JOURNAL OF
MEDICINAL
CHEMISTRY



Volume 37, Number 24 November 25, 1994

*Perspective*

# The Glycine Site on the NMDA Receptor: Structure–Activity Relationships and Therapeutic Potential

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*Received July 25, 1994*

## The NMDA Receptor

**Background.** The amino acid L-glutamate is the most important fast excitatory neurotransmitter in neuronal circuits in the mammalian central nervous system (CNS).[1,2] Almost all CNS neurons can be excited by L-glutamate, acting on a variety of different ligand-gated ion channel cell surface receptors. These are classified into two main categories, those for which the synthetic glutamate analogue *N*-methyl-D-aspartate is a potent excitant (NMDA receptors) and those on which NMDA is not active (non-NMDA). The unique glycine modulatory site on the NMDA receptor represents a novel target for medicinal chemistry which has attracted considerable interest since its discovery in 1987.[3,4] In both NMDA and non-NMDA classes of receptors, glutamate opens an ion channel which leads to a rapid influx of cations and a resulting discharge of the normally negative intracellular membrane potential. A third family of glutamate receptors, the "metabotropic glutamate receptors," are G-protein-coupled and represent targets of so far less well understood modulatory actions of glutamate in the CNS.[2]

NMDA receptors are widely distributed in brain and spinal cord, with the highest densities in cerebral cortex and hippocampus. The receptor when activated controls the opening of an ion channel which permits the entry of monovalent (mainly $Na^+$) and divalent (mainly $Ca^{2+}$) cations into target cells. An unusual feature of the NMDA receptor is that it is inoperative when target cells are in a resting state, as under such conditions of negative intracellular membrane potential the ion channel associated with the NMDA receptor is fully blocked by $Mg^{2+}$ ions. This block is voltage dependent, however, and is removed if the target cell is partially depolarized by activation of non-NMDA receptors or other excitatory inputs. Thus, the NMDA receptor mechanism has a "conditional" feature, making it potentially an important "logic gate" in CNS circuits, especially relevant in processes of learning and memory.[5] The NMDA receptor has another unusual feature, as excessive activation of the receptor can lead to over-excitation of the target neurons to the point of cell death, probably caused by an excess accumulation of intracellular $Ca^{2+}$. Much research has focused on the role of NMDA receptors in such "excitotoxic" cell death in recent years, as it seems likely that this mechanism contributes importantly to the permanent damage to the CNS that occurs when there is excessive release of L-glutamate following traumatic head or spinal cord injury, stroke, perinatal ischemia, or in hypoglycemic conditions. The discovery of potent NMDA receptor antagonists has shown that such drugs have the potential to protect the CNS from excitotoxic damage in these conditions, and several compounds of this type are currently undergoing clinical trials to assess their efficacy in the acute treatment of stroke and head injury.[6,7]

**Molecular Architecture.** The NMDA receptor is a complex multimeric protein of high molecular weight. Genes encoding two different varieties of subunit have been identified and cloned, but the exact subunit composition is not yet established. The NR-1 subunit is a protein of approximately 900 amino acid residues.[8] It is present in all NMDA receptors, although it can exist in at least seven different mRNA splicing isoforms.[9,10] When expressed alone in Xenopus oocytes the NR-1 subunit can form a functional NMDA receptor, although the cation channel has only a small conduc-

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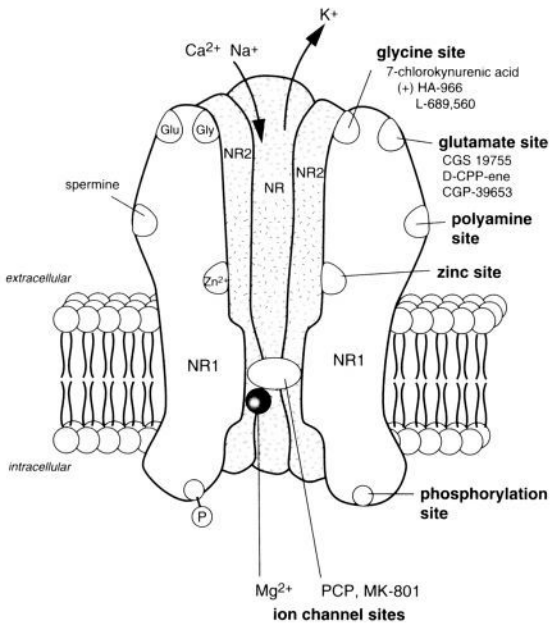


**Figure 1.** Model of the NMDA receptor showing sites for antagonist action.

tance by comparison with that seen in native receptors.[8] Four different genes encode a second subunit, the NR-2, these are large proteins of some 1500 amino acids designated NR-2A, -2B, -2C, and -2D.[11–13] The human genes corresponding to the rat NR-1 and NR-2A subunits were recently described.[14] It is necessary to have at least one NR-1 and one NR-2 subunit expressed together in oocytes *in vitro* to obtain an NMDA receptor with properties resembling native receptors. By analogy with other ligand-gated ion channel receptors (GABA, inhibitory glycine, nicotinic acetylcholine), it is likely that the functional NMDA receptor consists of a pentamer containing NR-1 and NR-2 subunits. The possibility exists that more than one type of NR-2 subunit can occur in a given receptor. The identification of multiple subtypes of NMDA receptors offers new possibilities for the development of subtype-selective agonist and antagonist drugs in the future.

**Drug Binding Sites.** The NMDA receptor possesses a variety of potential drug binding sites (Figure 1). In addition to the L-glutamate recognition site, which is the target for a number of synthetic competitive antagonist drugs (CGS 19755, *D*-CPP-ene) in the amino phosphonate acid series, MK-801 (dizocilpine) and the dissociative anæsthetics, phencyclidine and ketamine, act as noncompetitive antagonists binding at a site associated with the cation channel. There are also binding sites for $Mg^{2+}$, a separate divalent cation recognition site preferring $Zn^{2+}$, a polyamine (spermine, spermidine) binding site where these compounds up-regulate receptor function and a site which recognizes submicromolar concentrations of the amino acid glycine, which acts as a powerful up-regulator of receptor function.[15]

## The Glycine Binding Site

The stimulatory action of glycine on the NMDA receptor was discovered in 1987, when Johnson and Ascher showed that the magnitude of the electrophysiological response of cultured neurons to applied NMDA is greatly reduced or absent if glycine is rigorously excluded from the external medium.[15] Concentrations of glycine as low as 0.1 $\mu$M are almost sufficient to completely restore normal NMDA responses. The effect of glycine is so profound that some authors have described it as a "co-agonist" rather than merely a modulator of NMDA receptor function.[3,4]

***In Vitro* Properties.** Initially the strychnine-insensitive binding of [$^3$H]glycine to brain membranes or tissue sections was used for biochemical or autoradiographic mapping studies of the glycine/NMDA site.[16,17] More recently higher affinity antagonist radioligands have been developed which serve as more effective probes, including [$^3$H]-5,7-dichlorokynurenic acid (**13**)[18] and [$^3$H]L-689,560 (**21**).[19]

The glycine site on the NMDA receptor is clearly distinguishable from the previously described glycine receptor, a glycine-gated chloride ion channel which is important in inhibitory synaptic transmission in spinal cord and brainstem. The latter receptor is blocked by low concentrations of the convulsant alkaloid strychnine, whereas the glycine/NMDA site is strychnine-insensitive. The actions of glycine on the NMDA receptor are mimicked by other small neutral amino acids, notably by (*R*)-serine and (*R*)-alanine which have proved to be valuable research tools. (*R*)-Serine occurs normally in brain and may act as an endogenous activator of the NMDA receptor along with glycine. Not unexpectedly, there are interactions between the glycine site and the various other binding sites on the NMDA receptor. Thus, glycine enhanced the binding of [$^3$H]-glutamate to the NMDA receptor *in vitro*.[20,21] On the other hand, glycine reduced and the glycine site antagonist HA-966 (racemic **5**) increased the binding of competitive antagonists at the L-glutamate recognition site ([$^3$H]CGS-19755, [$^3$H]CPP).[22,23] The polyamines spermine and spermidine enhanced [$^3$H]glycine binding to the NMDA receptor, as did L-glutamate, and saturation binding experiments indicated that in each case this was due to an increase in [$^3$H]glycine binding affinity.[24] Glycine enhanced the ability of L-glutamate to promote the binding of [$^3$H]MK-801 or [$^3$H]TCP to the ion channel site, whereas the glycine site antagonist 7-chlorokynurenic acid (**12**) reduced the binding of the radioligands.[25–27] Zinc dose-dependently inhibited [$^3$H]-glycine binding, suggesting that zinc may inhibit NMDA receptor function by noncompetitively antagonizing glycine binding.[28] Grimwood and colleagues[29] reported a complex series of interactions between glycine and glutamate site ligands *in vitro*; the interaction being influenced by whether agonist or antagonist radioligands were used, and in part by possible steric interference between the binding of molecules at these two sites.

