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Review

# Pharmacology of ionotropic glutamate receptors: A structural perspective

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ABSTRACT

The impact of structural biology on the design of ligands (agonists, antagonists and modulators) for ionotropic glutamate receptors is reviewed.



**Contents**



## 1. Introduction

Ionotropic glutamate receptors (iGluRs) mediate the fast response to the major excitatory neurotransmitter of the mammalian central nervous system and play an essential role in its development and function. They are tetrameric, ligand-gated, cation-selective ion channels that are found throughout the animal kingdom and have homologs in plants and presumed ancestors in prokaryotes.[1] Many human neurological disorders, such as epilepsy or chronic neurodegenerative conditions, as well as stroke, can be linked to iGluRs. As such, their pharmacology, molecular physiology and expression have been studied in great detail.[2] Due to a wealth of recent data stemming from structural biology, they now belong to the best-understood types of receptors.[3–5] Important topics of pharmacology, such as agonism versus antagonism, allosteric modulation, or receptor desensitization can be beautifully illustrated using iGluRs. These crystallographic studies began with cleverly engineered soluble constructs representing the clamshell-like extracellular ligand-binding domain (LBD) of iGluRs[6] and recently culminated in a full-fledged structure of

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**Table 1**
X-ray structures of iGluR-LBDs and their ligands

| Type | PDB code | Ligand(s) | Functional | Mutant(s) | Citation |
|---|---|---|---|---|---|
| GluR0 | 1IIW | | apo | | 10 |
| GluR0 | 1II5 | Glutamic acid (**1**) | a | | 10 |
| GluR0 | 1IIT | L-Serine | a | | 10 |
| GluR0 | 2PYY | Glutamic acid (**1**) | a | | 11 |
| GluA2 | 1FTO | | apo | | 12 |
| GluA2 | 1MXV | $Zn^{2+}$ | apo | | 13 |
| GluA2 | 1MXW | $Zn^{2+}$ | apo | | 14 |
| GluA2 | 1MXX | $Zn^{2+}$ | apo | | 13 |
| GluA2 | 1MXY | $Zn^{2+}$ | apo | | 13 |
| GluA2 | 1MXZ | $Zn^{2+}$ | apo | | 13 |
| GluA2 | 1MY0 | $Zn^{2+}$ | apo | | 13 |
| GluA2 | 1MY1 | $Zn^{2+}$ | apo | | 13 |
| GluA2 | 1FTJ | Glutamic acid (**1**) | a | | 12 |
| GluA2 | 1FTK | Kainic acid (**17**) | a | | 12 |
| GluA2 | 1FW0 | Kainic acid (**17**) | a | | 12 |
| GluA2 | 1LB8 | AMPA (**10**) | a | L483Y, flop | 15 |
| GluA2 | 1LBB | Kainic acid (**17**) | a | N754D | 15 |
| GluA2 | 1M5B | 2-Me-TetAMPA (**8**) | a | | 16 |
| GluA2 | 1M5C | Br-HIBO (**13**) | a | Flop | 16 |
| GluA2 | 1M5D | Br-HIBO (**13**) | a | Flop | 16 |
| GluA2 | 1M5E | ACPA (**10**) | a | Flop | 16 |
| GluA2 | 1M5F | ACPA (**10**) | a | Flop | 16 |
| GluA2 | 1MM6 | Quisqualate (**14**) | a | | 17 |
| GluA2 | 1MM7 | Quisqualate (**14**), $Zn^{2+}$ | a | | 17 |
| GluA2 | 1MQD | (*S*)-Des-Me-AMPA (**5**), $Li_2SO_4$ | a | | 18 |
| GluA2 | 1MQG | Iodo-willardiine (**15e**) | a | | 14 |
| GluA2 | 1MQH | Bromo-willardiine (**15d**) | a | | 14 |
| GluA2 | 1MQI | Fluoro-willardiine (**15b**) | a | | 14 |
| GluA2 | 1MQJ | Willardiine (**15a**) | a | | 14 |
| GluA2 | 1MS7 | (*S*)-Des-Me-AMPA (**5**), $Zn(OAc)_2$ | a | | 18 |
| GluA2 | 1MXU | Bromo-willardiine (**15d**) | a | | 13 |
| GluA2 | 1MY2 | AMPA | a | | 14 |
| GluA2 | 1MY3 | Bromo-willardiine (**15d**) | a | | 14 |
| GluA2 | 1MY4 | Iodo-willardiine (**15e**) | a | | 14 |
| GluA2 | 1N0T | ATPO (**35**) | a | | 19 |
| GluA2 | 1NNK | ATPA (**6**) | a | | 20 |
| GluA2 | 1NNP | ATPA (**6**) | a | | 20 |
| GluA2 | 1P1N | Kainic acid (**17**) | a | L138T | 21 |
| GluA2 | 1P1O | Quisqualate (**14**) | a | L138T | 21 |
| GluA2 | 1P1Q | AMPA (**4**) | a | L138T | 21 |
| GluA2 | 1P1U | AMPA (**4**) | a | L138T | 21 |
| GluA2 | 1P1W | AMPA (**4**) | a | L94Y, L138T | 21 |
| GluA2 | 1SYH | CPW399 (**16**) | a | | 22 |
| GluA2 | 1SYI | CPW399 (**16**) | a | Y702F | 22 |
| GluA2 | 1WVJ | 4-AHCP (**11**) | a | | 23 |
| GluA2 | 1XHY | Kainic acid (**17**) | a | Y702F, flop | 22 |
| GluA2 | 2AIX | Thio-ATPA (**7**) | a | | * |
| GluA2 | 2AL4 | Quisqualate (**14**) | a | | 24 |
| GluA2 | 2ANJ | Kainic acid (**17**) | a | Y61W | 25 |
| GluA2 | 2GFE | Glutamic acid (**1**) | a | A476E S673D | 26 |
| GluA2 | 2I3V | Glutamic acid (**1**) | a | G725C | 27 |
| GluA2 | 2I3W | Glutamic acid (**1**) | a | G729C | 27 |
| GluA2 | 2P2A | BnTetAMPA (**9**) | a | | 28 |
| GluA2 | 2UXA | Glutamic acid (**1**) | a | Flip | 29 |
| GluA2 | 3B6Q | Glutamic acid (**1**) | a | T686A | 30 |
| GluA2 | 3B6T | Quisqualate (**14**) | a | T686A | 30 |
| GluA2 | 3B6W | Glutamic acid (**1**) | a | T686S | 30 |
| GluA2 | 3BFT | TDPA (**12**) | a | | 31 |
| GluA2 | 3BFU | TDPA (**12**) | a | | 31 |
| GluA2 | 3DP6 | Glutamic acid (**1**) | a | | 32 |
| GluA2 | 3LSL | Glutamic acid (**1**), piracetam (**74**) | a | | 33 |
| GluA2 | 2CMO | Glutamic acid (**1**), NS-1209 (**47**) | a, ant | | 34 |
| GluA2 | 3KGC | Glutamic acid (**1**), Z-200775 (**44**), LY-404187 (**71**) | a, ant, mod | | 7 |
| GluA2 | 1LBC | Glutamic acid (**1**), cyclothiazide (**61**) | a, mod | N775S | 15 |
| GluA2 | 2AL5 | Fluoro-willardiine (**15b**), aniracetam (**75**) | a, mod | | 24 |
| GluA2 | 2XHD | Glutamic acid (**1**), **73** | a, mod | | 35 |
| GluA2 | 3BBR | Glutamic acid (**1**), **70** | a, mod | | 36 |
| GluA2 | 3H6T | Glutamic acid (**1**), cyclothiazide (**61**) | a, mod | | 37 |
| GluA2 | 3H6U | Glutamic acid (**1**), NS-1493 (**67**) | a, mod | | 37 |
| GluA2 | 3H6V | Glutamic acid (**1**), NS-5206 (**68**) | a, mod | | 37 |
| GluA2 | 3H6W | Glutamic acid (**1**), NS-5217 (**69**) | a, mod | | 37 |
| GluA2 | 3IJO | Glutamic acid (**1**), althiazide (**65**) | a, mod | | 38 |
| GluA2 | 3IJX | Glutamic acid (**1**), IDRA-21 (**64**) | a, mod | | 38 |
| GluA2 | 3IK6 | Glutamic acid (**1**), chlorothiazide (**66**) | a, mod | | 38 |

