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Positive and Negative Allosteric Modulators of N-Methyl-D-Aspartate (NMDA) Receptors; Structure-Activity Relationships and Mechanisms of Action

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Abstract

Excitatory activity in the CNS is predominately mediated by L-glutamate through several families of L-glutamate neurotransmitter receptors. Of these, the N-methyl-D-aspartate receptor (NMDAR) family has many critical roles in CNS function and in various neuropathological and psychiatric conditions. Until recently, the types of compounds available to regulate NMDAR function have been quite limited in terms of mechanism of action, subtype selectivity, and biological effect. However, several new classes of NMDAR agents have now been identified that are positive or negative allosteric modulators (PAMs and NAMs, respectively) with various patterns of NMDAR subtype selectivity. These new agents act at several newly recognized binding sites on the NMDAR complex and offer significantly greater pharmacological control over NMDAR activity than previously available agents. The purpose of this review is to summarize the structure-activity relationships for these new NMDAR modulator drug classes and to describe the current understanding of their mechanisms of action.

1. Introduction

N-methyl-D-aspartate (NMDA) receptors are ligand-gated ion channels which, along with AMPA and kainate receptors, are activated by the brain's primary excitatory neurotransmitter, L-glutamate¹⁻⁴. The NMDA receptors (NMDARs) are known for their prominent roles in synaptic plasticity and in a number of neurological and psychiatric disorders including pain, stroke, epilepsy, schizophrenia, post-traumatic stress disorder, depression and various neurodegenerative diseases such as Alzheimer's and Parkinson's⁵⁻¹¹. Thus, NMDARs are a desirable pharmacological target and yet, few NMDAR-based drugs have been successful in the clinic due to adverse side effects from off-target activity or adverse effects from excessive NMDAR inhibition. Despite limited success to date, there

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remains significant potential for the development of useful agents that modify NMDAR activity. Since the different NMDAR subtypes have significantly varied physiological properties and anatomical distributions, agents that act at distinct NMDAR subtypes are expected to have markedly different therapeutic and adverse effects. Furthermore, the relatively recent identification of several distinct families of positive and negative allosteric modulators has revealed that there is a great potential to develop agents with highly specific activities that should provide advantages over prior agents^{12, 13}. These allosteric modulators differ from each other in several functional properties and in their subtype selectivity, thus greatly expanding the repertoire of NMDAR pharmacological modulation. In addition to developing therapeutics for the above-mentioned diseases, these agents are also powerful tools for defining the function of NMDARs, and their subtypes, in CNS function. These newer agents act at sites distinct from where the well characterized NAM ifenprodil is thought to bind¹⁴. As the pharmacology of ifenprodil-related agents has been well described over the years^{15, 16}, this review will focus mostly on the recent studies regarding compounds acting at novel allosteric sites that are distinct from the ifenprodil site.

The NMDAR is a heterotetrameric complex composed of subunits arising from seven homologous genes: GluN1, GluN2A-GluN2D and GluN3A-GluN3B^{3, 4}. These subunits form an ion channel pore through the plasma membrane that is permeable to Na⁺, K⁺, and Ca²⁺ and gated by the simultaneous binding of L-glutamate to the GluN2 subunit and glycine (or D-serine) binding to GluN1 or GluN3 subunits. The majority of NMDARs are believed to contain two GluN1 subunits and two GluN2 subunits. NMDAR subunits have a modular structure with distinct domains (Figure 1): the extracellular amino-terminal domain (ATD) – a modulatory domain which can bind zinc in the case of GluN2A, the ligand binding domains (LBD) which bind L-glutamate (GluN2A-D) or glycine (GluN1 or GluN3), the transmembrane domains (TMD) which forms the pore, and an intracellular C-terminal domain. The ATD is a bi-lobed, clamshell-like structure that can be either open or shut; the shut conformation inhibits receptor function^{14, 17}. The LBD forms a similar bi-lobed structure where the two extracellular segments (S1 and S2) form the two halves of the clamshell structure. S1 is the peptide sequence between the ATD and the first membrane-associated domain (M1) and S2 is the segment between the third and fourth membrane-associated domains (M3 and M4). Agonist binding in the center cavity of the LBD stabilizes a closed conformation whereas competitive antagonist binding prevents this closure¹⁸. The combined closing of the GluN1 and GluN2 LBDs allosterically transmits a change in the TMD corresponding to a higher probability of an open channel.

Allosteric modulators have several distinct advantages for the development of suitable therapeutic agents compared to the other classes of NMDAR pharmacological agents (agonists, competitive antagonists, and channel blockers). Enhanced subtype-selectivity generally minimizes off-target activity and unwanted side effects, and allosteric modulators bind to regions other than the highly conserved ligand binding sites or the channel pore. Thus, allosteric agents have greater potential for subtype-selectivity as has been found already. NAMs also have the potential of maximally inhibiting less than 100% of the agonist response, thus preserving some function and avoiding excessive blockade. Therefore, such partial NAMs should have a better safety profile than competitive antagonists and channel blockers that can potentially eliminate all activity. A partial agonist at the ligand binding site

also promotes intermediate activity levels, but unlike a NAM, a partial agonist could activate otherwise inactive receptors. Positive allosteric modulators (PAMs) also offer distinct advantages. For the treatment of NMDAR hypofunction as in schizophrenia, or possibly in other cases of cognitive dysfunction, augmenting NMDAR activity would be expected to restore proper function by increasing the activity of weakly-activated NMDAR-mediated signals. In contrast to PAMs, an NMDAR agonist would activate both appropriate and inappropriate receptors and thus increase system noise and potentially cause excitotoxicity.

Given these advantages of NMDAR allosteric agents, and the recent demonstration of multiple new classes of allosteric agents, there has been a resurgence in NMDAR drug development targeting PAMs and NAMs. This review will focus mostly on the recent studies regarding compounds acting at novel allosteric sites located outside of the ATD on the NMDAR. Structure-activity relationship (SAR) studies used in the development of these allosteric modulators and mechanistic studies aimed at identifying their binding sites and mode of action will be discussed.

2. Steroidal Based NMDAR NAMs and PAMs: Neurosteroids and Cholesterols

2.1 Neurosteroids

Neurosteroids and related cholesterols were among the first agents to be identified that can allosterically modulate ionotropic glutamate and GABA receptors^{19, 20}. Interestingly, some of these compounds are endogenous to neural tissue and they can display both PAM and NAM activity at NMDARs. Thus, they may represent natural ligands used to modulate NMDAR function. Using these steroid structures as starting points, medicinal chemistry efforts have gone on to identify synthetic agents which are now lead compounds being studied for different therapeutic applications. Of the various neurosteroids, two of the most extensively studied for their activity at NMDARs are pregnenolone sulfate **1** (20-oxo-5-pregnen-3 β -yl sulfate, commonly abbreviated to PS), which displays both PAM and NAM activity, and pregnanolone sulfate **2** (20-oxo-5 β -pregnan-3 α -yl sulfate, commonly abbreviated to 3 α 5 β S or PAS), which is a NAM (Figure 2a).

2.1.1 Neurosteroid PAM & NAM SAR

PS is a potentiator of NMDARs and an inhibitor of AMPA (AMPA), kainate (KAR) and GABA-A receptors²¹. PAS, on the other hand, displays inhibitory activity at NMDARs, KARs, and AMPARs²¹. This was found to be as a result of their respective geometry. PS has a more planar geometry than PAS as a result of the double bond in the second ring (Figure 2b). Investigations into the effect of the geometry were carried out with a number of derivatives with and without a double bond. It was found that more planar compounds tended to have PAM activity and those with a more 'bent' structure were generally inhibitors²². A summary of the most important SAR observations regarding PAS and PS derivatives can be found in Figure 2b. In general, some modifications of PAS did result in increased NAM potency, whereas PS PAM activity was not significantly improved. Both PAS and PS require a charged group at C3; PS PAM activity is dependent on a negatively charged substituent, but in the case of PAS, the group can be positively or negatively

charged. Replacement of the sulfate group with uncharged groups such as hydrogen or formate eliminates PAM activity in PS derivatives and NAM activity in PAS derivatives, while substitution with dicarboxylic acid esters of varied length, from hemioxylate to hemiglutarate, generally maintains activity²³. Replacement of the C3 sulfate of PAS with positively charged L-argininyl or 4-(trimethylammonio)butanoyl increases inhibitory activity²⁴. Although additions to ring **D** of PS reduced activity, some additions to ring **D** of PAS increased activity significantly^{25,26}. In particular, compound **3**, with an isobutyl chain instead of the acetyl group of PAS, has an IC₅₀ of 90 nM at GluN1/GluN2B receptors^{27a}. It was concluded that the inhibitory potency was directly related the lipophilicity of the compounds. Some of this requirement for lipophilicity may be needed for access to the PAS binding site; there is evidence that PAS can enter the membrane to get to its binding site²⁴.

2.1.2 Neurosteroid PAM activity and mechanism of action

PS inhibits GABA-A, glycine, AMPA and kainate receptor responses as well as those of NMDARs containing GluN2C and GluN2D subunits²²⁻²⁴. In addition to this general inhibitory activity, PS potentiates agonist responses at neuronal NMDARs^{25, 26} and NMDARs that have GluN2A or GluN2B subunits^{24, 27b}. Further studies demonstrated that PS has both PAM and NAM activity at each of the four GluN1/GluN2A-D receptors with PAM activity being dominant at GluN2A and GluN2B-containing receptors and NAM activity predominating at receptors containing GluN2C or GluN2D. When co-applied with agonist, PS potentiates steady-state responses of GluN1/GluN2A and GluN1/GluN2B receptors while inhibiting activity at GluN1/GluN2C and GluN2D receptors²⁴. However, when PS is pre-applied and rapidly replaced by agonist (sequential application), PS displays potentiating activity of the initial agonist response at GluN2C and GluN2D-containing receptors and enhanced potentiating activity at GluN2A- and GluN2B-containing receptors^{27b}. This is interpreted as reflecting the rapid loss of inhibitory activity and the persistence of PS potentiating activity when switching from PS to agonist at each of the four GluN1/GluN2 receptors. Taking advantage of these different drug application paradigms to distinguish NAM and PAM activity, PS was found to potentiate the different NMDARs with similar EC₅₀s (~80 μ M) but with greater maximal potentiation at receptors containing GluN2A or GluN2B subunits. In contrast, PS inhibitory activity was estimated to be several fold more potent at GluN2C and GluN2D than at GluN2A and GluN2B^{27b}.