While the binding data cited above mainly suggest the existence of positive allosteric cooperativity between the glutamate and glycine binding sites, this conclusion is not easily reconciled with results obtained from detailed kinetic studies made on voltage-clamped single neurons which suggested the existence of negative allosteric coupling between the sites.[30] It was proposed that the role of glycine may be to reduce or slow the rate of desensitization to L-glutamate otherwise seen on prolonged exposure to agonist.[30,31] In contrast, other electrophysiological studies, showing that HA-966 in-

creased the dissociation rate of glutamate from the NMDA receptor, provide support for the binding data.[32,33] Detailed kinetic studies also suggested that there are two glutamate and two glycine binding sites in each NMDA receptor unit.[34] This stoichiometry cannot yet be reconciled in detail with the molecular architecture of the receptor, although it is clear that the NR-1 subunit possesses both glutamate and glycine binding sites and homomeric receptors containing only NR-1 subunits show full glycine modulation.[8]

Further evidence for the location of the glycine site on the NR1 subunit has come from radioligand binding studies using [$^3$H]-**13**.[35a] Site-directed mutagenesis of the NR1 subunit has recently revealed that phenylalanine residues at positions 390, 392, and 466 are important determinants of the glycine binding site.[35b] Structural homology to bacterial amino acid binding proteins was suggested, where interactions between the amino group of the ligand and aromatic residues of the protein are thought to occur.[35b]

**Functional Role.** The NMDA receptor is unique in apparently requiring two agonists, and the functional significance of this feature remains obscure. Measurements of the level of glycine in human and animal cerebrospinal fluid indicate that it is normally present at concentrations of approximately 10 $\mu$M or above—much higher than those needed to activate NMDA receptors *in vitro*. Furthermore, when L-glutamate or NMDA is applied to single neurons in brain slice preparations, or to the intact brain in anesthetized animals, a full receptor response can be elicited without adding exogenous glycine—suggesting that glycine is normally present in supramaximal amounts in the extracellular milieu. However, different NMDA receptor subtypes differ in their sensitivity to glycine,[36] and measurements in CSF may not accurately reflect the true concentrations of the amino acid at synapses in brain. At mossy fiber inputs to the cerebellar granule cells, for example, there may be an unusually low extracellular glycine concentration because of the operation of local glycine uptake mechanisms, and at these synapses glycine potentiates glutamate responses.[37] Furthermore, Singh and co-workers[38] found that the intracerebral administration of the glycine agonist *R*-serine (but not the inactive *S*-serine) dose-dependently increased the potency of NMDA in inducing seizures in mice, suggesting that NMDA receptors may not normally be maximally activated by glycine in the intact brain. This was also the conclusion indicated by experiments which showed that local injections of glycine or (*R*)-serine into mouse brain led to increased levels of cyclic GMP in cerebellum—a response known to be elicited by NMDA receptor activation.[39] The effects of glycine and (*R*)-serine were blocked by HA-966.[39] It remains likely that the NMDA receptor is principally a glutamate receptor, i.e., it is activated under appropriate conditions by the pulsatile release of glutamate from nerve endings, while the overall responsiveness of the receptor mechanism or its ability to sustain chronic use may be determined by the prevailing levels of extracellular glycine.

## Structure–Activity Relationships

The identification of the novel NMDA-associated glycine site has stimulated intensive efforts to identify selective ligands, and many compounds are now available with selective high affinity. The major problem currently facing medicinal chemists is obtaining *in vivo* activity, since the majority of glycine-site ligands synthesized to date do not adequately penetrate the blood–brain barrier. Most attention has been directed toward antagonists, which provide alternatives to the established classes of NMDA receptor antagonists acting as channel blockers or competitively at the glutamate site. There has also been interest in glycine-site partial agonists of varying efficacy. High-efficacy partial agonist compounds may have potential as memory enhancers and low-efficacy compounds may have improved therapeutic ratios relative to channel blockers and glutamate-site antagonists. Agonists and partial agonists have been synthesized from α-amino acid structures and 1-amino-*N*-hydroxy-2-pyrrolidinone (racemic **5**, HA-966). Several classes of antagonists have been prepared using 2-carboxy-4-hydroxyquinoline (**11**, kynurenic acid) as a starting point. The structures of key compounds that have been developed are shown in Figures 2–9, and their biological activities are summarized in Table 1.

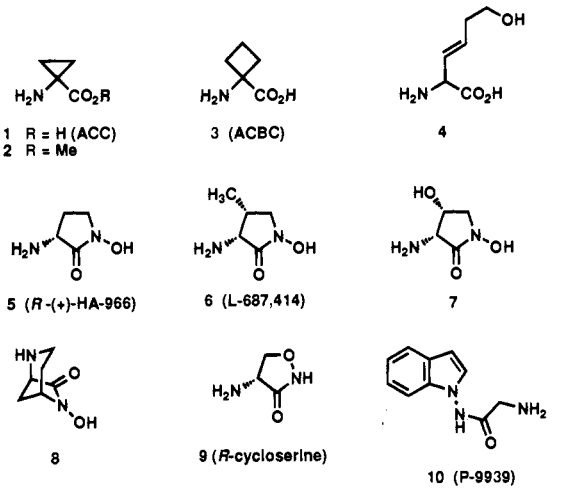


**Figure 2.** Structures of compounds acting as agonists or partial agonists at the glycine site.

**Amino Acid Derivatives.** As outlined above, glycine and other small neutral (*R*)-α-amino acids, such as alanine and serine, are enantioselective potent agonists with submicromolar affinities.[40] Cyclic amino acids such as the cyclopropane **1** (ACC) and cyclobutane **3** (ACBC, Figure 2) bind to the glycine site and have differing efficacies, **1** being essentially a full agonist.[41] Despite its high efficacy, **1** appears to act like an antagonist *in vivo*, since it blocks NMDA-induced seizures,[42] albeit weakly as a result of poor penetration of the blood–brain barrier.[43] The methyl ester **2** is a prodrug with improved activity *in vivo*, and anxiolytic activity has been demonstrated with **2** in forced swim and plus-maze tests.[44] Other than **1**–**3**, relatively little is known about the activities of unnatural amino acids. One interesting study has shown that the amino acid **4**, which has a bulky α-vinyl substituent, is an agonist which lacks enantiospecificity.[45]

**HA-966 Derivatives.** Following the initial recognition of HA-966 as a glycine site ligand,[46] the compound was resolved into its individual enantiomers.[47] This showed that the *R*-(+) enantiomer **5** (Figure 2) selectively binds to the glycine site and is anticonvulsant *in vivo*, whereas the *S*-enantiomer has sedative action,