**Table 1** (*continued*)

| Type | PDB code | Ligand(s) | Functional | Mutant(s) | Citation |
|---|---|---|---|---|---|
| GluA2 | 3IL1 | Glutamic acid (**1**), IDRA-21 (**64**) | a, mod | | 38 |
| GluA2 | 3ILT | Glutamic acid (**1**), trichlormethiazide (**63**) | a, mod | | 38 |
| GluA2 | 3ILU | Glutamic acid (**1**), hydroflumethiazide (**62**) | a, mod | | 38 |
| GluA2 | 3LSF | Glutamic acid (**1**), piracetam (**74**) | a, mod | | 33 |
| GluA2 | 1LB9 | DNQX (**40**) | ant | L483Y | 15 |
| GluA2 | 3B7D | CNQX (**41**) | ant | | 39 |
| GluA2 | 3H03 | UBP277 (**36a**) | ant | | 32 |
| GluA2 | 3H06 | UBP282 (**36b**) | ant | | 32 |
| GluA2 (functional receptor) | 3KG2 | ZK-200775 (**44**) | ant | N241E, N385D, N392Q, K410A, E413A, M414A, E416A, C589A | 7 |
| GluA2 | 3BKI | FQX (**46**) | ant, photoproduct | | 40 |
| GluA3 | 3DLN | Glutamic acid (**1**) | a | | 41 |
| GluA3 | 3DP4 | AMPA (**4**) | a | | 41 |
| GluA3 | 3M3K | Glutamic acid (**1**) | a | Flop | 42 |
| GluA3 | 3M3L | Glutamic acid (**1**), PEPA (**72**) | a, mod | | 42 |
| GluA4 | 3EN3 | Kainic acid (**17**) | a | | 43 |
| GluA4 | 3FAS | Glutamic acid (**1**) | a | Flip | 44 |
| GluA4 | 3FAT | AMPA (**4**) | a | Flip | 44 |
| GluA4 | 3KEI | Glutamic acid (**1**) | a | L651 V | 45 |
| GluA4 | 3KFM | Kainic acid (**17**) | a | L651 V | 45 |
| GluK1 | 3C31 | $Li^+$ | apo | | 46 |
| GluK1 | 3C32 | $Na^+$ | apo | | 46 |
| GluK1 | 3C33 | $K^+$ | apo | | 46 |
| GluK1 | 3C34 | $Rb^+$ | apo | | 46 |
| GluK1 | 3C35 | $Cs^+$ | apo | | 46 |
| GluK1 | 3C36 | $NH_4^+$ | apo | | 46 |
| GluK1 | 1TXF | Glutamic acid (**1**) | a | | 47 |
| GluK1 | 1YCJ | Glutamic acid (**1**) | a | | 48 |
| GluK1 | 2F36 | Glutamic acid (**1**) | a | | 49 |
| GluK1 | 2PBW | Domoic acid (**18**) | a | | 50 |
| GluK1 | 2WKY | 4-AHCP (**11**) | a | | 51 |
| GluK1 | 2ZNS | Glutamic acid (**1**) | a | | * |
| GluK1 | 2ZNT | Dysiherbaine A (**26a**) | a | | * |
| GluK1 | 2ZNU | Dysiherbaine A (**26a**) | a | | * |
| GluK1 | 3FUZ | Glutamic acid (**1**) | a | | * |
| GluK1 | 3FV1 | Dysiherbaine A (**26a**) | a | | * |
| GluK1 | 3FV2 | Neodysiherbaine A (**26b**) | a | | * |
| GluK1 | 3FVG | MSVIII-19 (**26c**) | a | | * |
| GluK1 | 3FVK | 8-Deoxy-neodysiherbaine A (**26d**) | a | | * |
| GluK1 | 3FVN | 9-Deoxy-neodysiherbaine A (**26e**) | a | | * |
| GluK1 | 3FVO | 8-Epi-neodysiherbaine A (**26f**) | a | | * |
| GluK1 | 3GBA | Dysiherbaine A (**26a**) | a | | 52 |
| GluK1 | 3GBB | MSVIII-19 (**26c**) | a | | 52 |
| GluK1 | 1VSO | ATPO (**35**) | ant | | 50 |
| GluK1 | 2F34 | UBP-310 (**36d**) | ant | | 49 |
| GluK1 | 2F35 | UBP-302 (**36c**) | ant | | 49 |
| GluK1 | 2OJT | UBP-310 (**36d**) | ant | | 53 |
| GluK1 | 2QS1 | UBP-315 (**36e**) | ant | | 52 |
| GluK1 | 2QS2 | UBP-318 (**36g**) | ant | | * |
| GluK1 | 2QS3 | UBP-316 (**36f**) | ant | | * |
| GluK1 | 2QS4 | LY-466195 (**37**) | ant | | * |
| GluK2 | 1S7Y | Glutamicacid (**1**) | a | | 47 |
| GluK2 | 1S9T | Quisqualate (**14**) | a | | 47 |
| GluK2 | 1SD3 | (2*S*,4*R*)-4-Methyl glutamic acid (**21**) | a | | 47 |
| GluK2 | 1TT1 | Kainic acid (**17**) | a | | 47 |
| GluK2 | 1YAE | Domoic acid (**18**) | a | | 54 |
| GluK2 | 2I0B | Glutamic acid (**1**) | a | K494E, I749L, Q753K, E757Q | 55 |
| GluK2 | 2I0C | Glutamic acid (**1**) | a | S775E, Y490C, L752C (crosslinked) | 55 |
| GluK2 | 3G3F | Glutamic acid (**1**) | a | | 56 |
| GluK2 | 3G3G | Glutamic acid (**1**) | a | K665R | 56 |
| GluK2 | 3G3H | Glutamic acid (**1**) | a | K665R, I749L, Q753K | 56 |
| GluK2 | 3G3I | Glutamic acid (**1**) | a | I442H, K494E, I749L, Q753K | 56 |
| GluK2 | 3G3J | Glutamic acid (**1**) | a | I442H, K494E, K665R, I749L, Q753K | 56 |
| GluK2 | 3G3K | Glutamic acid (**1**) | a | I442H, K494E, K665R, I749L, Q753K, E757Q | 56 |
| GluD2 | 2V3T | | apo | | 57 |
| GluD2 | 2V3U | D-Serine (**3**) | a | | 57 |
| GluN1 | 1PB7 | Glycine (**2**) | a | | 58 |
| GluN1 | 1PB8 | D-Serine (**3**) | a | | 58 |
| GluN1 | 1PB9 | D-cycloserine (**27**) | a | | 58 |
| GluN1 | 1Y1M | Cycloleucine (**31**) | a | | 59 |
| GluN1 | 1Y1Z | ACBC (**30**) | a | | 59 |