Under some conditions, PS appears as a weakly effective PAM. PS potentiation of GluN1/GluN2A and GluN1/GluN2B receptor responses declines during L-glutamate application, a response described as “dis-use dependent” potentiation²⁸. This may reflect a higher affinity of PS for the resting receptor state than the activated state and may also contribute to the greater potentiation seen with sequential PAM/agonist application mentioned above. Such dis-use dependency should result in greater potentiation of phasic, synaptic glutamate-mediated NMDAR responses over tonically-activated extrasynaptic NMDAR responses. However, this effect may be offset by reduced ability of PS to potentiate GluN2B-mediated responses to high agonist concentrations. PS causes a small increase in agonist potency at GluN2B-mediated responses^{24, 28}. Accordingly, at high L-glutamate concentrations, which would obscure a potentiating effect due to increased agonist potency, there is relatively little PS potentiation of GluN2B-mediated responses. Reduced PS potentiation in the presence of

high L-glutamate concentrations is also seen for neuronal NMDAR responses²⁶. Since synaptic responses see saturating agonist concentrations, while extrasynaptic receptors experience lower agonist concentrations, one might expect PS to preferentially potentiate extrasynaptic over synaptic GluN2B-containing receptors. Other than the small effect on L-glutamate potency at GluN2B-containing receptors, PS generally has little effect on agonist EC₅₀ and can potentiate responses due to saturating concentrations of agonist^{23, 24, 26}. Thus, PS potentiation must have additional mechanisms to increase NMDAR responses other than by increasing agonist potency.

PS slows the rates of both NMDAR desensitization and deactivation. In whole cell recordings of recombinant GluN1/GluN2A and GluN1/GluN2B receptors, PS slowed the macroscopic desensitization rate but did not change the extent of desensitization²⁹. PS also slowed the deactivation time that is related to L-glutamate unbinding^{28, 29}. Consistent with these findings, PS also prolongs NMDAR synaptic currents^{29, 30}, which are determined by NMDAR deactivation time³¹. Several of the physiological properties of PS along with other allosteric modulators are summarized in Table 1. Potencies of representative NAMs and PAMs are summarized in Tables 2 and 3, respectively.

NMDAR channel properties, as expected, are also changed by neurosteroids. Using the rate of MK-801 channel blockade as an index of open channels, PS increases open channel probability of GluN1/GluN2B receptors²⁸. Consistent with this finding, at single channels in isolated patches, PS increases the frequency of channel openings, has a small, variable effect on neuronal NMDAR mean channel open time, and has no effect on single channel conductance^{26, 32}. Similar results are found using cell-attached patches in which the receptor sees a relatively intact intracellular environment, except that in this condition, PS produces a more robust increase in the mean open time^{26, 33}. Thus, the effect of PS on mean open time depends upon an undiluted intracellular environment, but the effect on channel opening frequency does not. These results are consistent with the observation that the rapid loss of PS potentiation of NMDAR responses in outside-out patches is slowed by protein phosphatase inhibitors³⁴.

In addition to the NMDAR PAM activity of PS, there is also evidence for direct receptor activation when measuring calcium mobilization or ligand-induced receptor trafficking^{35, 36}. In this case, PS could possibly be acting as an agonist for mGluR-like activity of NMDARs^{36, 37}. Further SAR characterization of this agonist activity of PS would be interesting as it may be possible to have biased signaling of mGluR-like activity. Since the structurally related compound PAS can form nanoparticles³⁸, the role of possible PS nanoparticles in this additional activity may need to be evaluated.

2.1.3 Neurosteroid NAM activity and mechanism of action

PAS, like PS, has a general inhibitory action at ionotropic glutamate receptors - AMPARs, KARs, and NMDARs^{21, 23}, but without the additional NMDAR PAM activity. The inhibitory activity of PAS is voltage-independent and non-competitive with glutamate and glycine; it reduces the maximal agonist responses at each of the four GluN1a/GluN2 subtypes while minimally changing agonist potency^{24, 39}. PAS inhibition is also use-dependent with no evidence of binding to the resting state of the receptor in the absence of

agonist³⁹. Accordingly, PAS displays less inhibition of synaptic NMDAR responses than of steady-state NMDAR responses. This property could be useful in that PAS may weakly inhibit synaptic NMDAR currents while providing greater inhibition of tonically-activated extrasynaptic NMDAR currents that are thought to occur in pathological conditions. Among structural variations of the PAS structure, some agents were found to display use-independent inhibition in contrast to the use-dependent actions of PAS. Thus, these agents would be expected to differ in their actions on NMDAR responses due to phasic/synaptic or tonic/extrasynaptic activation⁴⁰.

In contrast to PS potentiation, PAS inhibition of GluN1/GluN2A receptors in cell-attached patches has no effect on mean open time but instead decreases open probability by increasing mean closed time by specifically increasing the duration of long-lived closed states related to desensitization⁴¹. Interestingly, under some recording conditions, the PAM PS can instead inhibit GluN1a/GluN2A responses. In this case, inhibition is associated with a reduction in mean open time and no change in mean closed time³³. Thus, the inhibitory actions of PS and PAS at GluN1a/GluN2A receptors appear to have distinct mechanisms.

2.1.4 Neurosteroid NAM and PAM Binding Sites

The neurosteroid NAM and PAM binding sites are thought to be located extracellularly and to be distinct from each other^{27b, 42}; neurosteroid NAMs do not display competitive interactions with neurosteroid PAMs⁴². Neurosteroids are able to access their modulatory binding site via the membrane. In cell-attached patch recordings, PS administration to the outside of the cell, and outside of the patch pipette, can potentiate NMDAR responses under the pipette²⁶. PS is not likely to be acting at an intracellular site since intracellular injections of PS do not reduce potentiation to externally applied PS^{27b, 42}. Thus, PS may be accessing the receptor through a membrane route as has been suggested for inhibitory neurosteroids⁴⁰. From this work, the neurosteroid binding sites are thought to be extracellular and may involve the transmembrane region of the receptor as has been suggested for GABA-A receptors⁴³.

The precise identification of the binding site responsible for PS PAM activity remains unknown but is thought to involve S2 (extracellular loop between M3 and M4) and M4. PS displays GluN2C-like inhibitory activity at chimeras wherein the GluN2A sequence has the S2 domain sequence (plus most of M3 and M4) replaced by GluN2C's corresponding sequence^{27b}. Similarly, it was found that a GluN2D construct in which the 84 amino acids containing the C-terminal third of S2 and M4 is replaced with the corresponding sequence from GluN2B displays GluN2B-like PS PAM activity⁴⁴. Replacing both of the S2 and M4 portions in GluN2D with the corresponding sequence from GluN2B is necessary for PAM activity in the chimeric construct. Conversely, replacing this segment in GluN2B with that from GluN2D eliminated PAM activity (but did not generate GluN2D-like NAM activity of PS or PAS). Thus, subunit-specific PS PAM activity appears to be defined by the C-terminal third of the S2 domain and M4 while PS/PAS NAM activity may involve additional upstream regions. This result is consistent with point mutation results; mutating D813A/D815A in GluN2A, which immediately precedes M4, prevents PS potentiation, but not PAS inhibition⁴⁵. Conversely, GluN2A-A651T (the homologous site of the Lurcher mutation at

the end of the highly conserved SYTANLAAF sequence in the M3/S2 linker region) reduces PAS inhibition but has no effect on PS potentiation. Likewise, mutations in this region of GluN1 and GluN2B, alter PAS potency³⁸. Thus, the PAS NAM binding site may be at the extracellular / membrane interface of M3/S2 or require allosteric interactions at this location.

2.2 Cholesterol derivatives

2.2.1 Cholesterol derivative PAM and NAM SAR

The endogenous compound 24(S)-hydroxycholesterol **5** (24(S)-HC) has a structure and PAM activity similar to that of PS but is more potent ($EC_{50} \sim 1 \mu M$) and thought to have a different binding site (Figure 3)⁴⁶. Receptor chimera studies indicate that PS requires transmembrane domains and some of the LBD whereas 24(S)-HC primarily requires just the transmembrane domains⁴⁷. The concept of different binding sites is also consistent with the observation that 24(S)-HC preapplication occludes the activity of the 24(S)-HC derivative SGE-201 (**6**, Figure 3), but not that of PS. While endogenous cholesterol **4**, from which 24(S)-HC is derived (Figure 3), helps to maintain baseline NMDAR responsiveness⁴⁸, it does not have potent PAM activity. 24(S)-HC is selective for NMDARs over AMPA and GABA-A receptors but does not distinguish between the four GluN1/GluN2 receptors. Further modification of the **D** ring alkyl chain by adding two methyl groups at C24 together with the hydroxyl group (SGE-201 **6**) increases PAM potency nearly ten-fold over 24(S)-HC. In contrast, 25-hydroxycholesterol (25-HC) (**7**) has very weak PAM activity and instead non-competitively blocks the potentiation by 24(S)-HC and SGE-201, but not by PS⁴⁹. This result again suggests that PS and 24(S)-HC have distinct binding sites and that 24(S)-HC and 25-HC may also have distinct binding sites.