**Table 1.** Activities of Glycine-Site NMDA Receptor Ligands

| | *In Vitro* | | | *In Vivo* | | | |
|---|---|---|---|---|---|---|---|
| | binding | NMDA functional activity | | | | | |
| no. | [$^3$H]Gly $IC_{50}$ ($\mu$M) | test | activity ($\mu$M) | model | route | $ED_{50}$ (mg/kg) | ref |
| Gly | 0.2 | [$^3$H]TCP, $EC_{50}$ | 0.2 | | | | 40a,b |
| (*R*)-Ser | 0.3 | [$^3$H]TCP, $EC_{50}$ | 0.2 | | | | 40a,b |
| 1 | 0.04 | [$^3$H]MK-801, $EC_{50}$ | 0.14 | NMDA seizure | ip | 234 | 41, 142 |
| **2** | >40 | | | NMDA seizure | ip | 46 | 42, 44 |
| | | | | forced swim | ip | 100 (MED) | |
| | | | | plus-maze | ip | 200 (MED) | |
| **3** | 19 | oocytes, $IC_{50}$ | 18.5 | | | | 143, 144 |
| (*R*)-**4** | 0.24 | [$^3$H]MK-801, $EC_{50}$ | 0.33 | | | | 45 |
| (*S*)-**4** | 0.75 | [$^3$H]MK-801, $EC_{50}$ | 0.93 | | | | 45 |
| **5** | 12.5 | cortical slice, $K_b$ | 55 | audiogenic seizure | ip | 53 | 48, 49 |
| | | cortical neurone, $K_i$ | 2.5 | NMDLA seizure | iv | 890 | 123, 124 |
| | | | | amphetamine hyperactivity | ip | 30 (MED) | |
| | | | | PCP hyperactivity | sc | 10–30 (MED) | |
| 6 | 1.4 | cortical slice, $K_b$ | 15 | audiogenic seizure | ip | 5.1 | 49, 111 |
| | | cortical neuron, $K_i$ | 0.65 | NMDLA seizure | iv | 20 | 145, 146 |
| | | | | | po | 44 | |
| | | | | PTZ seizure | iv | 13 | |
| | | | | electroshock | iv | 26 | |
| | | | | neuroprotection, rat MCAO | iv bolus + infusion | 13.5 + 13.5/h (MED) | |
| **7** | 1.3 | cortical neuron, $K_i$ | 0.75 | | | | 49 |
| **8** | 19 | cortical neuron, $K_i$ | 3.5 | | | | 49 |
| **9** | 2.3 | [$^3$H]TCP, $EC_{50}$ | 18.1 | elevation of cGMP | sc | 1.25–10 (biphasic) | 53, 54 |
| | | | | passive avoidance | ip | 3.0 | |
| **10** | 36 | [$^3$H]TCP, $EC_{50}$ | 3.6 | | | | 55 |
| | | [$^3$H]TCP, $IC_{50}$ | 780 | | | | |
| **11** | 41 | cortical slice, $K_b$ | 154 | audiogenic seizure | ip | >250 | 57, 59 |
| | | | | | icv | 20 nmol | 67 |
| **12** | 0.56 | cortical slice, $K_b$ | 7.0 | | | | 57 |
| **13** | 0.20 | cortical slice, $K_b$ | 3.0 | audiogenic seizure | ip | >250 | 58, 59 |
| | | | | | icv | 7.5 nmol | 67, 147 |
| | | | | separation-induced vocalization | ip | 67 | |
| **14** | 0.032 | cortical slice, $K_b$ | 0.41 | | | | 59 |
| **15** | 0.40 | myenteric plexus, guinea pig, $IC_{50}$ | 5 | neuroprotection, rat MCAO | iv | 20 | 61, 110 |
| **16** | 0.10 | | | | | | 62 |
| **17** | 0.36 | cortical slice, $K_b$ | 2.5 | | | | 63 |
| **18** | | | | audiogenic seizure | ip | 62 | 64 |
| **19** | 0.10 | | | neuroprotection, gerbil | ip | 10 (MED) | 65 |
| **20** | 0.13 | cortical slice, $K_b$ | 1.2 | audiogenic seizure | ip | >100 | 63, 67 |
| | | | | | icv | 35 nmol | |
| **21** | 0.0078 | cortical slice, $K_b$ | 0.13 | audiogenic seizure | ip | >100 | 19, 60 |
| | | | | | icv | 1.5 nmol | 66a, 67 |
| **22** | 0.019[a] | cortical slice, $K_b$ | 0.24 | audiogenic seizure | ip | 39 | 67 |
| | | | | | icv | 3.5 nmol | |
| **23** | | | | audiogenic seizure | ip | 32 | 67 |
| **24** | 0.032[a] | cortical slice, $K_b$ | 0.84 | audiogenic seizure | ip | 29 | 67 |
| | | | | | icv | 0.5 nmol | |
| **24a** | 0.008[b] | | | audiogenic seizure | ip | >100 | 66b |
| | | | | | icv | 3 $\mu$g | |
| **25** | 0.14 | cGMP cerebellar slice, $IC_{50}$ | 2.7 | audiogenic seizure | ip | 100 | 70, 73 |
| | | | | | icv | 0.09 $\mu$g | |
| **26** | 0.10 | | | audiogenic seizure | ip | 140 | 70 |
| | | | | audiogenic seizure + probenicid | ip | 45 | |
| **27** | 0.96 | oocytes, $IC_{50}$ | 3.2 | D-Ser-induced cGMP | ip | >50 | 71, 78 |
| | | | | | icb | 5.4 $\mu$g | |
| **28** | 0.68 | oocytes, $IC_{50}$ | 1.6 | harmaline-induced cGMP | ip | 100 (MED) | 71, 78 |
| **29** | 0.11 | | | | | | 74 |
| **30** | 0.027[a] | | | audiogenic seizure | ip | >100 | 73 |
| **31** | 0.80 | | | | | | 59, 75 |
| **32** | 0.41 | | | neuroprotection, gerbil | ip | 10 (MED) | 76 |
| **33** | 39 | | | | | | 59 |
| **34** | 4.8 | | | | | | 80b |
| **35** | 1.3 | | | | | | 80b |
| **36** | 1.0 | [$^3$H]GABA release, $IC_{50}$ | 0.4 | audiogenic seizure | ip | 0.1 | 67, 81 |
| | 0.17[a] | | | | | >10 | |
| | | cortical slice, $K_b$ | 1.2 | | icv | >20 nmol | |
| **37** | 3.1 | cortical slice, $K_b$ | 6.0 | | | | 59, 148 |
| **38** | | cortical neurone, $K_b$ | 0.005 | nociception, formalin paw | ip | 1–20 | 82, 87 |
| | | | | electroshock | ip | 5 | |
| **39** | 0.11 | | | | | | 83 |
| **40** | 6.0 | | | | | | 84b |
| **41** | 0.0026 | | | | | | 85 |

**Table 1** (Continued)

| | In Vitro | | | In Vivo | | | |
|---|---|---|---|---|---|---|---|
| | binding | NMDA functional activity | | | | | |
| no. | [$^3$H]Gly $IC_{50}$ ($\mu$M) | test | activity ($\mu$M) | model | route | $ED_{50}$ (mg/kg) | ref |
| **42** | 0.46 | | | | | | 80b, 86 |
| **43** | 0.12 | cortical slice, $IC_{50}$ | 11 | electroshock | ip | >100 | 86 |
| **44** | 0.41[a] | cortical slice, $K_b$ | 6.7 | audiogenic seizure | ip | 13.2 | 89 |
| **45** | 6.4[a] | cortical slice, $K_b$ | 23 | audiogenic seizure | ip | 12.5 | 90, 149 |
| | | | | PTZ seizure | ip | 43 | |
| **46** | 0.17[a] | cortical slice, $K_b$ | 1.4 | audiogenic seizure | ip | >20 | 90 |
| **47** | 0.42[a] | cortical slice, $K_b$ | 3.4 | audiogenic seizure | ip | 4.1 | 90 |
| **48** | 0.011 | spinal cord, $IC_{50}$ | 2.1 | | | | 92 |
| **49** | 0.0069[a] | cortical slice, $K_b$ | 0.091 | | | | 88 |
| **50** | 0.0020[a] | cortical slice, $K_b$ | 0.028 | audiogenic seizure | ip | 0.9 | 91 |
| | | | | | po | 0.9 | |
| **51** | 0.0014[a] | cortical slice, $K_b$ | 0.0050 | audiogenic seizure | ip | 0.8 | 91 |
| | | | | | po | 0.9 | |
| **52** | 0.031 | | | neuroprotection, gerbil | ip | 20 (MED) | 94 |