(*continued on next page*)

**Table 1** (*continued*)

| Type | PDB code | Ligand(s) | Functional | Mutant(s) | Citation |
|---|---|---|---|---|---|
| GluN1 | 1Y20 | ACPC (**29**) | a | | 59 |
| GluN1 | 1PBQ | 5,7-Dichlorokynureniuc acid (**54**) | ant | | 58 |
| GluN1/GluN2A | 2A5T | Glycine (**2**), glutamic acid (**1**) | a, a | | 60 |
| GluN2A | 2A5S | Glutamic acid (**1**) | a | | 60 |
| GluA2 ATD | 3H5V | | | | 61 |
| GluA2 ATD | 3H5W | | | | 61 |
| GluK2 ATD | 3H6G | | | | 62 |
| GluK2 ATD | 3H6H | | | | 62 |
| GluA2 ATD | 3HSY | | | | * |

The abbreviations a, ant, and mod denote agonists, antagonists and modulators, respectively. Structures marked with * have not yet been published. Structures in boldface are archetypical or especially instructive.

GluA2—one of the most formidable achievements in structural biology to date.[7]

The purpose of this article is to provide an overview of the various small molecule ligands, that is, agonists, antagonists and modulators, that target iGluRs and that have been structurally characterized together with their binding sites. We will not attempt to comprehensively review the pharmacology of iGluRs (a truly monumental task) but rather discuss recent crystallographic results that allow for the interpretation of the available structure–activity relationships and the design of new and improved molecules. Computational investigations, which provide a complementary approach, are beyond the scope of this review.[8] The same is true for the extensive pharmacology and emerging structural biology of metabotropic glutamate receptors (mGluRs), which mediate the slow response of neurons to glutamate. Since our review is 'ligand-centric', we will only briefly touch on the wealth of biophysical and biochemical data that have been accumulated to elucidate important receptor functions, such as activation, desensitization and trafficking, and we refer the reader to excellent accounts on these topics.[1,2]

Table 1, which is at the heart of this review, lists the names and functional roles of the ligands and their corresponding accession numbers in the Protein DataBank (pdb)[9] in the order of their receptor subtypes. References with further details on the origin, pharmacology, and medical use of the ligands are also provided. The corresponding chemical structures can be found in Figures 4–13. For good measure, we also list the known *apo* structures of receptor LBDs and the structures of amino-terminal domains (vide infra). Occasionally, we will discuss popular agonists and antagonists for iGluRs that have not (yet) been crystallized with their receptors. Their binding mode can be often inferred from the existing structural data.

## 2. An overview of iGluRs

Historically, iGluRs have been grouped according to their distinct responses to certain small molecule agonists, namely AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid), kainate, and NMDA (*N*-methyl-D-aspartate).[1,2] This classification had been upheld in light of more recent sequence data although the pharmacologically defined boundary between AMPA and kainate receptors has blurred over time and now they are often grouped into the so-called 'non-NMDA receptor' superfamily. According to a modern nomenclature,[63] the iGluRs comprise AMPA-type receptors (GluA1–GluA4), kainate receptors (GluK1–GluK5) and NMDA receptors (GluN1, GluN2A–GluN2D, GluN3A–GluN3B) (Fig. 1). Two orphan members of the iGluR gene family, GluD1 and GluD2, are also known, but their physiological role remains somewhat mysterious. In addition to this, a prokaryotic, potassium-selective glutamate receptor (GluR0) has been cloned and structurally characterized.[64]

AMPA receptors are the workhorses of fast synaptic transmission and are widely expressed throughout the mammalian central nervous system. Potentiation of synaptic responses is achieved through insertion of an increasing number of AMPA receptors at the post-synaptic membrane or the modulation of single channel conductance.[65] By contrast, kainate receptors assume more of a modulatory role and are active both on the postsynaptic and presynaptic side. They are also involved in generating synaptic plasticity but have a narrower distribution.[66] NMDA receptors primarily function as coincidence detectors and are critical for learning and memory. They are inactive at resting membrane potentials due to a voltage-dependent block of the channel pore by magnesium ions. Activation of AMPA receptors and membrane depolarization releases this inhibition. Unlike most AMPA and kainate receptors, NMDA receptors are permeable to calcium ions which triggers various intracellular signaling cascades.[67] Alternative splicing and RNA editing, whose most notable effects are on trafficking and $Ca^{2+}$ permeability, complicate the genetic and functional analysis of iGluRs.[68,69]

