen at the Royal Danish School of Pharmacy. She joined the Biochemistry Department at Ferrosan A/S in 1987 and was engaged by NeuroSearch A/S in 1989, where she is head of the Neurochemistry Department. Her research focuses on receptor/ligand interactions and identification of novel ligands for ionotropic receptors, such as glutamate, GABA, and nicotinic acetylcholine receptors.

**Ulf Madsen** achieved his Ph.D. degree in 1988 in the field of excitatory amino acids. His research involves design and synthesis of heterocyclic glutamate analogues, conformational studies by high-resolution NMR techniques, computer-based molecular modeling studies, and in vitro pharmacological investigations. Following a research visit at University of Sydney he returned to the Department of Medicinal Chemistry, the Royal Danish School of Pharmacy, from 1994 as associate professor. His research has led to the development of different series of potent and specific agonists for different receptor subtypes, new antagonists, functional partial agonists, and enhancers of excitatory activity, the two latter examples representing possible new therapeutic strategies in the areas of neurodegenerative and neuropsychiatric disorders.

**Povl Krogsgaard-Larsen** achieved his Ph.D. degree in 1970 in natural products chemistry and his D.Sc. degree in 1980, based on the design of specific $GABA_A$ agonists and GABA uptake inhibitors. Both degrees were obtained at the Royal Danish School of Pharmacy, where he established medicinal chemistry as a subject area. Since 1986 he has been professor of medicinal chemistry at this school. He was chairman of the biotechnological research center PharmaBiotec during the period 1987−1999. At present, he is director of the research center NeuroScience PharmaBiotec and Graduate School of Drug Research. He was awarded honorary doctor degrees at University of Strasbourg in 1992 and at University of Uppsala in 2000. He has received a number of Danish and international research awards and prizes for his design and pharmacological characterization of $GABA_A$ agonists and antagonists, GABA uptake inhibitors, glutamate receptor agonists and antagonists, and different types of ligands for muscarinic and nicotinic acetylcholine receptors. He is a member of the boards of directors of the Alfred Benzon Foundation and the Carlsberg Foundation, and since 1986 he has been member of the Royal Danish Academy of Science and Letters.

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