2.2.2 Cholesterol PAM & NAM mechanism of action

The mechanism of action of SGE201 and 24(S)-HC is to increase open channel probability, as indicated by an acceleration of the rate of MK-801 open channel blockade of NMDARs. Also, like PS, SGE201 can access its binding site from the outside through the plasma membrane but not from the intracellular side⁴⁹. The onset and offset of PAM activity is very slow, which is consistent with SGE201 accessing its binding site through the membrane. PAM activity is associated with a small increase in L-glutamate potency, but this may be due to an increase in agonist efficacy rather than an increase in agonist affinity.

3. Non-steroidal NMDAR NAMs

3.1 GluN2A-selective NAMs

3.1.1 Sulfonamide series—Bettini and colleagues⁵⁰ identified the first highly selective non-competitive GluN2A inhibitors which were also the first ligands later reported to bind at the LBD GluN1/GluN2 dimer interface⁵¹. The prototype is TCN-201 **8** (Figure 4a). Although the inhibitory activity of TCN-201 is reversed by high concentrations of glycine in a manner largely consistent with a competitive antagonist⁵⁰, Schild analysis indicates that the compound is a non-competitive antagonist that reduces glycine and D-serine affinity^{52, 53}. In the initial study, five lead compounds were identified in a high-throughput screen using a FLIPR®/Ca²⁺ assay, with all fully blocking human recombinant GluN2A

receptors. A number of structurally similar analogues were identified and tested in order to gather SAR information. TCN-201 (**8**) was one of the lead compounds, and one of the more successful of the series, displaying submicromolar potency ($IC_{50} \sim 100$ nM) with >300-fold selectivity for GluN2A over GluN2B, GluN2C, and GluN2D^{50, 53}.

3.1.1.1 Sulfonamide series NAM SAR—Replacing ring **B** of TCN-201 with various cycloalkyl or heteroaryl moieties had little effect on activity, perhaps indicating a larger space was available than the one currently occupied (Figure 4a). With respect to ring **C**, the 3-Cl was recently shown to be essential with its removal drastically reducing activity⁵⁴. The 4-F on the other hand, was found to be superfluous with its removal marginally increasing activity. The 3-Br analogue (**9**, $IC_{50}=204$ nM) was reportedly 2.5-fold more potent than TCN-201 ($IC_{50}=512$ nM) against GluN1/GluN2A receptors (Figure 4b).

Although TCN-201 has been used in a small number of studies to investigate the role of GluN2A in physiological processes⁵⁵⁻⁵⁷, its use as a tool in native systems has been limited due to poor aqueous solubility. Consequently, a SAR study was initiated with the aim of improving potency and drug-like properties whilst maintaining GluN2A selectivity⁵⁸. Keeping the phenylsulfonamide ring constant, it was found that replacing the phenylhydrazide portion of the molecule with a 2-(methylthiazol-5-yl)methanamine moiety did not significantly affect activity (Figure 4c). To lower lipophilicity, the central benzene ring (**A** in Figure 4a) was replaced with either a pyrazine or pyridine ring. Encouragingly, the resultant analogues displayed significantly improved potency and the pyrazine-containing derivatives had superior pharmacokinetic properties. Further observations included: (i) an improvement in potency on addition of a methyl group to the pyrazine ring (i.e. $R^4 = Me$), (ii) swapping the 3-Cl and 4-F substituents on the benzenesulfonamide ring resulted in a large drop in activity, and (iii) alkylation of the sulfonamide nitrogen was detrimental ($R^5 = Me$). Considering GluN2A potency, selectivity, solubility and other pharmacokinetic properties, MPX-004 **10** ($IC_{50} = 79$ nM) and MPX-007 **11** ($IC_{50} = 27$ nM) were selected for further investigation. Although MPX-004 had a lower antagonist potency at GluN2A than MPX-007, it was more selective over the other GluN2 subtypes of the NMDAR (Figure 4c).

3.1.1.2 Sulfonamide series: LBD-interface NAM binding site and mechanism of action—The structural basis of both glycine reversal of NAM activity and GluN2A selectivity of TCN-201 and MPX-004/MPX-007 are now understood⁵¹. Site-directed mutagenesis and crystallography studies indicate that the TCN-201 binding site is located at the dimer interface between the two ligand binding domains (Figure 5) at a site partially overlapping with the PAM GNE-6901 binding site (discussed below). In the binding pocket, both TCN-201 and a related compound (compound **6** from Bettini and colleagues⁵⁰) were found to fold back on themselves with stacking of the middle and the halogenated rings^{51, 59} (Figure 6). The middle ring also interacts with the GluN2A-specific residue V783 which accounts for TCN-201's selectivity; this residue is replaced by phenylalanine in GluN2B and leucine in GluN2C and GluN2D which sterically hinder NAM binding. When the NAM is bound, GluN2A V783 is displaced and, in turn, interacts sterically with GluN1 F754. This latter interaction is unfavorable and can be accommodated by glycine unbinding and

opening of the GluN1 LBD to allow GluN1 F754 to move away from GluN2A V783⁵¹. Thus, the NAMs stabilize the open, inactive GluN1 LBD conformation. Using a GluN1 construct in which the LBD is locked in the closed, active conformation by incorporating disulfide bonds across the LBD cleft, confirms that opening of the GluN1 LBD is necessary for TCN-201/MPX-004/MPX-007 inhibition⁵¹.

3.2. GluN2C/D selective NAMs

3.2.1 The quinazoline-4-one series—A series of non-competitive inhibitors containing a quinazoline-4-one ring system (e.g. QNZ46, **15**, Figure 7d) was found to be selective for GluN2C/D- over GluN2A/B-containing NMDARs⁶⁰. Compounds with an (*E*)-3-phenyl-2-styrylquinazoline-4(3*H*)-one backbone were identified during a high throughput screen looking for allosteric NMDAR antagonists. Lead compounds **12** and **13** displayed IC₅₀ values of 9 μ M and 5 μ M against GluN2D, respectively (Figure 7a). However, these hits were structurally similar to the non-competitive AMPAR antagonist CP-465022 (**14**)⁶¹ so selectivity over AMPARs was predicted to be an issue (Figure 7b). When tested, both compounds proved inactive at kainate receptors (KARs), but had only 4 to 5-fold selectivity for NMDARs over AMPARs.

3.2.1.1 Quinazoline series NAM SAR—An SAR study was subsequently conducted with the aim of optimizing potency and selectivity toward GluN2D containing NMDARs. The study consisted of making systematic modifications to the ring substituents; the optimal substituent and position was determined for each of the three rings in turn (Figure 7c). Analogues without substituents on any of the three rings were inactive at both NMDARs and AMPARs. The optimal substituent on ring **A** was found to be a *para*-carboxylic acid ($R^3 = p\text{-CO}_2\text{H}$), regardless of what substituents were present on the other two rings. With respect to ring **B**, a nitro group proved best with the *ortho* and *meta* analogues ($R^4 = o\text{-}$ or $m\text{-NO}_2$) being roughly equipotent versus GluN2D. Interestingly, the *para*-nitro derivative ($R^4 = p\text{-NO}_2$) was less active but displayed better selectivity for GluN2D over GluN2A and AMPARs. Keeping the favored carboxylate and nitro groups constant on rings **A** and **B** ($R^3 = p\text{-CO}_2\text{H}$, $R^4 = m\text{-NO}_2$), attention turned to optimizing ring **C**. Substitution at the 6-position with either a methoxy or iodo group proved most beneficial to potency and selectivity ($R^5 = 6\text{-OMe}$ or 6-I). Having extensively explored the substitution on each ring, the effect of modifying the backbone itself was examined. Removal of ring **A** resulted in complete loss of inhibition while reduction of the styryl linker to the corresponding phenethyl analogue reduced potency 10-fold. Replacement of ring **A** with larger aromatic systems was tolerated to some extent, suggesting space in the binding pocket for a larger hydrophobic group. From the series, compounds **15** (QNZ46) and **16** were the most potent and selective compounds for GluN2C/D-containing NMDARs when tested using a two-electrode voltage clamp (TEVC) assay (Figure 7d); **15** had an IC₅₀ value of 3 μ M at GluN2D and 6 μ M at GluN2C in comparison to >200 μ M at GluN2A/B and AMPAR, while **16** had an IC₅₀ of 2 μ M at GluN2C and 1 μ M at GluN2D and is over 300-fold selective for GluN2D over GluN2A/B and AMPARs.

Although at times it was difficult to pin down which substitution patterns were controlling potency and selectivity, the fact that there were variations in selectivity suggests the potential

to achieve selectivity over GluN2A/2B-containing NMDARs, AMPARs and KARs. Overall the series indicated a promising starting point for achieving potent NAMs that were selective for GluN2C and GluN2D versus GluN2A and GluN2B.

3.2.1.2 Quinazoline NAM series: QNZ46 binding site and mechanism of

action—The subtype-selectivity of QNZ46 is due to residues located in S2, but it has not been possible to define the binding site precisely. By taking advantage of the differential activity of QNZ46 on GluN2A and GluN2D, chimera and point mutations studies were able to identify a cluster of residues in the GluN2 S2 domain that are important for QNZ46 activity⁶². Since these residues have a relatively weak effect on QNZ46 activity, it was suggested that these residues may not be directly contributing to the binding site but may instead be contributing to QNZ46's actions. These critical residues are located on the lower portion of the S2 domain near the membrane and the linker sequences between the LBD and the TMDs. These residues are thus positioned where they may influence channel gating through interacting with the linkers to M1 and M3 (Figure 8). It is possible that QNZ46 binds in a site that is conserved among subunits, but the ability of binding to transduce inhibition involves GluN2-specific residues downstream.