[a] $IC_{50}$ values for displacement of [$^3$H]L-689,560 (**21**) binding. [b] $IC_{50}$ value for displacement of [$^3$H]-**13** binding.

occurring by an unknown mechanism.[48] A program of optimization of **5** showed highly restrictive structure–activity requirements, with only small changes being tolerated.[49] Only the 4-*cis*-substituted derivatives **6** and **7** had improved affinity. The activity of the rigid bicycle **8** suggests that the 4-methyl and 4-hydroxy substituents in **6** and **7** probably increase affinity by a conformational effect, resulting in a higher proportion of the axial amino conformer of the pyrrolidone ring.[50] This conclusion is consistent with an analysis of the binding conformations of flexible acyclic amino acids at the glycine site.[51] Compounds **5** (*R*-(+)-HA-966)[52] and **6** (L-687,414) are weak efficacy (<10% of glycine) partial agonists but are active after systemic administration in a variety of animal models (see Table 1) and have therefore become useful tools for exploration of the *in vivo* roles of the glycine site.[4] The structurally related amino hydroxamate, the antibacterial agent (*R*)-cycloserine (**9**), is also a systemically active partial agonist, but with higher efficacy than **4**.[53] (*R*)-Cycloserine acts *in vivo* as an agonist to stimulate cGMP production and is effective in enhancing memory in rodent models.[54] No derivatives or analogues of **9** have been reported to have activity at the glycine site. The glycinamide **10**, despite having modest affinity for the glycine site, may represent a new lead towards more structurally diverse partial agonists.[55]

**Kynurenic Acid Analogues.** Immediately following the discovery of glycine's action at the NMDA receptor, it was recognized by several groups that kynurenic acid (**11**, Figure 3), an NMDA antagonist of hitherto unknown mechanism, acts at the glycine site.[56] The 2-carboxyquinoline structure of **11** has proven highly amenable to optimization strategies, and several series of bicyclic heteroaromatic antagonists with considerably improved affinity and selectivity have been developed.

A key early discovery was the beneficial effect of 7-chloro substitution (**12**),[57] which selectively enhances affinity for the glycine site by 70-fold. Notably, a substituent (usually chlorine) is also required at the corresponding position in all subsequently developed high-affinity compounds. Extensive optimization of substitution led to 5,7-dichloro (**13**)[58] and 5-iodo-7-chloro (**14**)[59] derivatives, the latter displacing [$^3$H]glycine binding with an $IC_{50}$ value of 32 nM.[60] Affinity for the glycine site is associated with lipophilic, optimally sized 5- and 7-substituents as shown by a quantitative

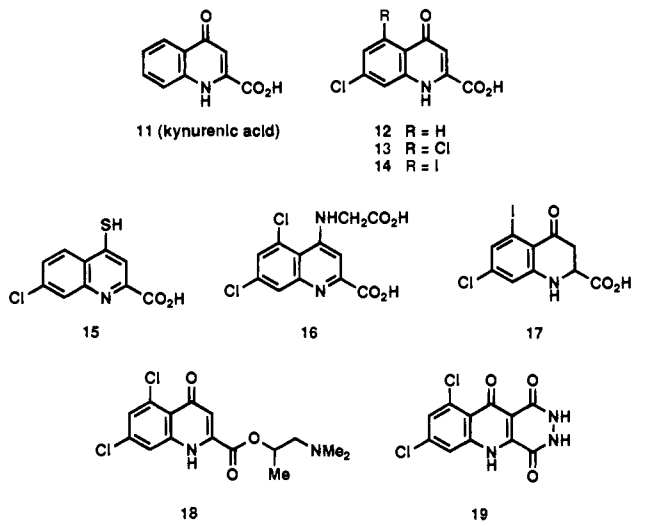


**Figure 3.** Structures of kynurenic acid-derived glycine antagonists.

structure–affinity relationship on a wide group of compounds.[59] Modification of the 4-position carbonyl group results in retention of affinity in thiokynurenates (e.g., **15**)[61] and the 4-carboxymethylamino derivative (**16**).[62] The heterocyclic ring of **14** can be saturated, and the resulting analogue (**17**) has 10-fold reduced affinity despite being conformationally flexible and containing a chiral center at C-2.[63] Water-soluble prodrug esters (e.g., **18**)[64] and a cyclic hydrazide (**19**)[65] have been reported.

Development of the dihydrokynurenate series (**17**) led to a highly potent series of *trans*-4-substituted 2-carboxytetrahydroquinolines (**20**–**24**, Figure 4), with affinities in the nanomolar range.[60,66] The 4-substituent was optimized[66a] to provide the phenylurea (**21**, L-689,-560) ($IC_{50}$ = 7.8 nM for [$^3$H]glycine binding), which has been radiolabeled at the 4′-position[19] to provide a high-affinity radioligand for the glycine site. The absolute stereochemistry required for activity in the tetrahydroquinolines is 2*R* (in common with the amino acid center in agonists and partial agonists), 4*S*. The conformational and stereochemical properties of the tetrahydroquinolines provide important insights into structure–activity relationships, revealing in particular a bulk tolerance site placed approximately 5 Å above the plane of the quinoline ring.[63,66a] These studies define

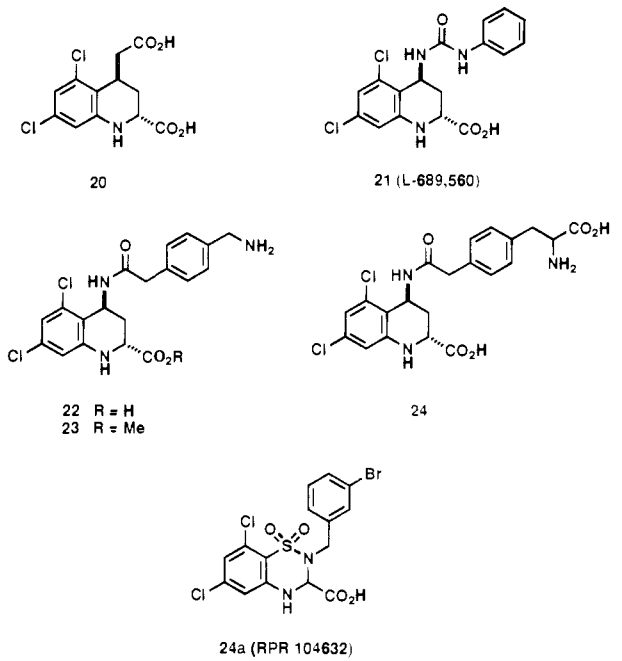


**Figure 4.** Structures of tetrahydroquinoline-derived glycine antagonists.

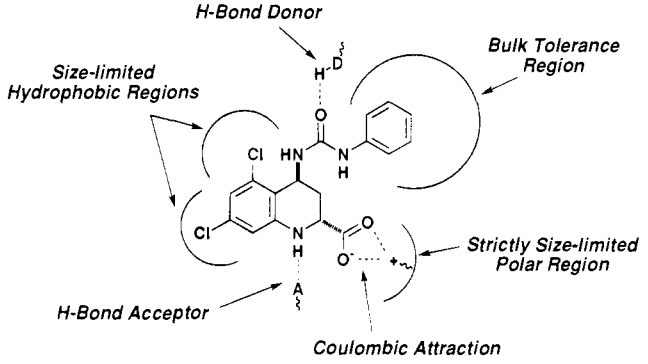


**Figure 5.** Putative pharmacophore for glycine site antagonist L-689,560 (**21**).

a putative pharmacophore for antagonist binding (Figure 5). The heterocyclic ring of kynurenic acid has recently been modified to a benzothiadiazine 1,1-dioxide (**24a**, RPR 104632)[66b] which displays high enantioselectivity. The structure of **24a** is consistent with the pharmacophore in Figure 5, with the 2-(3-bromobenzyl) substituent gaining access to the bulk tolerance pocket.