Ionotropic glutamate receptors function as a complex of four individual subunits. Whereas the NMDA receptors are obligate heterotetramers, the AMPA and kainate receptors can exist as homo- and heterotetramers within one subfamily. For example, the major kainate receptor in the human brain consists of GluK2 and GluK5.[70] NMDA receptors are composed of subunits that bind to glycine or D-serine (NR1 and NR3) and subunits that are activated by glutamate (NR2). NR1 and NR2A are the most abundant forms in the fully developed human brain.[71]

Each individual subunit of an iGluR features an extracellular amino-terminal domain (ATD), which is involved in subfamily-specific tetramerization, an extracellular LBD that interacts with the neurotransmitter, a transmembrane domain (TMD) that forms the cation-selective pore and a cytoplasmatic carboxy-terminal domain (CTD) involved in receptor localization and modulation. The ATD and LBD of iGLuRs have often been compared to clamshells (or a Venus fly-traps). Starting from the N-terminus, the polypeptide first forms the ATD followed by a part of both lobes of the LBD clamshell, then dips into the membrane as transmembrane helix 1, the pore loop, and transmembrane helix 2, then rises again to the extracellular side to complete the remainder of the LBD clamshell, and finally crosses the membrane once more as transmembrane helix 1 to end up as a CTD of varying length. Structurally, the LBD clamshell can be easily dissected in an 'upper lobe' (relative to the cell membrane), which is also known as D1, and a 'lower lobe', known as D2.

## 3. The three-dimensional structure of iGluRs and the LBD clamshell

Very recently, the whole crystal structure of a functional tetrameric AMPA receptor complete with its ATD, LBD and TMD was

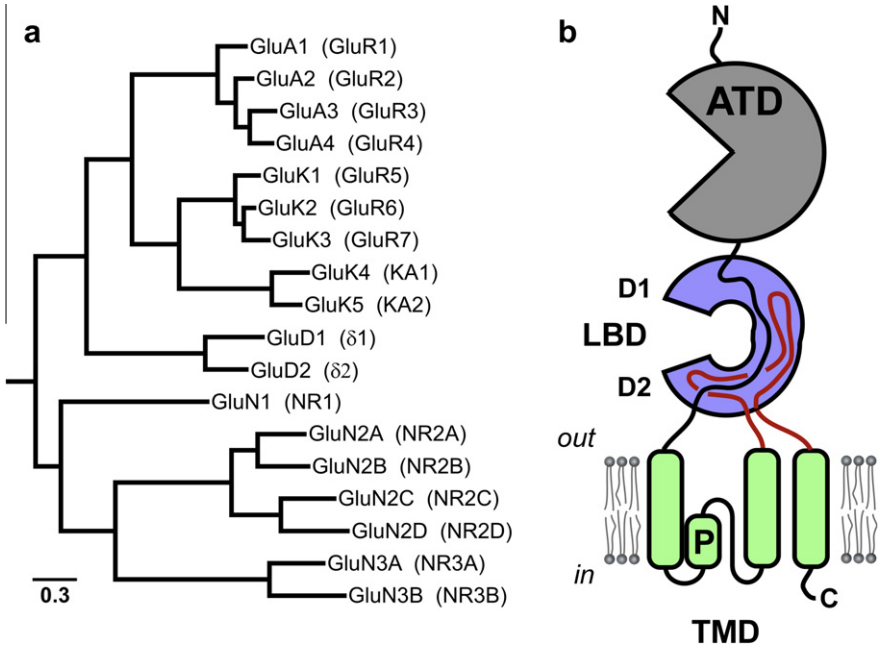


**Figure 1.** (a) Modern (and previously used) nomenclature and maximum likelihood phylogeny of human iGluRs. The branch lengths are proportional to the amount of inferred evolutionary change. Sequences were aligned with Muscle[72] and phylogeny was calculated using FastTree.[73] (b) Domain structure of eukaryotic iGluRs. ADT = amino-terminal domain, LBD = ligand-binding domain, D1 = upper lobe of the LBD, D2 = lower lobe of the LBD, TMD = transmembrane domain, P = pore helix.

disclosed (pdb 3KG2, Fig. 2).[7] This structure reveals that homotetrameric iGluRs are approximately $C_4$-symmetric in their TMDs and $C_2$-symmetric with respect to extracellular domains. The first two helices and re-entrant loop of the TMD closely resemble those of potassium channels. Due to this 'symmetry mismatch' (or, rather, reduction in symmetry), the four sequence-identical chains of iGluRs fall into two conformational subtypes, which is reflected in two types of LBD clamshells. Although the fold of these clamshells is very similar overall, they are oriented at different angles with respect to the $C_2$-axis of symmetry and it is the ipsilateral clamshells that form a dimer exclusively via contacts in the D1 lobes. Upon activation, the clamshells close and the separation of their D2 lobes increases. This movement is mechanically linked to the second transmembrane helix and opens a gate in the TMD. It also creates mechanical tension within the LBD multimer, which is alleviated by subsequent separation of the D1 lobes, a process that results in receptor desensitization and channel inactivation.[27]

The crystallization and functional analysis of individual LBD clamshells has been greatly aided by soluble constructs (so called S1–S2 constructs) that capture most of their essential structural features and have allowed for the development of the above gating model. Coordinates of these constructs are available for two prokaryotic glutamate receptors, the AMPA receptors GluA2, GluA3, and GluA4, the kainate receptors GluK1 and GluK2, the NMDA receptor subunits GluN1, GluN3 (glycine sites), and GuN2A (glutamate site), as well as the orphan receptor GluD2. These have been