QNZ46 NAM activity is mechanistically distinct from the TCN-201-related GluN2A inhibitors discussed above. Unlike TCN, QNZ46 has minimal and distinctly different effects on agonist potency⁶². Whereas TCN inhibits by reducing glycine potency, QNZ46 at GluN1/GluN2D receptors increases L-glutamate potency 2-fold and has a smaller effect at increasing glycine potency. A notable feature of QNZ46 inhibition that provides a hint as to the inhibitory mechanism is the requirement of L-glutamate binding. QNZ46/glycine pre-incubation followed by L-glutamate plus QNZ46/glycine application results in a transient peak response followed by a steady-state inhibition. These and other experiments led to the idea that L-glutamate, but not glycine, is necessary for QNZ46 binding. Also, QNZ46 unbinding may be partially necessary for L-glutamate unbinding since QNZ46 presence slows receptor deactivation due to L-glutamate (but not glycine) removal. This property can account for the 2-fold increase in L-glutamate potency by QNZ46. These findings led to the model that QNZ46 inhibits NMDAR function by binding somewhere in/near the lower lobe of S2 at a binding site exposed by L-glutamate binding. LBD cleft closure due to L-glutamate binding is thought to pull on the LBD's S2-M3 linker to open the channel. NAM binding in this region may block the ability of the S2-M3 linker to move in response to LBD cleft closure thus keeping the channel closed and at the same time stabilizing the closed LBD conformation. This mechanism thus accounts for the ability of a NAM to increase agonist potency while blocking channel activation.

3.2.2 The Pyrazoline series—The pyrazoline scaffold was found to have GluN2C/D NAM activity from a high throughput screen. The initial hit, **17** (Figure 9a), had an IC₅₀ of 2.7 and 5.4 μ M at GluN2D and GluN2C respectively, with marginal selectivity over the other NMDAR subunits (IC₅₀ = 78 μ M at GluN2A and 19 μ M at GluN2B)⁶³. As for QNZ46, residues in the S2 domain near the membrane are important for the subtype-selectivity of these compounds, but the precise binding site remains to be defined. An in-depth SAR study resulted in the development of several compounds with IC₅₀ values in the

100-500 nM range with 50- to 200-fold selectivity for GluN2C- and GluN2D- over GluN2A or GluN2B-containing NMDARs.⁶⁴ These compounds also showed minimal off-target activity when tested against AMPA, kainate, glycine, serotonin, GABA, and nicotinic receptors.

3.2.2.1 The Pyrazoline series: SAR—During SAR studies, systematic modifications were made to rings **A**, **B**, and **C** of **17** (Figure 9b). With respect to ring **A**, electron-withdrawing groups at the *para*- position were found to bestow the best activity, with a chloro substituent ($R^1 = \text{Cl}$) proving optimal. Replacement of ring **A** with various heterocyclic rings including furan and thiophene reduced activity drastically. The introduction of electron-withdrawing substituents to ring **B** also enhanced activity with *meta*- and *para*- substituted derivatives (e.g. $R^2 = m$ - or *p*-Cl) proving roughly equipotent. In contrast, the addition of substituents to ring **C** (R^3) had a detrimental effect, although the resultant analogues showed variability with regard to selectivity for GluN2A over GluN2B containing receptors, thereby suggesting a potential for optimizing selectivity. Having thoroughly investigated rings **A**, **B**, and **C**, attention was focused on the acyl chain. Incorporation of a double bond had little effect on potency as did extension of the chain from succinic ($n = 1$) to glutaric acid ($n = 2$). Swapping the terminal acid ($R^4 = \text{CO}_2\text{H}$) for a hydroxymethyl ($R^4 = \text{CH}_2\text{OH}$) greatly improved selectivity for GluN2D over GluN2A whilst maintaining similar activity at the other NMDAR subtypes. However, replacement of the carboxylic acid with an amide ($R^4 = \text{CONH}_2$) reduced both potency and selectivity while a fluoro group ($R^4 = \text{F}$) resulted in all activity being lost. Compound **18** (DQP-26) is representative of one of the more successful compounds with IC_{50}s of 0.77 and 0.44 μM at GluN2C and GluN2D respectively, with ca. 50-fold selectivity over GluN2A- and GluN2B-containing receptors. Enantiomeric separation showed that the *S*-enantiomer of **18** was 11-fold more potent at GluN2D than the *R*-enantiomer and had improved selectivity for GluN2C and GluN2D over GluN2A and GluN2B.

3.2.2.2 The Pyrazoline series: DQP-1105 binding site and mechanism of action—DQP-1105 (**19**, Figure 9) and QNZ46 partially share the same structural determinants in the receptor that are responsible for the GluN2C/GluN2D selectivity of these compounds^{62, 63}. Thus, they may have overlapping binding sites and a similar mechanism of action. From GluN2A/GluN2D chimera studies, sequences in the N-terminal third of the S2 domain are necessary for DQP-1105 inhibitory activity. Of the GluN2D-specific residues in this region, mutating Q701 and L705 to the corresponding amino acids in GluN2A significantly reduce inhibition by DQP-1105 and QNZ46. Another similarity between these two classes of compounds is that their binding requires L-glutamate binding and thus they act as use-dependent inhibitors. DQP-1105 decreases open probability by increasing the mean shut time without affecting mean open time and has minimal effects on channel conductance. Thus, the non-competitive inhibition appears to be through reducing the probability of channel activation and not by reducing the stability of the open state.

3.2.3 GluN2C/D selective NAMs: The Iminothiazolidinone series and their SAR—A novel series of NAMs showing a slight preference for GluN2C/D containing NMDARs was identified from a medium-throughput screen⁶⁵. The lead compound, **20**,

consisted of an iminothiazolidinone ring attached to a thiophene via an acetamide linker (Figure 10a). Alterations to the substituents on the thiophene ring suggested that the ethyl group at R³ was favorable, with its removal (R³ = H) or replacement with methyl (R³ = Me) proving deleterious to activity (Figure 10b). The introduction of alkyl substituents at R² (e.g. Me or Et) also proved detrimental. In contrast, the methyl ester could be replaced with an ethyl ester (R¹ = Et) without adversely affecting potency. With shortening or removal of the ethyl group at R³ decreasing activity, the authors hypothesized that a hydrophobic pocket may exist at this position. To explore this theory, the thiophene was replaced with various bicyclic thiophene rings. Several of these analogues (e.g. **21**, Figure 10c) proved quite potent in a TEVC assay, with the incorporation of heteroatoms (S or O) into the ring giving potent inhibitors with marginal selectivity for GluN2D over GluN2A. For example, **21** displayed an IC₅₀ of 0.8 μM against both GluN2C and GluN2D compared to 6.2 μM and 12.2 μM for GluN2A and GluN2B, respectively.

Changes to the aminothiazolidinone ring, namely changing the heteroatoms, were not well tolerated. For example, replacement of the imine with a carbonyl (i.e. Y = O) or methylation of both nitrogen atoms (i.e. X = Y = NMe) led to all inhibitory activity being lost. This suggested that hydrogen bond donor groups were essential for NAM activity. Although none of the compounds were particularly selective, some did show submaximal inhibition at saturating concentrations. Thus, as proposed for some of the naphthoic acid based NMDAR NAMs (section 4.1.2)⁶⁶, inhibitory activity may provide neuroprotection without risking excessive blockade. In addition, the neuroprotective effect of another potent compound, **22**, was demonstrated in an assay using cultured hippocampal neurons challenged with NMDA.

3.2.4 GluN2C/D selective NAMs: The *N*-aryl benzamide series and their SAR

—A screen of 100,000 compounds, which had already resulted in the discovery of a series of GluN2C selective PAMs (see section 5.3.1), identified *N*-aryl benzamide **23** as a novel NAM of GluN2C/2D containing NMDARs (Figure 11a)⁶⁷. When tested on recombinant NMDARs in *Xenopus* oocytes, **23** displayed IC₅₀s of 2.6 μM and 1.4 μM versus GluN2C and GluN2D respectively, with >400-fold selectivity for these subunits over GluN2A/B. However, despite promising activity, **23** had poor aqueous solubility so an SAR optimization study was carried out.

48 analogues of **23** were synthesized and tested leading to a number of general SAR observations, the most important of which are summarized in Figure 11b. Replacing the carbamothioate in **23** with a carbamate improved aqueous solubility but decreased both activity and selectivity. However, replacement of the naphthalene with an indole ring restored low micromolar potency whilst retaining improved solubility. Shortening or extending the alkyl component of the carbamate to either *N,N*-dimethyl or *N,N*-diisopropyl was found to reduce potency. A variety of substituents were introduced to the indole ring, but all were found to reduce activity or abolish it completely. Overall, NAB-14 (**24**) displayed the best combination of activity, selectivity and aqueous solubility (Figure 11c).

4. NMDAR modulator families with NAM and PAM activity with varying selectivity

4.1 Phenanthrene, Naphthalene and Coumarin Carboxylic Acids

4.1.1 The phenanthrene series and their SAR—The phenanthrene series of PAMs and NAMs were identified through a small in-house compound screen on NMDARs⁶⁸. These compounds displayed several distinct, novel patterns of activity with NAM and/or PAM activity and varied subtype-selectivity. The lead compound, 9-iodophenanthrene-3-carboxylic acid, **25** (UBP512) (Figure 12a) was found to potentiate GluN2A, have virtually no activity at GluN2B, and inhibit GluN2C/D responses ($IC_{50} \sim 50 \mu M$). UB512 potentiation of GluN1/GluN2A responses increased with higher agonist concentrations, showing that the mechanism of potentiation is not by increasing agonist potency. A series of analogues were subsequently synthesized to try and exploit this selectivity whilst improving the activity. A SAR study was carried out investigating substitutions at the 9-position and modifications to the acidic group at the 3-position (Figure 12b).