While it has proved possible to optimise *in vitro* affinity to nanomolar levels, it is clear from the *in vivo* data (Table 1) that the majority of the compounds derived from **11** have little or no activity following systemic administration in relevant tests for central nervous system activity. Consequently, the focus of current effort has moved to devising strategies to improve brain penetration and bioavailability. Activity in the DBA/2 mouse audiogenic seizure model is observed following intracerebroventricular (icv), but not intraperitoneal (ip), dosing of **11**, **12**, **20**, **21**,[67] and **24a**.[66b] Modest activity was found after ip dosing of the tetrahydroquinolines **22** ($ED_{50}$ 39 mg/kg) and **24** ($ED_{50}$ 29 mg/kg) and the prodrugs **18** and **23**. The poor brain penetration of these antagonists was further confirmed by measurements of plasma and brain levels of **22**.[67] Preliminary reports suggest that the cyclic diacyl hydrazide (**19**) is active in the gerbil model of ischaemia following ip dosing.[65] This promising result suggests a

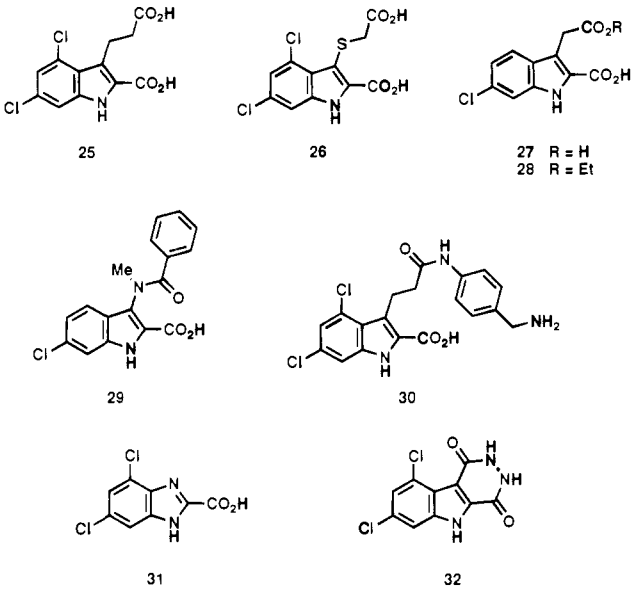


**Figure 6.** Structures of glycine antagonists derived from 2-carboxyindole.

strategy of carboxyl replacement, but with the exception of **19**, modifications of the 2-carboxyl have to date resulted in loss of affinity for the receptor.[68]

**2-Carboxyindoles and Derivatives.** The essential kynurenate-type glycine antagonist pharmacophore is also found in indole-2-carboxylic acid.[69] 6-Chloro substitution enhances binding, in common with the kynurenates, and a number of workers have shown that introduction of hydrogen-bond-accepting 3-substituents leads to improved activity.[70-74] The propanoic acid (**25**, Figure 6) is the prototypical compound of this class, having affinity comparable to the corresponding kynurenic acid (**13**).[70] Substantial structural tolerance at the 3-position of the indoles is shown by compounds **29** and **30**, suggesting a broadly parallel structure–activity requirement to the 4-position of the tetrahydroquinoline series (**20**–**24**), a conclusion supported by molecular modeling studies.[66a,73,74] Benzimidazoles (e.g., **31**)[59,75] and the cyclic diacyl hydrazide (**32**)[76] also bind to the glycine site.

In common with the kynurenates, the 2-carboxyindoles are potent in *in vivo* models after icv[77] but not systemic[70,73] administration. The weak activity of **26** in the DBA/2 mouse audiogenic seizure model ($ED_{50}$ 140 mg/kg ip) is improved upon pretreatment with the uricosuric agent probenicid ($ED_{50}$ 45 mg/kg ip).[70] Probenicid may act to reduce efflux of the antagonist from the brain via carboxylic acid transporters. However, measured brain levels of compound **27** (SC-50132) demonstrate poor CNS penetration and rapid elimination from mouse brain ($T_{1/2}$ 35 min).[78] The diacyl hydrazide **32**, in common with its analogue **19**, is reported active in the gerbil model of neuroprotection at 10 mg/kg ip.[76]

**Quinoxaline Derivatives.** Quinoxaline-2,3-diones **34** (CNQX, Figure 7) and **35** (DNQX) were introduced as the first antagonists of the AMPA-subtype of non-NMDA excitatory amino acid receptor[79] and were subsequently shown[80] to have comparable affinities for the glycine site. Efforts to improve the glycine vs AMPA selectivity in this series have focused on both aromatic substitution (**36**–**38**) and on modification of the heterocyclic ring (**39**–**41**). Affinity, but not selectivity, is

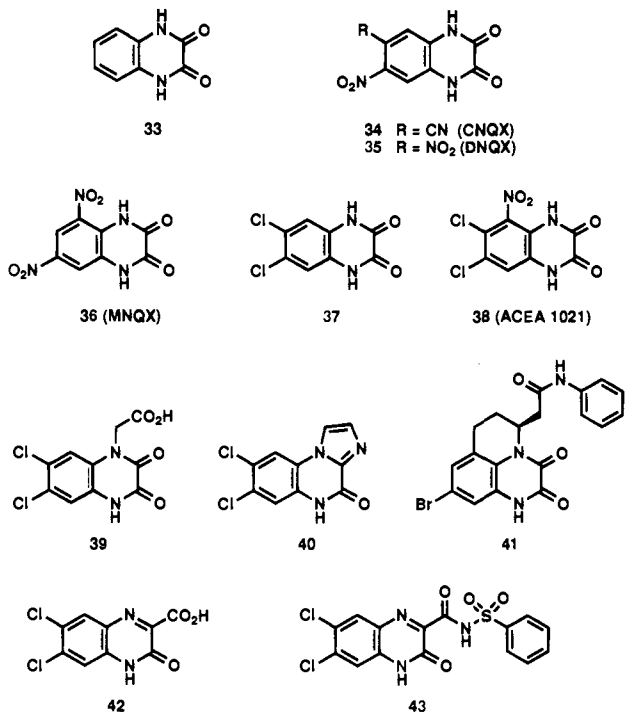


**Figure 7.** Structures of glycine antagonists derived from quinoxaline-2,3-dione.

improved by aromatic disubstitution with electron-withdrawing groups at the 5,7-(**36**)[81] and 6,7-(**37**)[59,80] positions. Preliminary reports have appeared showing very significantly improved affinity and $10^3$-fold selectivity for the glycine site vs the AMPA receptor with the trisubstituted compound **38** (ACEA 1021).[82] Substitution of one of the nitrogen atoms is tolerated, and the acetic acid **39** has good affinity and 20-fold selectivity over AMPA receptors.[83] The tricyclic imidazoquinoxaline **40** is not very potent or selective,[84] but the alternative tricyclic quinoxalinedione **41** successfully incorporates structural features from the 4- and 5-positions of the tetrahydroquinoline series (**21**, **22**) to provide nanomolar affinity ($K_i$ 2.6 nM for inhibition of [$^3$H]-**13** binding).[85] The high binding affinity of **41** is particularly interesting in that it does not possess the acidic functionality, present in both kynurenates and unsubstituted quinoxalinediones such as **38** ($pK_a$ 5.4[82c]), which is suggested for receptor interaction by the putative pharmacophore (Figure 5). The quinoxalic acid (**42**) lacks selectivity for glycine vs AMPA sites,[80] and while the acyl sulfonamide (**43**) has comparable selectivity, its improved affinity demonstrates, in common with **21**, **30**, and **41**, the presence of a bulk-tolerance region on the glycine antagonist recognition site.[86]