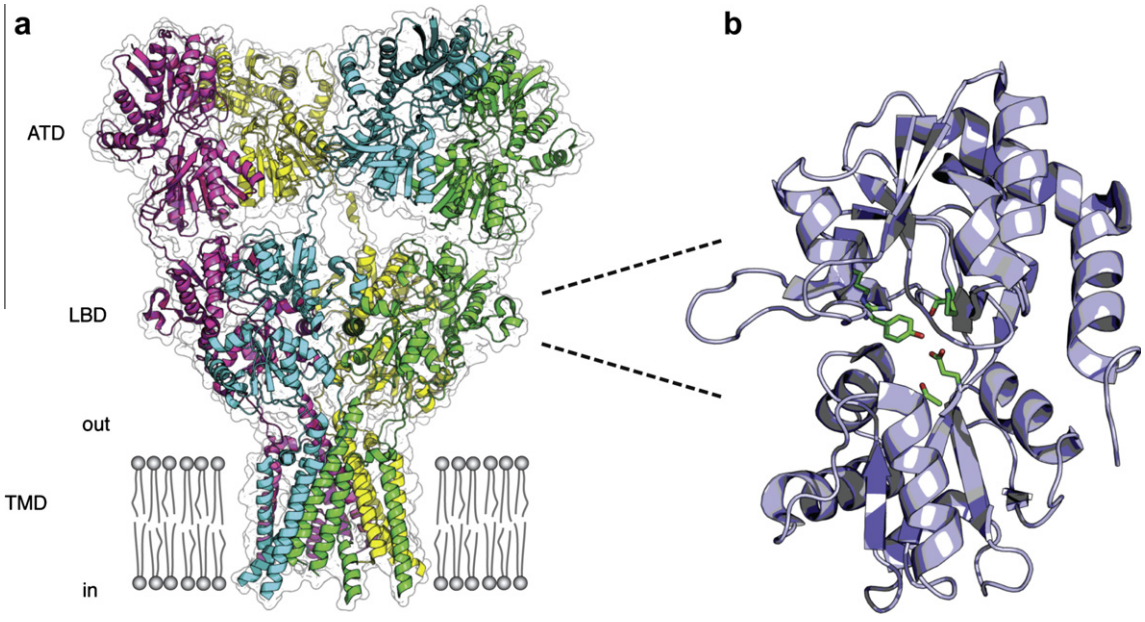


**Figure 2.** (a) Overall structure of iGluRs exemplified by GluA2 (pdb 3KG2). (b) Close-up of a clamshell with important residues highlighted (GluA2 S1S2 from pdb 1FTM).

crystallized as monomers or dimers and with a variety of agonists, antagonists and—in the case of AMPA receptors—several modulators that are know to influence receptor desensitization. A handful of *apo* structures are also available (Table 1).

Since the human vocabulary concerning clamshells is somewhat limited compared with our ability to describe our own anatomy, we occasionally find it useful to speak of the LBD in terms of a human head and mouth. A 'nose'-like loop can be found in all crystal structures of iGluR LBDs and we orient the clamshell in Figures 2 and 4 such that the nose protrudes to the left. We prefer this orientation for the comparative analysis of the X-ray structures since the 'mouth' (or cleft) of the LBD points towards the spectator and provides a perspective of glutamate and its congeners that corresponds to Figure 3 (vide infra). The cleft of the clamshell is lined by 'lips', which can be fully closed or partially open to accommodate portions of a ligand.

## 4. The binding mode of endogenous ligands: glutamate, glycine and D-serine

The binding mode and conformation of glutamate is highly conserved in AMPA, kainate and NMDA receptors (Fig. 3). The most conspicuous interaction is a salt-bridge between a positively charged arginine side chain protruding from the roof of D1 and the negatively charged C1-carboxylate of glutamate (**A** in Fig. 3c). This interaction is found in all functional eukaryotic iGluRs and mutation of the Arg residue leads to complete loss of channel function.[74] The protonated alpha-amino group of the ligand primarily interacts with a conserved glutamate residue at the C-terminal end of an antiparallel beta sheet on the D2 lobe (**D** in Fig. 3c). Conservative mutants wherein this residue is replaced with an aspartate have much lower affinity for the neurotransmitter.[75–77] Together, these highly conserved residues form what we call the 'amino acid-clamp', which brings the two lobes of the clamshell together upon binding of the ligand (Fig. 3b). In addition to these primary interactions, there is an aromatic residue on D1 that forms the ceiling of the binding site and is in hydrophobic contact with the beta-methylene (C3) of glutamate and its derivatives (**B** in Fig. 3c). This residue is a tyrosine in all structurally characterized AMPA/kainate receptors, a phenylalanine in GluN1 and a histidine in GluN2A. A hydrogen bond involving the ligand's ammonium group and the backbone carbonyl of a proline residue provides additional affinity to the D1 lobe (**C** in Fig. 3c). On the other side, the C5-carboxylate of the glutamate ligand mostly interacts with a threonine residue that sits at the C-terminal end of the so-called 'helix F' in the D2 lobe (Fig 4a). In all structurally characterized iGluRs (with the exception of GluR0), this helix features a Ser-Thr or Ala-Thr motif at its tip (**E** in Fig. 3c). Interestingly, the helix dipole is oriented with its positive end toward the negatively charged carboxylate of glutamate or corresponding bioisosteric groups in agonists.

Glycine and D-serine bind to the GluN1 and GluN3 LBDs in a very similar manner (Fig. 3d). The canonical amino acid-clamp is present, save that the glutamate in position **D** is replaced by an aspartate (cf. pdb 1PB7). The aromatic lid is a phenylalanine side chain and the proline backbone-carbonyl interacts with the ammonium group as usual. The helix dipole of helix F now points toward the alpha-amino carboxylate. Overall, this creates a more compressed binding pocket, in accordance with the smaller ligands it accommodates. The hydroxymethyl side chain of D-serine, which has been recently identified as an endogenous ligand released by glial cells,[78–80] interacts with the serine side chain that is part of the Ser-Thr motif on top of this helix (cf. pdb 1PB8).

## 5. Binding modes of agonists and antagonists

A large number of agonists and antagonists bound to iGluR LBDs have been crystallized and several trends can be deduced from the data obtained. Generally, agonists bind deeply within the cleft of the clamshell and allow the clamshell to close to a large degree. The degree of clamshell-closure more or less correlates with the efficacy of the agonists (Fig. 4).[6] Accordingly, partial agonists, such as substituted willardiines (**15**, for AMPA receptors) or domoic acid (**18**, for kainate receptor), close the clamshell to a lesser degree than full agonists. This is a somewhat simplified picture since the ligand-induced response and coupling to the TMD is complex, the clamshells function as dimers of unsymmetrical dimers, and heteromultimeric iGluRs