Alkyl substituents at the 9-position appeared to promote potentiating activity; increasing the length or size of the alkyl chain increased NMDAR PAM activity. Introduction of a polar group into the side chain, however, promoted antagonism. Inserting a CH_2 linker between the ring and carboxylic acid also promoted antagonism rather than potentiation⁶⁹. Some of these compounds also had mixed subunit selectivity; UB710 (**26**), with a cyclopropyl group at the 9-position, potentiated GluN2A/B and weakly inhibited GluN2C/D-containing receptors at higher concentrations⁶⁸. UB646 (**27**), with a large hydrophobic iso-hexyl group, was found to be a pan potentiator (Figure 12c).

4.1.2 Naphthoic acid series and their SAR—In a follow up study to the phenanthrene-based research, compounds with a naphthoic acid core were synthesized to probe the importance of the three rings to activity (Figure 13a). Although many of the compounds in this series displayed NAM activity, the addition of a long chain alkyl group at R^4 led to potentiation. As found for the phenanthrenes, extending the alkyl side-chain from propyl to hexyl progressively increased PAM activity. One of the most effective pan PAMs in the series, UB684 (**28**), had an *i*-hexyl substituent at the R^4 position (Figure 13b)⁷⁰. In the absence of an alkyl side-chain, the naphthoic acid derivatives were predominately non-selective NAMs whose potency across the GluN2 subunits was increased by halogen substituents at R^2 and R^5 , a hydroxy group at R^1 , and a phenyl ring at R^5 in a generally additive manner (Figure 13a). Thus, increasing potency reduced selectivity such that 1-bromo-2-hydroxy-6-phenyl-3-naphthoic acid, **29** (UBP618), is a non-selective NAM with an $IC_{50} \sim 2 \mu M$ ⁶⁶. The carboxylic acid was found to be crucial to activity; removing it from one of the more potent analogues eliminated activity. Phenyl substituents at R^5 afforded NAMs that cannot maximally inhibit NMDARs (maximal inhibition of 60 – 90%), thus potentially generating antagonists that might not excessively inhibit function (Figure 13a)⁶⁸.

The 3,5-dihydroxy derivative of 2-naphthoic acid, UB551 (**30**), showed PAM activity on GluN2D but NAM activity on GluN2A-C and is therefore a lead for the development of GluN2D selective PAMs (Figure 13b)⁶⁸.

4.1.2.1 Naphthoic acid series PAMs: UBP684 mechanism of action—The naphthoic acid derivative UBP684, 6-(4-methylpent-1-yl)-2-naphthoic acid (**28**), displays robust potentiation of each of the four GluN1/GluN2 receptors and was thus selected for further mechanistic studies^{70, 71}. UBP684 increases open probability in a use-independent manner in the presence of saturating concentrations of agonist⁷⁰. Potentiation is associated with a minor increase in L-glutamate potency at GluN2A and a decrease in L-glutamate potency at GluN2C and GluN2D. Despite these opposite actions of UBP684 on L-glutamate potency at GluN2A and GluN2D, UBP684 potentiates the steady-state response, and slows the deactivation upon L-glutamate removal, at both of these receptors⁷⁰. The potentiation of steady-state responses appears to be distinct from the slowing of deactivation; the potentiation response is lost in dialyzed cells from whole-cell recordings, whereas the slowed deactivation remains⁷¹. Thus, like PAM activity of PS, the intracellular environment can differentially affect the potentiation mechanisms of UBP684.

Channel analysis⁷¹ indicates that potentiation by UBP684 is due to an increase in open probability by increasing mean open time and reducing the long-lived shut times with no change in the single channel conductance. PAM activity appears to specifically affect gating steps governed by the GluN2 subunit's LBD, a result that is consistent with the finding that steady-state PAM activity requires a conformational change in the GluN2 LBD, but not that of GluN1's LBD^{70, 71}. Studies of pH effects on PAM activity revealed that the PAM activity of UBP684, PS, CIQ, and GNE-8324 were all reduced at high pH, with UBP684 and GNE-8324 even becoming NAMs at pH 8.4⁷⁰. This suggests the possibility that these PAMs stabilize a receptor conformation that is intermediate between the fully-protonated, inhibited receptor and the de-protonated, maximally responsive receptor.

4.1.2.2 Naphthoic / phenanthroic acid series PAM binding site(s)—The binding site for the naphthoic / phenanthroic acid series of PAMs is not known but appears to be either at the LBD dimer interface or closer to the TMD. Removal of both the GluN1 and GluN2 ATDs does not eliminate the potentiating activity of UBP512⁶⁸. These agents also do not compete with L-glutamate or glycine and cannot mimic either agonist; thus, they are not binding in the ligand-binding cleft of the LBD of GluN1 and GluN2. They also do not have voltage-dependent activity and do not compete with ketamine for the channel binding site, thus they do not appear to bind in the central pore of the channel. In GluN2A/GluN2C chimeras, UBP512 PAM activity is associated with S2. Thus, the PAM binding site may overlap with the PS PAM binding site that involves S2 and M4 and/or the LBD dimer interface site that involves S1 and S2. Residues in M4 are critical for UBP512/UBP684 PAM activity (Figure 8), hence M4 may be contributing to PAM binding or the transduction of PAM activity.

4.1.3 Coumarin-3-carboxylic acid series NAMs / PAMs and their SAR—The previously described SAR studies on 2-naphthoic acid derivatives led to the identification of a structurally related coumarin (**32**, UBP608, Figure 14a) which displayed NAM activity with weak selectivity toward GluN2A containing NMDARs (IC_{50} = 19, 90, 68, and 426 μ M at GluN2A-D, respectively)⁶⁶. With respect to SAR, the 6-bromo group was found to be important with its removal proving detrimental to activity (Figure 14b)⁷². Whilst the 6-

bromo substituent could be replaced with an iodo to afford a derivative with similar activity and selectivity, the introduction of a more polar 2-carboxyvinyl moiety (**33**, UBP656) significantly reduced potency across all 4 subtypes (Figure 14c). The introduction of a bromo group at the 8-position enhanced activity but led to reduced GluN2A selectivity. Interestingly, 4-methyl substitution of UBP608, yielding **34** (UBP714), turned the compound from a NAM to a weak PAM (Figure 14c). UBP714 displayed low levels of potentiation at GluN2A, GluN2B and GluN2D⁷³. UBP714 also enhanced NMDAR EPSPs evoked in hippocampal slices.

4.1.4 Coumarin 3-carboxylic acid / naphthoic / phenanthroic NAM binding site(s) and mechanisms of action.—NAM activity of the naphthoic acid and phenanthroic acid derivatives is voltage-independent, and use-independent^{66, 68}. The IC₅₀ of UBP552 (**31**, Figure 13) is increased only 3-fold in the presence of 150-fold higher L-glutamate concentration and 30-fold higher glycine concentration, thus the NAM activity is non-competitive and unlike TCN-201 which behaves largely as a competitive glycine antagonist. Channel analysis indicates that 2-naphthoic acid decreases mean open time and increases mean closed time⁷⁴. This action appears to be due to stabilizing closed states and making it more difficult to open the channel. The NAM binding site is unknown, but NAM activity remains after removal of the ATD of both GluN1 and GluN2⁶⁸. These agents also cannot substitute for, or compete with, either L-glutamate or glycine, so they do not appear to bind within the LBD cleft. GluN2A/2C selectivity of UBP618 requires residues in the S1 domain.

5. Non-steroidal NMDAR PAMs

5.1 Non-selective PAMs

5.1.1 Benzenesulfonamide—The non-selective PAM GNE-9278 (**35**), which potentiates each of the GluN1/GluN2 NMDARs in the low micromolar range, was recently reported by Wang and colleagues (Figure 15)⁷⁵. This compound potentiates by increasing the potency of both L-glutamate and glycine and by increasing the peak response in the presence of saturating concentrations of agonists. At higher concentrations, GNE-9278 slows deactivation upon L-glutamate removal. Consistent with a strong allosteric interaction between the agonist binding site and the PAM binding site, GNE-9278 binding is thought to be dependent upon agonist binding. The effect on channel properties is unknown. The structural determinants for activity of this compound include residues T550 and D552 in the GluN1 pre-M1 helix at a site near to where residues on GluN2C/D are important for CIQ activity (Figure 8). Binding at this site can then influence gating at the nearby extracellular end of M3.

5.2. GluN2A selective PAMS

5.2.1 Thiazole series

Recent studies have now provided selective GluN2A PAMs with reasonable potency and drug-like properties. These agents bind at the same GluN1/GluN2 LBD dimer interface as the TCN-201 family of NAMS^{59, 76}. The research involved hit identification via high-

throughput screening for GluN2A PAM activity, followed by lead optimization using a combination of X-ray crystallography, structure-based design and SAR studies. The lead compound, designated GNE-3476 (**36**)⁷⁶, displayed low micromolar potency at GluN2A, some activity as an AMPAR PAM, and weak activity at GluN2B-containing receptors (Figure 16a). Further studies confirmed that the compound was acting as an allosteric modulator and identified several structurally-related compounds that display 10-fold to 100-fold selectivity for GluN2A over GluN2B and still greater selectivity over GluN2C/D containing NMDARs⁵⁹.

5.2.1.1 Thiazole series GluN2A PAM SAR

Compounds were tested for their ability to potentiate either NMDARs or AMPARs using a calcium imaging assay and evaluated for their P-glycoprotein efflux ratio (P-gp ER) to identify favorable CNS properties. Initial attempts to optimize the GluN2A PAM activity indicated that shortening the butyl chain and adding a fluoro group at the *para*- position of the aniline ring enhanced PAM activity at GluN2A. A crystal structure of one of these optimized analogues bound to the receptor was obtained and showed the binding site to be located at the dimer interface of the GluN1-GluN2A LBD⁷⁶. This turned out to be the same site where PAM binding is observed in AMPARs⁷⁷, explaining the poor selectivity over AMPARs for the initial lead. This binding site also has significant overlap with the TCN-201 NAM binding site discussed above. Thus, ligands at this site can either be NAMs or PAMs depending upon the specific interactions within the binding pocket.