*In vivo* data on the quinoxaline compounds are limited, but suggest, in common with the kynurenate and indole classes of glycine antagonists, that CNS penetration is poor following systemic dosing. One exception may be the quinoxalinedione **38** where preliminary reports of systemic activity have appeared.[87] The reported *in vivo* activity of **36** in preventing audiogenic seizures in DBA/2 mice[81] has not been substantiated,[67] and the acyl sulfonamide **43** is inactive in preventing electroshock seizure at a dose of 100 mg/kg ip.[86]

**2-Quinolone Derivatives.** Based on the existing pharmacophore for the glycine site, a series of 2-quinolone derivatives has been designed[88] with the goal of

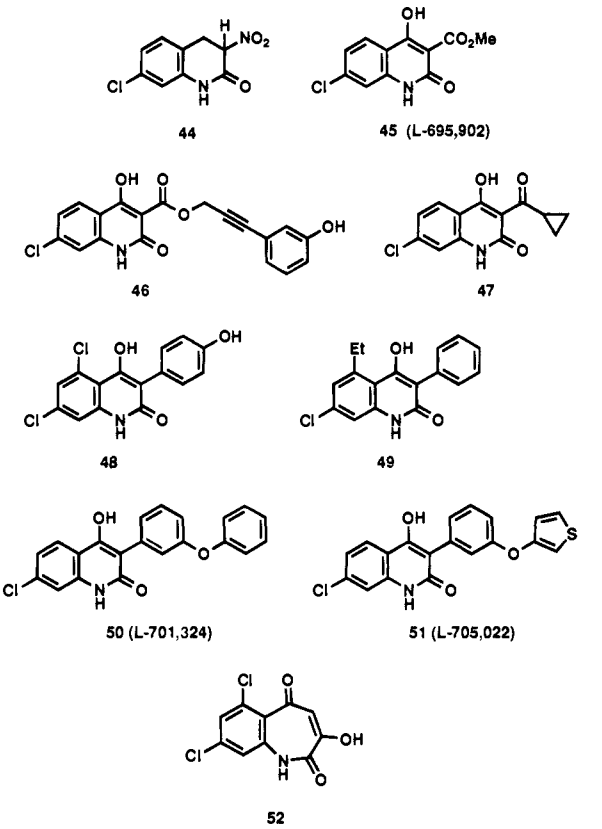


**Figure 8.** Structures of glycine antagonists derived from 2-quinolone.

combining structural features present in the kynurenic acids and quinoxalinediones. The 2-quinolones hold the requisite acidic functionality within the fused heterocyclic ring, delocalized to the 2-carbonyl. The 3,4-dihydro-3-nitro-2-quinolone **44** (Figure 8) has comparable antagonist activity at the glycine and AMPA sites and blocks audiogenic seizure after ip administration ($ED_{50}$ 13.2 mg/kg).[89] Compound **44** is the only systemically active combined NMDA/AMPA receptor antagonist and may be predicted to possess good neuroprotective properties.

The 4-hydroxy-2-quinolone series **45**–**51**,[90–92] where *in vitro* affinity has been shown to be strongly dependent on the nature of the 3-substituent, represent the only class of glycine antagonists to date with consistent *in vivo* activity (Table 1). The 3-acyl series was optimized to provide ester **45** (L-695,902) and cyclopropyl ketone **47**, both compounds having good anticonvulsant activity in the DBA/2 mouse model.[90] Affinity for the receptor can be optimized in esters such as the arylpropargyl derivative **46**, which take advantage of the bulk tolerance site exposed in earlier compounds (e.g., **21**, **30**, and **43**). Introduction of 3-phenyl substitution in **48**[92] and **49**[88] led to enhanced affinity, which can be rationalized by an ion–dipole interaction between the 3-phenyl ring $\pi$-system and a positively charged group on the receptor.[88] Very recently, further optimization, based on combining the structural features contained in **46** and **49**, has led to a breakthrough in systemic activity in a specific class of 3′-(aryloxy) and 3′-(arylmethyl)-3-phenyl derivatives exemplified by **50** (L-701,324, Figure 8) and **51** (L-705,022).[91] Compounds **50** and **51** are the most potent glycine antagonists, both *in vitro* and *in vivo*, yet described. The similar anticonvulsant activities of **50** and **51** following ip and po

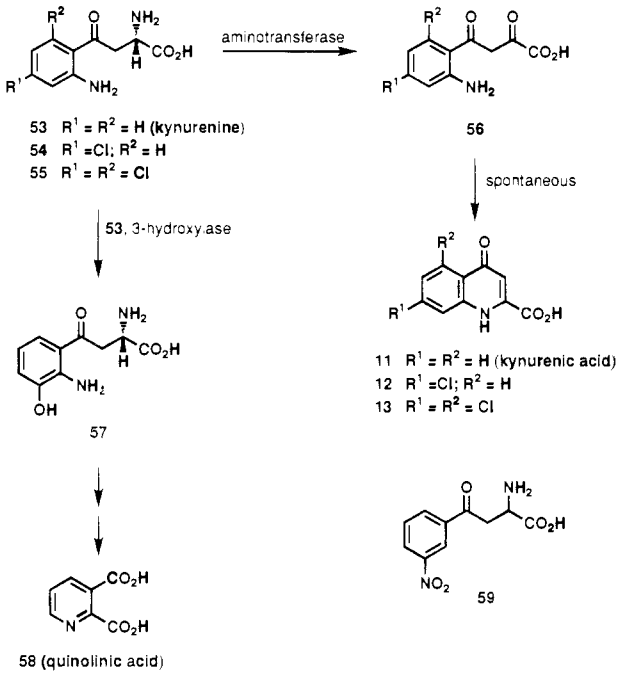


**Figure 9.** Kynurenine metabolism pathway.

administration suggest high oral bioavailability, and compounds of this class appear to be the current tools of choice for exploration of the role of glycine-site antagonism *in vivo*.

The physicochemical features underlying the improved anticonvulsant activity of the 4-hydroxy-2-quinolones **45**–**51** relative to carboxylic acids such as **21** are not entirely clear. Both classes are extensively ionized at physiological pH ($pK_a$ values of **45**–**51** are in the range 4.4–5.5), and there is no clear dependence of activity on hydrophobicity.[91] The reduced hydrogen-bonding potential of **50** relative to **21** may be important for penetration of the blood–brain barrier. Incorporation of a $\beta$-dicarbonyl system as a vinylogous carboxylic acid bioisostere, allowing delocalization of the negative charge over five atoms in the corresponding anions, may also assist cell membrane permeability. A parallel to these $\beta$-dicarbonyls is found in peptidomimetics containing cyclopyrrolone functionality as vinylogous amide bioisosteres which also show improved transport properties.[93] It is interesting to note that the benzazepine **52**, which also has $\beta$-dicarbonyl acidic functionality, has been reported active in the gerbil model of global ischæmia.[94] Benzazepines such as **52** may also possess antagonist activity at non-NMDA receptors.[95]

**Kynurenines.** Manipulation of the L-kynurenine (**53**, Figure 9) pathway of L-tryptophan metabolism has received attention because kynurenic acid (**11**), an antagonist, and quinolinic acid (**58**), an agonist, are products of the route which have opposing effects on NMDA receptors.[96] It has been shown that the chloro derivatives **54** and **55** are substrates for kynurenine aminotransferase and **53** is converted to 7-chlorokynurenic acid (**12**) *in vivo*.[97] Since **53** crosses the blood–brain barrier via the large amino acid transport system, compounds **54** and **55** may act as CNS available prodrugs of **12** and **13**. Modulation of the balance of the endogenous metabolites **11** and **58** can be accomplished with specific enzyme inhibitors, for example racemic *m*-nitrobenzoylalanine (**59**), which blocks kynurenine-3-hydroxylase with an $IC_{50}$ value of 0.9 $\mu$M. High doses of **59** (50–800 mg/kg ip) administered to rats raise hippocampal extracellular levels of **11** by up to 14-fold, and this is accompanied by decreased motor activity and duration of electroshock induced convulsions.[98] The site of action of **59** is not necessarily in the brain, since peripheral inhibition of the pathway can contribute to the raised levels of **11**.