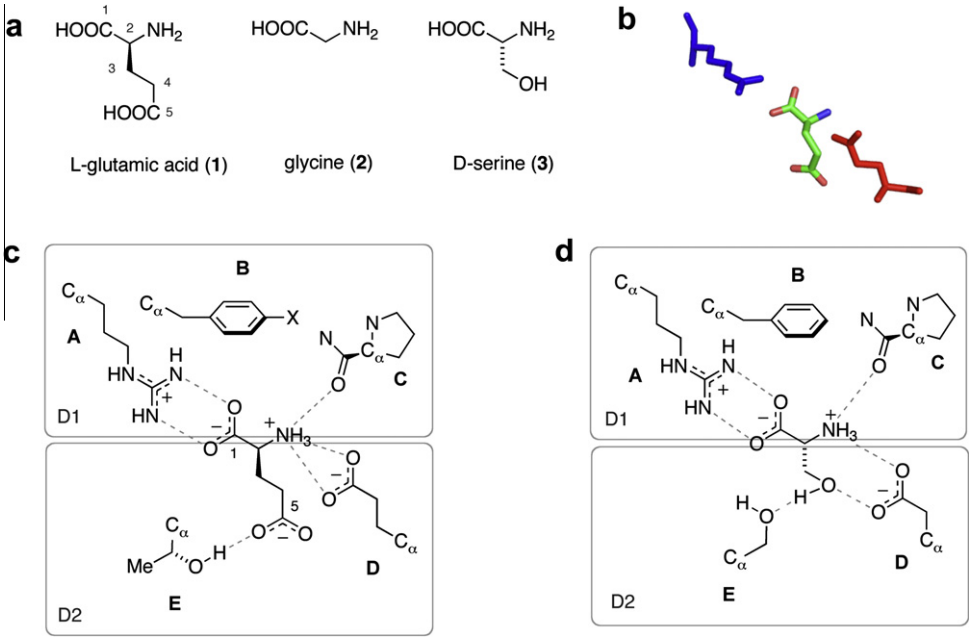


**Figure 3.** (a) Endogenous ligands for AMPA, kainate and NMDA receptors. (b) The amino acid-clamp. (c) Schematic diagram of the major interactions of glutamate with residues on D1 and D2. (d) Schematic diagram of the glycine and D-serine binding site.

must be taken into account. It should be noted that in partially closed LBDs there are several 'exit tunnels' that allow substituents to protrude between the lips.

By contrast, competitive antagonists strongly bind to interaction sites **A**–**C** on D1 and to a lesser extent to residues on D2. In most cases, they literally pry the clamshell open (Fig. 4b).[6] This 'foot in the door' mechanism explains how relatively minor changes can turn an agonist into an antagonist and provides a general paradigm for antagonists in clamshell-containing receptors.

## 6. Agonists for AMPA/kainate receptors

Structurally, agonists and partial agonists of AMPA and kainate receptors are more or less directly derived from glutamate. They invariably feature the alpha-amino acid moiety of the neurotransmitter and bind to the amino acid-clamp in the canonical fashion (cf. Fig. 3b and c). Their other end interacts with helix F and other residues of D2, sometimes mediated by water molecules. There is considerable structural variety with respect to this part of the molecules and there are several positions where the parent glutamate backbone can be substituted without loosing agonist activity. The configuration at these positions is crucial and can be easily explained by the available X-ray structures.

Figure 5 provides an overview of agonists for AMPA-kainate receptors, which can be classified as follows:

(a) Glutamate derivatives wherein C4 and C5 have been replaced by flat, conformationally rigid heterocycles (Fig. 5; **2**–**16**). All of these compounds bind with their alpha-amino moiety in the usual fashion and interact with helix F on D2 with their heterocyclic moieties (**E** in Fig. 3). These are in most cases negatively charged and function as bioisosters of the C5-carboxylate. The classical agonist AMPA (**4**) and its derivatives (**5**–**10**), as well as 4-ACP