The pharmacokinetic properties of these lead compounds were generally favorable for use in the CNS; moderate log *D* (<3) and low topographical polar surface area (<90 Å²) values were recorded, however, poor metabolic stability was observed, in particular as a result of *N*-dealkylation. Consequently, one aim of the optimization study was to replace the *N*-ethyl aniline with a more metabolically stable group (Figure 16b). Although various aryl and heteroaryl moieties were explored, a 3-trifluoromethyl pyrazole was identified as a good candidate. Modelling studies suggested that substituents at the 5-position of this heterocycle could occupy the same binding pocket as the *N*-ethyl group in the lead compounds. Various groups were subsequently investigated with a 5-chloro moiety proving optimal for GluN2A activity and selectivity.

Another aim of the study was to explore a water-filled pocket proximal to the thiadiazole-core nitrogen which had been identified from the crystal structure. While AMPARs have a similar pocket in their equivalent site it is relatively small, meaning a large group could potentially enhance selectivity for NMDARs over AMPARs. To investigate this, the thiadiazole core was replaced with a thiazole thereby allowing substituents to be introduced to the 3-position of the ring (Figure 16b). This change was found to moderately improve GluN2A PAM activity on its own. The introduction of polar groups to the 3-position generally improved GluN2A activity and selectivity but increased the P-gp ER, making the resultant analogues less effective at crossing the blood brain barrier (BBB). Achieving a balance between activity, selectivity, metabolic stability and ability to cross the BBB proved challenging. However, by utilizing a cyclopropyl nitrile substituent at the 3-position of the thiazole ring a balance was eventually achieved. Lastly, the addition of a trifluoromethyl

group to the 2-position of the thiazole core improved selectivity over AMPARs and afforded GNE-0723 (**37**), the most successful compound in the series (Figure 16c). The large hydrophobic group is believed to be incompatible with the polar serine and asparagine residues in the equivalent site of the AMPAR, explaining the 250-fold selectivity for GluN2A over AMPARs. This selectivity was achieved without compromising potency (EC_{50} = 0.021 μ M) or metabolic stability.

A crystal structure of GNE-0723 bound to GluN2A⁵⁹ showed, as predicted, that the conformation of the *trans*-cyclopropyl enables the nitrile moiety to occupy the water-filled pocket. Selectivity over GluN2B/C/D was also achieved; GNE-0723 was ~300-fold more selective for GluN2A over GluN2C and GluN2D and yet more selective over GluN2B with weak potentiation at 100 μ M. A later optimization campaign, using GNE-0723 as a lead compound, saw a pyridopyrimidinone replace the previous thiazolopyrimidinone core. This led to GNE-5729 (**38**), which displayed an improved *in vivo* pharmacokinetic profile (Figure 16c)⁷⁸.

5.2.1.2 Thiazole series GluN2A PAM binding site and mechanism of action

Precisely how GNE-6901/GNE-0723 (**39/37**, Figure 16c) potentiate GluN1/GluN2A receptor responses is not known, but they appear to stabilize the agonist-bound conformation. These compounds increase agonist efficacy and have variable effects on agonist potency⁵⁹. Crystallographic studies indicate that, like TCN-201, GNE-6901 selectivity for GluN2A is due to V783. Replacing this residue in GluN2A with the corresponding residue from GluN2B (phenylalanine) essentially eliminates GNE-6901 PAM activity and substituting valine for this residue in GluN2B enables PAM activity (but of several-fold lower potency than GluN2A)⁵⁹. A potentiation mechanism is suggested by the observation that AMPAR potentiators that bind at this site have been shown to reduce desensitization and slow deactivation by stabilizing the AMPAR LBD dimer interface and the agonist-bound LBD in its closed cleft conformation⁷⁷. Consistent with this mechanism, binding of GNE-8324 (**40**, Figure 16c) to GluN1/GluN2A causes a marked slowing of deactivation associated with L-glutamate removal but not with glycine removal. Interestingly, however, the closely related compound GNE-6901 only causes a modest slowing of deactivation following L-glutamate removal⁵⁹. GNE-8324 also causes a greater increase in L-glutamate potency than does GNE-6901, and reciprocally, increasing concentrations of L-glutamate increases GNE-8324 potency but minimally increases GNE-6901 potency. Thus, GNE-8324 PAM activity is consistent with a stabilization of the closed, L-glutamate bound GluN2A LBD conformation, but PAM activity of GNE-6901 appears to require an additional mechanism for increasing agonist responses.

In contrast to the NAM activity of TCN-201, the PAM activity of GNE-6901 is not associated with a displacement of the side chains of GluN2A's V783 and, instead, is associated with a movement of GluN1 Y535 and GluN2A E530 side chains⁵⁹. The GluN1 Y535 residue interacts with the GluN2 hinge region and mutating this residue affects deactivation upon L-glutamate removal and open channel probability. Thus, GNE-6901 potentiation may involve this residue⁵¹ although this could not be confirmed by mutational

analysis⁵⁹. Presently, single channel studies of GNE compound activity have not been reported, but such studies should help reveal the mechanism of action.

5.3. GluN2C selective PAMs

5.3.1 The pyrrolidinone series

The first GluN2C-selective PAM was obtained from a series of pyrrolidinones^{79, 80}. The most active analogues of these allosteric potentiators were over 100-fold selective for receptors containing GluN2C over GluN2A, B and D. The lead compound – identified through library screening/bioinformatics searches – was pyrrolidinone **41** (Figure 17a).

5.3.1.1 The pyrrolidinone series: SAR

To gather SAR information and optimize PAM activity on GluN2C, a large number of structural analogues of **41** were synthesized and tested (Figure 17b). Interestingly, these modifications revealed a relatively flat SAR indicating that most of the structure was already optimized. For example, PAM activity was either lowered or abolished completely by: (i) changing the position or type of substituents on ring **A**, (ii) replacing ring **B** with various aryl or heteroaryl systems, (iii) modifying the enol or (iv) shortening or lengthening the alkyl linker (Figure 17b). However, changes to R¹ indicated that there was room for larger substituents at this position with either a phenyl, 3-pyridyl or 4-pyridyl ring bestowing moderately better activity (interestingly the 2-pyridyl derivative was inactive). Changing the methyl ester on ring **A** to an ethyl ester was tolerated, but bulkier esters proved detrimental. The space surrounding ring **B** was systematically explored by the addition of various substituents (e.g. R³ = F, Cl, Me, OMe). This identified the 6-position as being optimal with a methyl group giving the best activity. Finally, methylation of the indole nitrogen abolished activity thus suggesting a hydrogen bond donor role for the indole hydrogen (Figure 17b). Combining these observations gave compound **42** (PYD-111), which was the most potent of the series, selectively potentiating GluN2C-containing receptors up to 219% with an EC₅₀ of 4.3 ± 0.3 μM. On separation of the enantiomers for a representative analogue from the series, it was found that the activity of the compounds originates solely from one enantiomer. Although relatively few alterations were made to the original molecule the activity was significantly enhanced.

5.3.1.2 The pyrrolidinone series: PYD-106 binding site and mechanism of action

The mechanism of action of an analogue from the series, PYD-106 (**43**, Figure 17c), has been proposed. PYD-106 potentiation of GluN1/GluN2C NMDAR responses appears to be predominately through increasing agonist efficacy rather than a change in agonist potency⁷⁹. Thus, under saturating agonist concentrations, PYD-106 can increase the maximal receptor response. There is, however, some effect on agonist potency; PYD-106 causes a small increase in glycine potency and a small reduction in L-glutamate potency. Single channel analysis indicates that PYD-106 does not change channel conductance, and instead potentiates GluN1/GluN2C receptor responses by increasing mean open time and may also increase opening frequency. Thus, PYD-106 stabilizes an open channel state of the receptor complex.

The activity of PYD-106 is notable because it is unusual to identify compounds that can distinguish GluN2C from GluN2D. This suggests that PYD-106 binds to site with greater GluN2 sequence variability. By using a series of GluN2A/GluN2C chimeras, the ATD, S1 and the ATD-S1 linker were identified as being important for PYD-106 activity⁷⁹. Subsequent evaluation of an extensive panel of point mutations then lead to the proposal of a binding pocket between the ATD and S1 that could accommodate PYD-106 (Figure 18). Further point mutations based on the docking in a homology model provided additional support for this GluN2 ATD/S1 binding pocket being the PYD-106 binding site. This binding site is likely to influence communication between the ATD and the LBD and thus appears to represent a novel mechanism of NMDAR potentiation. Structural studies are needed to confirm the PYD-106 binding site and to determine how binding influences receptor conformation.

5.3.2. GluN2C/D PAMs: The tetrahydroisoquinoline series (CIQ)

A series of tetrahydroisoquinoline derivatives has been reported as selective potentiators of GluN2C and GluN2D containing NMDARs⁸¹. The lead compound for this series was CIQ (**44**, Figure 19a), which displayed an EC₅₀ of 3 μ M and enhanced receptor responses ~2-fold⁸². To gather SAR information and improve activity, a number of structural analogues of CIQ were synthesized and tested. Evaluation of the ability of these compounds to potentiate GluN2 subunits was carried out using both calcium imaging assays and TEVC recordings.