## Therapeutic Potential

**Neuroprotection.** Many studies in the past decade have shown that competitive and noncompetitive NMDA receptor antagonists acting at other sites on the NMDA receptor have powerful neuroprotective properties.[6,7] It is therefore not surprising that noncompetitive NMDA receptor antagonists which act at the glycine site should exhibit similar properties. Addition of the glycine antagonists 7-chlorokynurenic acid (**12**) and HA-966 (racemic **5**) was able to protect against NMDA or hypoxia-mediated cell death in primary cultures of neonatal rodent neurons *in vitro*.[99–101] In this model, the glycine antagonists were as effective as MK-801, i.e., they were capable of providing complete protection. Similarly, **12** and the related analogue 7-chlorothiokynurenic acid (**15**) were able to protect cerebellar granule cells in culture from cell death elicited by L-glutamate.[102] Lipton[103] found that **12** protected rat retinal ganglion cells *in vitro* from cell death elicited by the HIV coat protein gp120, a model which had previously been shown to respond to MK-801. A number of studies have shown that intracerebrally administered glycine antagonists (racemic **5**, **12**, and **15**) offered protection against local excitotoxic damage elicited by the intracerebral injection of NMDA agonists into rat striatum[104,105] and others reported similar protection after administering racemic **5** systemically.[106,107]

Most importantly, like other NMDA receptor antagonists, the glycine/NMDA antagonists can protect against neuronal damage in animal models of cerebral ischemia. Thus, intracerebrally administered **12** protected rat hippocampal neurons against damage induced by transient forebrain ischæmia,[108] and the partial agonist 1-aminocyclopropanecarboxylic acid (**1**) protected against damage in gerbil brain elicited by transient forebrain ischæmia,[109] a finding which is surprising in view of the relatively high agonist efficacy of this compound.[41,52] In an animal model of stroke, involving occlusion of the middle cerebral artery in rat brain, the systemically administered glycine antagonists 7-chlorothiokynurenic acid (**15**) and L-687,414 (**6**) yielded levels of protection similar to those observed in similar experiments using MK-801 or other NMDA receptor antagonists.[110,111] Overall, the glycine antagonists appear to be equally effective as other NMDA antagonists as neuroprotective agents, and they may offer advantages over other NMDA antagonists in terms of their side effect profile in the acute treatment of stroke and other conditions of cerebral ischæmia (see below). It has been suggested that NMDA antagonists might also slow the course of a variety of chronic neurodegenerative diseases (e.g., Parkinson's disease, Huntington's disease, motor neuron disease, Alzheimer's disease) in which excitotoxic mechanisms may be involved. It is not clear, however, whether the glycine antagonists or other NMDA antagonists would be suitable for chronic use because of their possible effects on cognitive and motor function.

**Anticonvulsant Effects.** Various glycine antagonists are able to protect against seizures elicited by photostimulation in baboons (L-687,414, **6**),[112] by auditory stimulation in DBA-2 mice (**22**, **45** and **50**)[67,90,91] by NMDA in mice ((+)-HA-966, **5**),[47] by electroshock in mice (**5**),[107] and by morphine in mice (ACEA-1011, 5-chloro-7-trifluoromethylquinoxaline-2,3-dione).[113] The glycine site partial agonist (*R*)-cycloserine (**9**) is also capable of protecting against electroshock-induced seizures in rats,[114] but these results are more difficult to understand as 7-chlorokynurenic acid (**12**) blocked the effect of **9** and was itself inactive as an anticonvulsant. Similarly puzzling are reports that very high doses of systemically administered glycine or (*R*)-serine (>1 g/kg po) enhanced the anticonvulsant effects of conventional antiepileptic drugs in rats, and that these effects were blocked by **12** (icv),[115] or that (*R*)-serine and **9** increased focal seizure thresholds in a rat model of focal epilepsy.[116]

As NMDA receptor antagonists may be of more potential therapeutic importance for their ability to prevent the processes leading to the development of epilepsy, rather than in the control of seizures, it is important to note that Croucher and Bradford[117] found that locally administered **12** could retard the development of epileptic seizures in rats after repeated daily electrical stimulation of the amygdala.

**Anxiolytic and Antipsychotic Effects.** Anxiolytic effects were first reported for the noncompetitive NMDA receptor antagonist MK-801[118] and have since been observed in animal models with many other NMDA antagonists. The glycine antagonists share this property, with positive effects reported for HA-966 in conflict, social interaction, and elevated plus-maze models.[119,120] In addition, (+)-HA-966 (**5**) seemed to lack some of the undesirable side effects associated with conventional anxiolytic benzodiazepines, including motor incoordination and ataxia, and showed no adverse interactions with ethanol.[120] The high efficacy partial agonist 1-aminopropanecarboxylic acid (**1**) is reported to be effective in the elevated plus-maze anxiolytic model in rat,[121] and positive effects with this compound and with HA-966 and **12** have been found in a novel anxiolytic model, the rat potentiated startle test.[122] It is too early to judge whether these effects predict a useful anti-anxiety potential in humans or whether the glycine antagonists may exhibit a more acceptable side-effect profile than conventional anxiolytics.

A selective interaction between glutamate and dopaminergic mechanisms involving NMDA receptors in the limbic forebrain is suggested by the finding that (+)-HA-966 (**5**) and 5,7-dichlorokynurenic acid (**13**) were able to selectively antagonize the stimulant effects of *d*-amphetamine on dopamine synthesis in rat nucleus accumbens but not in striatum.[123] (+)-HA-966 also blocked the behavioral syndrome elicited by local administration of amphetamine to nucleus accumbens, but not that elicited by administration of amphetamine into the striatum.[123] Surprisingly, (+)-HA-966 was also found to block the stimulant effects of phencyclidine and MK-801 on dopamine turnover in rat nucleus accumbens and the accompanying behavioral arousal.[124] This is currently difficult to interpret as phencyclidine and MK-801 are noncompetitive NMDA receptor antagonists—yet their effects can be blocked by another noncompetitive NMDA antagonist ((+)-HA-966). This may reflect differences in the drug responsiveness of different NMDA receptor subtypes, or the existence of some other as yet undiscovered pharmacology in MK-801 and phencyclidine. These findings were reinforced by the observation that the discriminative stimulus properties of (+)-HA-966 in rats were clearly distinguished from those elicited by the psychotomimetic drug phencyclidine. Thus, animals trained to recognize either drug as a discriminative stimulus failed to cross-generalize to the other, whereas earlier studies had shown a clear cross-generalization between phencyclidine and MK-801.[125] The findings suggest a potential application of glycine antagonists as "atypical neuroleptics" in the treatment of schizophrenia and other psychoses.