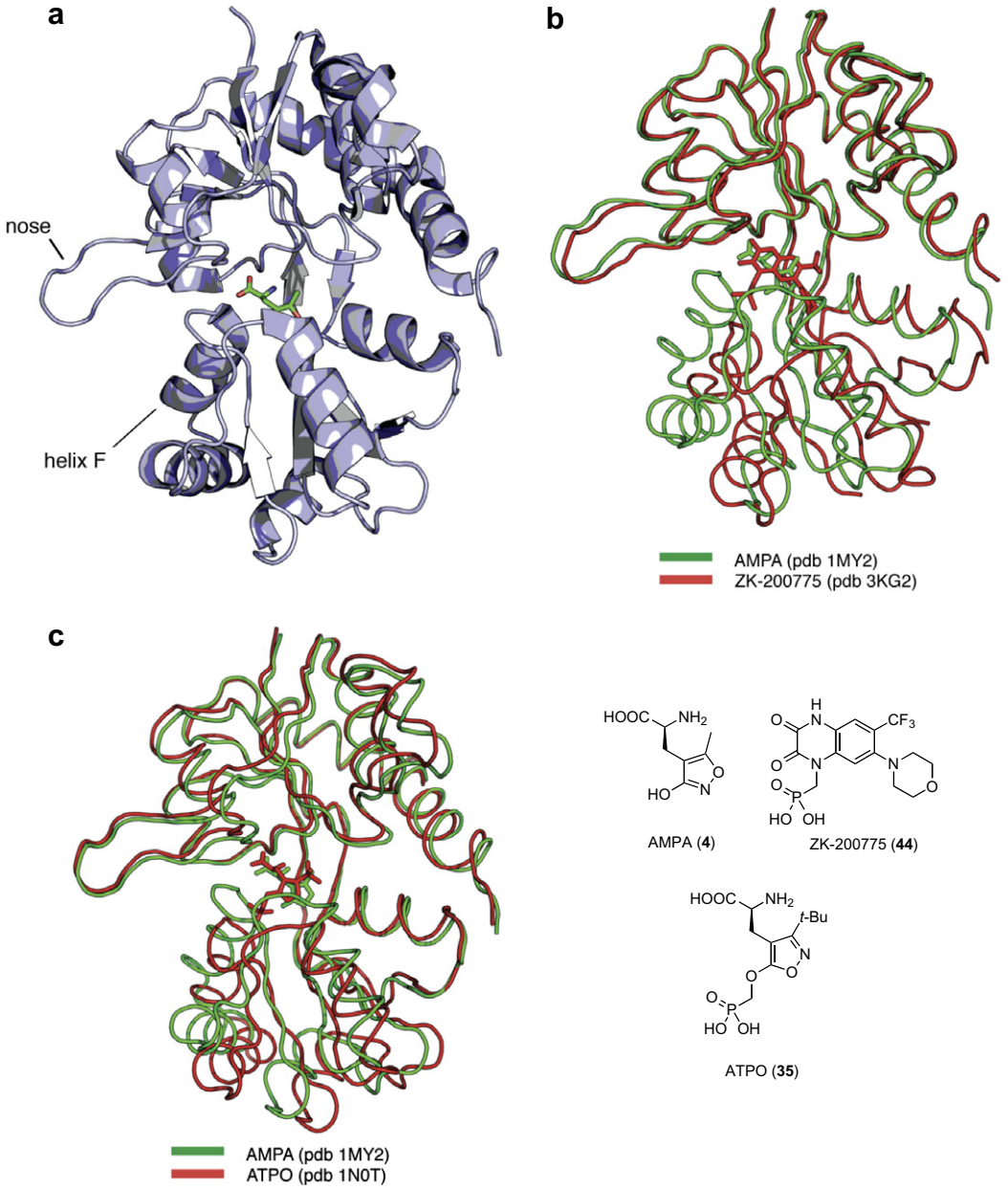


**Figure 4.** (a) Binding mode of a typical agonist, AMPA, to GluA2 (pdb 1MY2). (b) The foot-in-the-door mechanism of antagonism. Structure of the GluA2 LBD bound to AMPA (pdb 1MY2), a selective agonist, overlaid with the LBD bound to ZK-200775 (**44**), a selective antagonist (pdb 3KG2). (c) Structure of the GluA2 LBD bound to glutamate (pdb 1MY2) overlaid with the GluA2 LBD occupied by the AMPA-derived antagonist ATPO (pdb 1N0T).

(**11**), TDBA (**12**), bromo-HIBO (**13**), and the natural products quisqualic acid (**14**) and willardiine (**15a**) belong to this class. A series of substituted willardiines has been prepared with increasingly bulky halogen substituents (**15b–e**). These progressively loose potency as the substituents prevent closure of the clamshell to an increasing degree.[14]

Many derivatives of AMPA have been synthesized and the structure–activity relationships of this class of agonists are welldefined. The isoxazole ring can be substituted with a variety of residues at position 5′, which can even result in selectivity within receptor families. For instance, the bulky *tert*-butylsubstituent on the heterocycle in ATPA (**7**) provides very high selectivity for GluK1.[81,82]

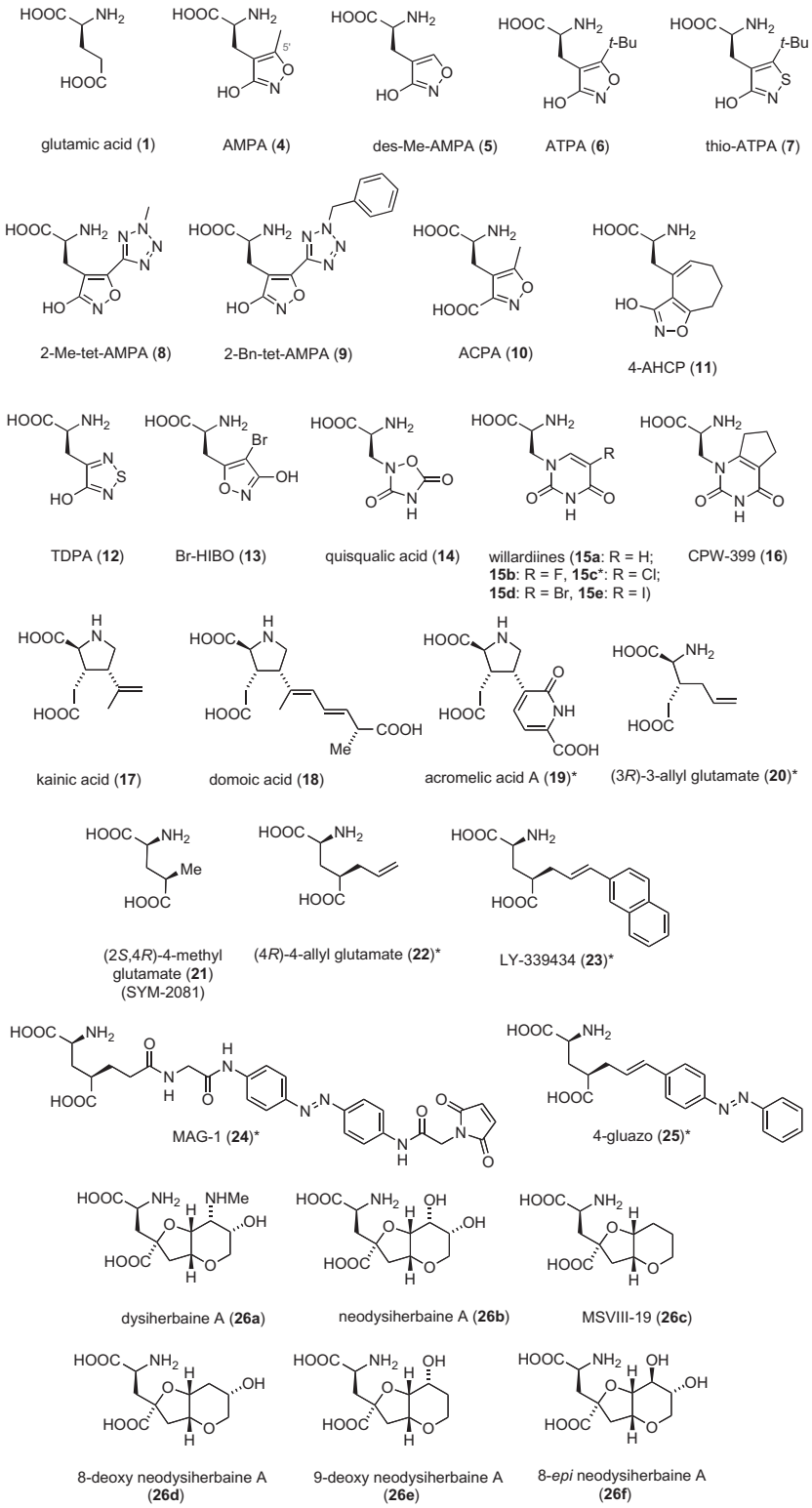


**Figure 5.** Chemical structures of AMPA/kainate receptor agonists. Compounds marked with an asterisk have not yet been crystallized with their receptors.