5.3.2.1 GluN2C/D PAMs: The tetrahydroisoquinoline series: SAR

The main skeleton of the structure was found to be essential with removal of ring **B** and the ether linker abolishing activity (Figure 19b)⁸¹. Shortening the linker to a single methylene carbon or replacing it with either a thioether or ethyl linker led to a similar outcome. Altering the position and substituents on ring **B** established that a *p*-OMe group was optimal for PAM activity. Replacing ring **A** with a variety of aromatic and heteroaromatic systems was detrimental, although a 2-thiophene replacement showed some potential as a starting point for gaining selectivity for GluN2C- over GluN2D-containing NMDARs. The amide between ring **A** and the tetrahydroisoquinoline core was also important with its replacement with various other linkers (e.g. urea or sulfonamide) either reducing activity or abolishing it completely. Altering the position and substituents on ring **A** showed that *meta*- substitution gave the strongest potentiation and, of those tested, halogen substituents gave the best activity. With respect to ring **C**, introducing substituents to the 3-position reduced PAM activity, potentially due to an undesirable steric clash with the nearby ether linker. Additionally, substituents at the 6-position abolished activity suggesting a steric restriction around this area of the binding site. Interestingly, 5-OMe derivatives were found to be more active than their 4,5-diOMe counterparts. The most potent compound of the series, **45** (EC₅₀ = 0.3 μ M at GluN2C and GluN2D), was obtained by placing an *O*-benzyl group at the 5-position of ring **C**, thereby suggesting a hydrophobic pocket exists at this area of the binding site (Figure 19c). As all the compounds were tested as racemic mixtures for ease of synthesis, it was suspected that only one enantiomer may be responsible for the potentiating effect. Stereoselective synthesis and testing of the enantiomers of CIQ (**44**) revealed that the potentiating activity arises from a single enantiomer, namely (+)-CIQ (the absolute stereochemistry of this enantiomer has yet to be confirmed)⁸³.

Further structural modification of the tetrahydroisoquinoline backbone led to the development of compounds, which displayed PAM activity at GluN2B as well as GluN2C and GluN2D containing NMDARs⁸⁴. Removing the 4-OMe group from ring **C** of CIQ and replacing the 5-OMe with a branched isopropyl ether afforded a PAM (**46**) which had activity at GluN2B/C/D subunits (Figure 20a). SAR studies on **46** established that various other branched or cycloalkyl ethers (e.g. $R^1 = i\text{-Bu}$, $c\text{Hex}$) were tolerated at the 5-position of ring **C** (Figure 20b). Furthermore, it was found that PAM activity could be increased significantly by changing the linker between ring **A** and the tetrahydroisoquinoline core from amide to thioamide (i.e. $X = S$). Enantiomeric separation revealed that the *S*-(-) enantiomer of **46** was active at GluN2B/C/D while the *R*-(+) enantiomer was only active at GluN2C/D. *S*-(-)-**46** was one of the most active PAMs to be developed with EC₅₀'s of 0.32, 0.48, and 0.48 μM at GluN2B/C/D subunits respectively. Additionally, this series of compounds displayed selectivity for NMDARs over AMPARs and KARs (Figure 20).

5.3.2.2 GluN2C/D PAMs: The tetrahydroisoquinoline series; CIQ binding site and mechanism of action

The potentiation action of CIQ on GluN1/GluN2D involves residues in the linker between the GluN2D ATD and S1, and GluN2D specificity involves Thr592 in M1⁸² (see Figure 8). Further chimera and point mutation analysis suggests the involvement of the ATD linker may be to facilitate potentiation but that residues immediately preceding M1 (pre-M1 cuff helix which lies parallel to the membrane) and several residues within M1, are specifically necessary for CIQ actions⁸⁵. This putative binding site location is thus well positioned to affect gating by modulating the interactions between pre-M1/M1 and M3.

The mechanism of potentiation by CIQ is distinct from that found for UBP684 and GNE-6901 (see sections 4.1.2.1 and 5.2.1.2). CIQ increases open probability, but does not increase the mean open time⁸². Instead, CIQ decreases mean shut time. Thus, CIQ appears to enhance a pre-gating step by lowering the energy barrier for channel opening but does not appear to stabilize the open state. These results are consistent with a minimal effect on agonist potency and receptor deactivation time. CIQ appears to bind either in the absence or presence of agonist.

6. Conclusion

In recent years, significant progress has been made in the development of selective allosteric modulators binding to newly-identified sites on NMDARs. These new binding sites have already shown great potential as targets for both therapy and tool development; the apparent presence of a diverse range of binding pockets has meant that selective ligands for several of the subtypes have been identified, with both potentiating and inhibitory activity. There are now NAMs selective for GluN2A, GluN2B and GluN2C/D and PAMs selective for GluN2A, GluN2C, and GluN2D. Although there are a number of compounds that are of adequate potency for pharmacological characterization and potential starting points for therapeutic investigations, there is still a need for new compound development. Future studies should focus on 1) increasing the structural diversity of lead compounds, 2) improving pharmacodynamic properties and optimizing the balance between water solubility and

lipophilicity (this has been challenging in some of the SAR studies described herein) in order to improve bioavailability, 3) improving selectivity for individual GluN2 subunits, 4) developing compounds that are selective for triheteromeric NMDARs (i.e. those containing two GluN1 and two different GluN2 subunits), and 5) developing related agents to distinguish NMDARs containing GluN3 subunits. The finding that PYD106 is ineffective on heterotrimeric GluN2C-containing NMDARs⁷⁹, suggests that it may be possible to develop other agents that can further distinguish triheteromeric from diheteromeric receptors.

These endeavors would be aided by structural information obtained from protein-ligand complexes. For agents that bind in the LBD interface, recent crystallography studies have characterized the binding sites in detail. Other agents that bind near or in the membrane or between the ATD and LBD await such detailed structural information, but recent AMPAR structures with homologous binding sites can already provide some insights⁸⁶. As we determine the exact mechanisms of action and establish the structures of the binding pockets, it should become possible to tap into the potential of these new NMDAR binding sites even further, with compounds of increased potency and selectivity.

The new classes of drugs represented by these varied NMDAR NAMs and PAMs should offer significantly greater pharmacological control over NMDAR modulation than the previously available competitive antagonists and channel blockers. In addition to the improved targeting of specific subtypes, these agents have a surprising diversity in physiological properties at the receptor level that lead to distinct effects at the level of synaptic transmission and neuronal network function. These varied properties mean that it is possible to pharmacologically target distinct NMDAR populations in specific physiological/pathological conditions. These agents differ in their pH-sensitivity, thus can have a differential effect under pathological acidosis. They differ in their use/disuse-dependency and hence they can preferentially affect responses due to phasic or tonic agonist exposure. These agents can also differ in their effects on agonist potency, agonist efficacy and agonist deactivation kinetics. Reciprocally, some NAMs/PAMs differ in how their modulatory activity is affected by high and low agonist concentrations. Thus, an agent can potentially target pathological conditions that are excitotoxic due to chronic, low concentrations of extracellular glutamate, but minimally affect the phasic, high concentration glutamate exposure seen in synaptic transmission. With evidence that phosphorylation state and other intracellular factors can alter modulator activity, it may be possible to develop agents that target receptors on cells with specific intracellular conditions. Furthermore, modulators that slow NMDAR deactivation time could specifically enhance the response to repetitive synaptic activity that occurs during burst neuronal firing.

To date, medicinal chemistry has focused on compounds with improved selectivity and potency. Present efforts are to improve solubility, brain penetration, and pharmacokinetic/toxicity properties. But a remaining challenge will be to understand the structure-function properties of these allosteric modulators that underlie their diverse and specific physiological properties, thus enabling targeted drug design for optimal activity. Overall, there is significant potential to develop NMDAR NAMs and PAMs with improved properties for a variety of indications such as pain, epilepsy, neuroprotection, cognitive enhancement, and conditions of NMDAR hypofunction such as schizophrenia.

Author biographies

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David E Jane is a Professor of Chemical Pharmacology at the University of Bristol (UK) since 2008. Since 1986 his research has involved the development of pharmacological tools for glutamate receptors to understand their role in the central nervous system in health and disease. He has developed subtype selective agonists and antagonists for ionotropic and metabotropic glutamate receptors, which are in widespread use by the neuroscience community. Recent interests include the development of subtype selective allosteric modulators and competitive antagonists for NMDA and kainate receptors. He is author of more than 130 papers and 6 patents.

Daniel Monaghan is a Professor of Pharmacology and Experimental Neuroscience at the University of Nebraska Medical Center. He obtained his BS degree in Life Sciences at the Univ. Nebraska, Lincoln and then a PhD at the University of California, Irvine (UCI), Psychobiology Department (1985) for work in Dr. Carl Cotman's laboratory on glutamate receptor identification. After a postdoctoral fellowship and an Assistant Professorship in the

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Abbreviations

24(S)-HC	24(S)-hydroxycholesterol
3α5βS	20-oxo-5 β -pregnan-3 α -yl sulfate
AMPA	AMPA receptor, named for the agonist α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATD	amino terminal domain
BBB	blood brain barrier
EPSP	excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
GluN1,2,3	Glutamate receptor subunit, NMDA type 1-3
KAR	kainate receptor
LBD	ligand-binding domain
M1-M4	membrane-associated domain 1-4
NAM	negative allosteric modulator
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
PAM	positive allosteric modulator
PAS	pregnanolone sulfate or 20-oxo-5 β -pregnan-3 α -yl sulfate
P-gp ER	P-glycoprotein efflux ratio
PS	pregnenolone sulfate or 20-oxo-5-pregnen-3 β -yl sulfate
S1	segment 1
S2	segment 2
SAR	structure activity relationship
TEVC	two-electrode voltage clamp
TMD	transmembrane domain

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Figure 1.