**Cognitive Enhancement.** Glutamate acting through the NMDA receptor has been suggested to play a key role in the processes of neuronal plasticity involved in the laying down of memories.[2] It is not surprising, therefore, that glycine agonists have been proposed as potential cognitive enhancers. Particular attention has focussed on the compound (*R*)-cycloserine (**9**), which acts selectively as a partial agonist on the glycine site with an efficacy of 50−80% of that seen with glycine itself.[52,53] (*R*)-Cycloserine was found to facilitate the retention of a learned task (footshock avoidance) in mice and improved the impaired memory performance of old mice in this test.[126] (*R*)-Cycloserine also improved the impaired spatial learning ability of rats that had been treated with the cholinergic antagonist scopolamine in a water maze task,[127] reversed the spatial memory impairment seen in rats with hippocampal brain lesions,[128] and facilitated hippocampus-dependent learning in a rabbit model.[129] However, (*R*)-cycloserine failed to reverse scopolamine-induced cognitive impairments in a monkey study,[130] and initial reports of a clinical trial of this compound in Alzheimer's disease (more than 400 patients) were entirely negative.[131] Although cognitive enhancement remains a potential therapeutic target, the negative human results so far available may dampen enthusiasm.

**Pain.** Several lines of evidence suggest that NMDA receptors may play a key role in the development of persistent pain and hyperalgesia following nerve or tissue injury.[132] The glycine antagonists 7-chlorokynurenic acid (**12**)[133] and ACEA-1011 (5-chloro-7-(trifluoromethyl)quinoxaline-2,3-dione)[134] reduced the late stage of pain sensitivity following subcutaneous injection of formalin into the hind paw in mice. (+)-HA-966 (**5**) also enhanced the antinociceptive effects of nonpeptide substance P receptor antagonists in this model.[135] These finding suggest a potential therapeutic use of glycine antagonists in the treatment of persistent pain and hyperalgesia.

**Side Effect Profile.** It is clear that glycine antagonists share many of the properties of other NMDA antagonists which target different sites on the receptor. There are, however, indications that the glycine-targeted antagonists may have a more benign side-effect profile. Thus, these compounds do not cause the profound behavioral arousal accompanied by large activation of cerebral glucose metabolism that are seen with noncompetitive antagonists of the phencyclidine or MK-801 group and at higher doses with the competi-

tive NMDA antagonists.[136,137] Furthermore, the glycine antagonists studied so far have failed to cause the transient neuropathological changes seen in neurons in certain areas of the brain after phencyclidine or MK-801 administration.[138-140] These effects, involving swelling of neurons and the appearance of fluid-filled vacuoles, have caused concern about the potential neuronal damage that NMDA antagonists might cause in clinical use. The neuropathological changes are restricted to small regions in the limbic forebrain and seem to accompany the intense metabolic and behavioral arousal elicited by MK-801 and related compounds;[137] their absence in the glycine antagonists is clearly an advantage. Furthermore, MK-801 and other channel blockers elicit an arousal of autonomic nervous system function which leads to elevated heart rate and blood pressure at neuroprotective doses, a potentially serious complication in the clinical use of these compounds in stroke patients; these effects are also not seen with glycine antagonists.[140]

Reference has already been made to the lack of cross-generalization between phencyclidine and (+)-HA-966 (**5**) as discriminative cues in animal behavior tests,[125] suggesting that the glycine compounds will not share the liability for psychotomimetic effects seen with the channel blocking NMDA antagonists. This has also been confirmed using a glycine antagonist from a different chemical class, the quinolinediones (L-695,902, **45**).[141] There is also less motor incoordination and ataxia with the glycine antagonists at neuroprotective dose levels. Overall, the profile of these compounds in animal models appears to be more acceptable than those of other NMDA antagonists, although it is well-known that animal models are not always reliable predictors of drug effects in human subjects, and this potential advantage remains to be substantiated by clinical experience. The acceptability of the side-effect profile of the glycine antagonists in clinical use will also depend on the nature of the treatment regime. A temporary drug-induced loss of memory, accompanied by sedation, may be acceptable in the acute treatment of life-threatening stroke or head injury, but would not be tolerated in a compound to be used for the chronic treatment of neurological or mental illness.

## Future Directions

Preclinical studies to date suggest considerable therapeutic potential for glycine-site ligands in CNS disorders. The clinical assessment of these compounds, however, is only just beginning, principally because of the problems of identifying suitably potent candidate compounds which readily cross the blood-brain barrier. The task of designing molecules to interact with a brain amino acid recognition site, and to reach this site *in vivo*, has provided a significant test of the ingenuity of medicinal chemists. Current potent glycine antagonists, based on kynurenic acid and quinoxalinedione, show that the acidic group can be modified and hydrophobic binding can be introduced, but a 3-substituted aniline moiety is retained as an essential feature. It is clear that the acidic group, which is necessary for high receptor affinity, also results in poor brain penetration. Within the molecular framework of these existing compounds, design of alternative structures is clearly feasible. However significant improvements in CNS penetrability and bioavailability are likely to require novel, more structurally diverse lead molecules. Further insights into the physicochemical properties influencing brain penetration, such as hydrogen bonding properties and hydrophobicity, may assist future synthetic chemistry. Prodrug or "soft drug" strategies have not been explored to any great extent.

The identification of NMDA receptor subtypes has been a highly significant advance which is expected to have a major impact on drug discovery efforts, particularly in the search for antagonists with improved specificity and side effect profiles. Differences in receptor subtype affinity may help to explain the varied and sometimes puzzling biological activities of glycine-site and other classes of NMDA antagonists. When known, the subunit constitutions of native receptors, together with their regional distribution and abundance in the brain, is expected to provide specific targets for future drug discovery programmes. It is too early to say which of the several known sites for antagonist action on the NMDA receptor (Figure 1) will provide viable approaches to subtype specificity. Because of the problem of poor brain penetration in both glycine-site and glutamate-site antagonists, other sites, such as the ion channel and polyamine sites, or as yet undiscovered modulatory sites, should also be considered as potential targets for receptor subtype specificity.

## Conclusions

The available evidence, based largely upon studies with the bioavailable partial agonists (+)-HA-966 (**5**), L-687,414 (**6**), and (*R*)-cycloserine (**9**), suggests that NMDA receptor antagonists acting at the glycine site have broad therapeutic potential and offer a highly attractive target for CNS drug discovery. In comparison with NMDA receptor antagonists acting competitively at the glutamate site or uncompetitively as channel blockers, glycine antagonists may have significantly improved side-effect profiles. Many classes of glycine antagonists with high affinity and selectivity have now been synthesized, but most of these lack activity in the central nervous system following systemic dosing. The recently described compounds ACEA 1021 (**38**) and L-701,324 (**50**) appear to have the best systemic activity to date and are expected to provide further impetus for the evaluation of the glycine site *in vivo*. Despite the progress made so far, the challenge remains for medicinal chemists to design further molecules which freely penetrate the blood–brain barrier.

## Biographies

**Paul D. Leeson** received his Ph.D. in Organic Chemistry from Cambridge University, England, working on the synthesis of indole alkaloids with Dr. J. Harley-Mason. After postdoctoral studies on the chemical mechanisms of bioluminescence with Prof. F. McCapra at Sussex University, he joined Smith Kline & French Research Laboratories in Welwyn, England. In 1985 he joined the Merck Sharp & Dohme Neuroscience Research Centre, where he is currently Associate Director of Medicinal Chemistry. His research interests are in the design and synthesis of selective agonists and antagonists for neurotransmitter and hormone receptors.

**Leslie L. Iversen** received his Ph.D. in Pharmacology from Cambridge University, England, and did postdoctoral work in the United States with Dr. J. Axelrod (NIH) and E. Kravitz (Harvard). From 1971 to 1983 he was Director of the Medical Research Council Neurochemical Pharmacology Unit in Cambridge, and since 1983 he has been Director of the Merck Sharp & Dohme Neuroscience Research Centre. He has worked on

the neurochemistry and neuropharmacology of brain neurotransmitters and neuropeptides and has published more than 300 papers in this area.

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