NMDA receptor structure. (Top) Crystal structure of the tetrameric NMDA receptor complex of *Xenopus laevis* without intracellular C-terminals⁸⁷ (PDB: 4TLM). The two GluN1 backbones are shown in green/blue and the two GluN2B backbones are in yellow/red. (Bottom row) A single GluN1 subunit in the same orientation as the blue GluN1 subunit in the top, left panel. Middle panel showing this subunit's secondary structure (beta sheets, blue / alpha helix, red) and right panel: stick representation of backbone and side chains with the ATD - green, S1 and linker to M1- red, S2 and linker to M4 - blue, M1 – yellow, M2 -

orange, M3 - magenta, and M4 - cyan. The three domain levels are shown on the left for ATD, LBD, and transmembrane (TM) domain.

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Figure 2:

(a) Lead compounds **1** (PS) and **2** (PAS); (b) General SAR; (c) NAM **3**.

Figure 3:
Structures of cholesterol (4), 24(S)-HC (5), SGE-201 (6) and 25-HC (7).

Figure 4:

(a) Structure of lead compound **8** (TCN-201); (b) compound **9**; (c) Most selective compound for GluN2A (**9**, MPX-004) and most potent compound at GluN2A (**10**, MPX-007).

Figure 5.

Allosteric modulator binding sites on the NMDA receptor. (Left panel) Crystal structure of the tetrameric NMDA receptor complex of *Xenopus laevis* without intracellular C-terminals⁸⁷ (PDB: 4TLM). The two GluN1 backbones are shown in green/blue and the two GluN2B backbones are in yellow/red. Homologous amino acid residues which are involved in the binding and/or actions of allosteric modulators are shown as space-filled: PYD-1 – light blue; GNE-6901/TCN-201 - yellow; UBP512 - pink; QNZ46/DQP-1105 - orange; GNE-9278 - green; CIQ - purple. Also shown are space-filled ligands with CPK coloring: Ro25-6981 bound in the ATD, glycine-site agonist 1-aminocyclopropanecarboxylic acid bound in the GluN1 LBD, and glutamate-site agonist trans-1-aminocyclobutane-1,3-dicarboxylic acid bound in the GluN2B LBD. (Right) LBD dimer with homologous residues that interact with TCN-201 are shown as space-filled in blue (GluN1) and yellow (GluN2). Bound glycine and L-glutamate are shown in CPK coloring.

Figure 6. (A,B) GluN1/GluN2A LBD dimer with bound TCN-201 (yellow), L-glutamate (green), and glycine (light blue) PDB: 5I56⁵¹. (C) TCN-201 binding pocket with interacting residues shown in stick mode.

Figure 7:

(a) Lead compounds **12** and **13**; (b) CP 465022 (**14**); (c) General SAR observations; (d) Representative compounds **15** (QNZ46) and **16**.

Figure 8.

Allosteric sites associated in or near the TM domain. The two GluN1 backbones are shown in green/blue and the two GluN2B backbones are in yellow/red. Homologous amino acid residues which are involved in the binding and/or actions of allosteric modulators are shown as space-filled: UBP512 - pink; QNZ46/DQP-1105 - orange; GNE-9278 - green; CIQ - purple. UBP512 and PS activity also involves residues in the S2 domain (not shown).

Figure 9:

(a) Lead compound **17**; (b) General SAR observations; (c) Representative compounds **18** (DQP-26) and **19** (DQP-1105).

Figure 10:

(a) Lead compound **20**; (b) General SAR observations; (c) Compounds **21** and **22**.

Figure 11:

(a) Lead compound **23**; (b) General SAR observations; (c) Compound **24** (NAB-14).

Figure 12:

(a) Lead compound **25**; (b) General SAR observations; (c) **26** (UBP710), **27** (UBP646).

Figure 13:

(a) General SAR observations; (c) Representative naphthalene derivatives from the series: **28** (UBP684), **29** (UBP618), **30** (UBP551) and **31** (UBP552).

Figure 14:

(a) Lead compound **32** (UBP608); (b) General SAR observations; (c) Representative coumarin derivatives from the series: **33** (UBP656) and **34** (UBP714).

35 (GNE-9278)

Figure 15:
Structure of GNE-9278 (**35**).

Figure 16:

(a) Lead compound GNE 3476 (**36**); (b) Summary of SAR study; (c) Structures of GNE-0723 (**37**), GNE-5729 (**38**), GNE-6901 (**39**) and GNE-8324 (**40**).

Figure 17:
(a) Lead compound **41**; (b) General structure for SAR studies; (c) Compounds **42** (PYD-111) and **43** (PYD-106).

Figure 18.

Residues important for PYD-106 GluN1/GluN2C-selective PAM activity. (Left) Residues important for NAM/PAM activity are shown in the tetrameric complex with GluN1 backbones in blue/green and GluN2 backbones in red/yellow (PDB: 4TLM). Residues homologous to those important for PYD-106 GluN2C-selectivity are shown on a single GluN2 structure as space-filled in light blue. These sites oppose each other across the ATD/LBD interface.

Figure 19:

(a) Lead compound **44** (CIQ); (b) General SAR observations; (c) Compound **45**.

Figure 20:

(a) Lead GluN2B/C/D PAM **46**; (b) General SAR observations; (c) Active enantiomer of **46**.

Table 1:

Functional characteristics of NMDA receptor allosteric modulators

PAM or NAM*	Effect on agonist potency		Deactivation	Use dependency	Single-channel properties			pH	Ref
	Glutamate	Glycine			P _O	MOT	MST	Low	High
GENE-6901 (GluN2A)	No change	No change	No change	-	-	-	-	-	[59]
GENE-8324 (GluN2A)	↑	No change	Slow (Glu)	-	-	-	-	PAM	NAM [59,70]
GENE-9278 (GluN2A)	↑	↑	Slowed (Glu)	Use-dependent?	↑	↑	↓	PAM	NAM [70,71]
UBP684 (GluN2A)	↑	No change	Slowed	Use-independent	-	-	-	PAM	NAM [70]
UBP684 (GluN2D)	↓	No change	Slowed (Glu)	Use-independent	-	-	-	PAM	NAM [70]
UBP753 (GluN2D)	↓	No change	No change	Use-independent	-	-	-	PAM	NAM [70]
PYD-106 (GluN2C)	↓	↑	Faster (Glu)	-	↑	↑	No change	-	- [79]
CIQ (GluN2D)	No change	Small ↑	No change	Use-independent	↑	No change	↓	-	- [82,85]
PS (GluN2A)	No change	No change	Slowed	-	↑	↑	No change	↑ PAM	↓ PAM [24,33,45,29]
PS (GluN2B)	Small ↑	Small ↑	Slowed	Dis-use dependent	↑	↑	No change	↑ PAM	↓ PAM [44,28,24,70]
Spermine (GluN2B)	↑	↑	Slowed (Gly)	-	-	-	-	↑ PAM	↓ PAM [88, 89]
SGE-201 (GluN2A)	↑	No change	-	-	↑	-	-	-	- [49]
*PAS (GluN2A)	↓	↓	-	Use-dependent	↓	No change	↑	-	- [24,41]
*DQP-1105 (GluN2D)	-	-	-	Use-dependent	↓	No change	↑	-	- [63]
*QNZ-46 (GluN2D)	-	-	Slowed	Use-dependent	-	-	-	-	- [62]

P_O, open probability; MOT, mean open time; MST, mean shut time; -, not available.

Table 2:

Potency of negative allosteric modulators at different GluN1/GluN2 NMDARs

NAMs	IC ₅₀ (μM)				Ref
	GluN2A	GluN2B	GluN2C	GluN2D	
TCN-201 ^c	0.11 ^a	50	-	>>30	[50]
	0.446 ^a		-	-	[52]
	0.32 ^a	>>10	>>10	>>10	[53,58]
	034 ^{a,b}		-	-	
MPX-004	0.198/0.079 ^b	-	-	-	[58]
MPX-007	0.143/0.027 ^{a,b}	-	-	-	[58]
Ifenprodil ^b	39.5	0.11	29.1	75.9	[90]
Ro 25-6981	52	0.009	-	-	[91]
EVT-101	-	0.012	-	-	[92]
QNZ-46	229	>300	6	3	[60]
	182	193	7.1	3.9	[62]
DQP-1105	-	113	7.0	2.7	[63]
PAS	62	38	12	14	[24]
UBP608	18.6	90	68	426	[68]
UBP618	1.8	2.4	2.0	2.4	[68]
UBP551	9.7	9.4	15	10 (PAM)	[68]

IC₅₀ values were determined by TEVC using *Xenopus* oocytes unless noted otherwise; -, data not available^a 3 μM glycine was used for IC₅₀ determination^b IC₅₀ was calculated at human NMDA receptors^c As determined by Ca²⁺ influx assay using HEK cells

Table 3:

Potency of positive allosteric modulators at different GluN1/GluN2 NMDARs

PAMs	EC ₅₀ (μM)				Ref
	GluN2A	GluN2B	GluN2C	GluN2D	
GNE-6901	8.5				[59]
GNE-8324	2.43 ^a	-	-	-	[59]
GNE-9278	3.2	15.7	6.6	6.7	[75]
Spermine	-	127	-	-	[89]
		81 ^c			[93]
		125 ^d			[88]
PS	21	33	112/NAM	118 /NAM	[24]
	34 ^b	63 ^b	83 ^b	78 ^b	[27]
CIQ	>10	>10	2.7	2.8	[82]
PYD-106	N.A.	N.A.	13	N.A.	[79]
UBP512	~100	N.A.	NAM	NAM	[68]
UBP684	28	34	37	29	[70]
R-(+)-138	-	-	1.7	2.0	[84]
S-(-)-138	-	0.32	0.48	0.48	[84]

EC₅₀ values were determined by TEVC in *Xenopus* oocytes unless noted otherwise; -, data not available^a Determined by Ca²⁺ influx assay using HEK cells^b Determined in HEK cells^c spinal cord neurons^d hippocampal neurons

N.A. – no activity