Bn-Tet-AMPA, which has a slimmer and more elongated substituent, was found to be selective for GluA2-4.[28] The benzyl substituent of the latter substituent protrudes into a region of the clamshell that is not occupied by other agonists (cf. pdb 2P2A). ACPA (**10**) and 4-AHCP (**11**) are expanded and enlarged versions of AMPA that still function as agonists.

(b) L-Glutamate derivatives wherein C3 has been substituted and is D-configured (Fig. 5; **17**–**20**). Compounds of this type often feature a secondary amine as part of a pyrrolidine ring and resemble the natural excitotoxin kainic acid (**17**), which is in itself a conformationally rigidified version of glutamate. Interestingly, the selectivity of kainic acid itself for kainate receptors is rather poor. Indeed, X-ray structures exist for kainic acid bound to GluA2 and GluK2 (pdb 2ANJ and 1TT1). The natural products domoic acid (**18**) and acromelic acid (**19**) are kainic acid derivatives with elongated and heterocyclic side chains, respectively. Only **18** has been cocrystallized with the GluK1 and GluK2 LBDs but it is unlikely that acromelic acid A (**19**) and its many natural and synthetic variants[83–85] will yield surprises. Interestingly, the long unsaturated side chain of the partial agonist **18** protrudes out of the closed clamshell of GluK2 and is partially solvent exposed (pdb 1YAE). Simple glutamate derivatives alkylated in position 3, such as **20**, have been barely explored although the pharmacological profile of **20** looks promising.[86] Similarly, N-substituted derivatives of L-glutamate, such as *N*-methyl-L-glutamate, have not been systematically evaluated to date[87,88] although the crystal structures suggest that some substituents could be tolerated.

(c) Compounds wherein C4 of glutamate has been substituted and is L-configured (Fig. 5 and **21**–**26**). These glutamate derivatives, such as 4-methyl glutamate (**21**) or LY-339434 (**23**), are often selective for kainate receptors.[86] Their substituents at C4 point towards the cleft of the clamshell and can be quite large. They have guided the development of several azobenzene derivatives (e.g., L-MAG1, **24** and 4-GluAzo, **25**), which have been used to convert iGluRs into photoreceptors.[89,90] The extremely potent seizurogenic natural product dysiherbaine A (**26a**) and its natural and semisynthetic derivatives (**26b**–**f**) also belong to this class, demonstrating that the glutamate scaffold can tolerate a quaternary center at the C4 position. The relatively complex heterobicyclic ring system of the dysiherbaines is deeply buried in the LBD of GluK1 (e.g., pdb 3FV1) and its exact composition determines selectivity for GluK1 versus GluK2.[5]

Figure 6 shows the conformation of bound glutamate and several agonists in a Newman projection along C2–C3. It is apparent that this conformation is highly conserved and the C1–C2–C3–C4 dihedral angle is always close to 180° with all agonists and in all receptors. By contrast the C2–C3–C4–C5 dihedral angle varies to a larger degree and is ca. 60° for glutamate and ca. 90° for kainate, respectively, in GluA2 (pdb 1FTJ, 2ANJ).

Despite the large number of agonist-bound X-ray structures, it is difficult to come up with selectivity rules for AMPA versus kainate receptors, in accordance with their diminishing pharmacological discrimination. Looking at the structure of selective agonists bound to their receptor LBD, it seems that kainate receptors can 'chew a bigger chunk'. However, when the structures of GluA2 and GluK1 bound to the same ligand are overlaid, there seems to be little difference with respect to the peptide backbone (glutamate: pdb 1FTJ and pdb 3FUZ, kainate: pdb 1FW0 and pdb 1TT1).

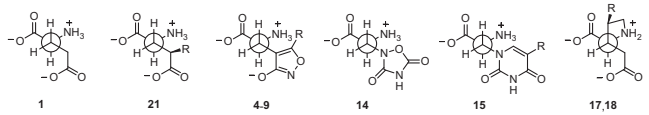


**Figure 6.** Conformation of selected agonists in the binding site.

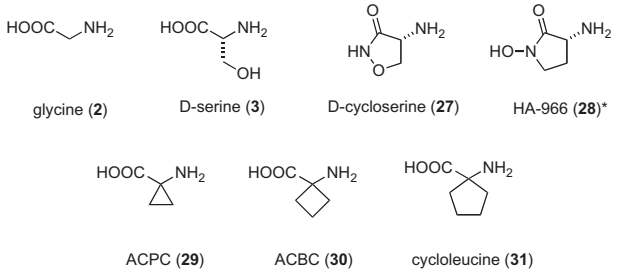


**Figure 7.** Agonists targeting the glycine site of NMDA receptors (GluN1 and GluN3). Compounds marked with an asterisk have not yet been crystallized with their receptors.

Therefore, selectivity has to be assessed on a case-to-case basis and the exact arrangement of non-conserved residues lining the clamshell cleft has to be taken into account. For instance, specific residues have been identified that confer selectivity, for example, for ATPA to GluK1[82] and for Bn-tet-AMPA (**9**) to GluA2-4.[28]

## 7. Agonists for NMDA receptors

Selective agonists of NMDA receptors are usually simple amino acids or amino acid derivatives. Of course, one needs to distinguish between compounds that primarily target the glycine sites (GluN1 or GluN3) and those that bind preferentially to the glutamate sites (GluN2), with their various subtypes. Whereas a wide range of agonists for glycine sites have been crystallized together with their receptor LBDs (Fig. 7; **3**, **27** and **29**–**31**), there are only two structures of the glutamate site (GluN2A), both with glutamate itself (pdb 2A5T, 2A5S). None of the well-established synthetic agonists shown in Figure 8 have been crystallized with their receptors. In particular, there is no structure to date featuring NMDA (**32**), which would be useful in explaining the high selectivity of the eponymous ligand.

## 8. Antagonists for AMPA/kainate receptors

Antagonists of AMPA and kainate receptors show a much wider structural variety than agonists and can belong to one of two major structural classes (Fig. 9):

(a) Expanded versions of agonists (Fig. 9; **35**–**39**). These bear the canonical alpha-amino acid motif of agonists but have their negatively charged other end moved further away. As elongated molecules, they still interact with the amino acid-clamp and the top of helix F thus prying the clamshell open. The phosphonate ATPO (**35**), derived from ATPA, and the willardiine derivatives **36a**–**g** are typical representatives of this category. A number of kainate receptor-selective *cis*-decahydroisoquinolines, such as LY-466195 (**37**), tezampanel (**38**) or LY-294486 (**39**), also belong to this group. They are, in a sense, ring-expanded and homologated versions of kainic acid. LY-466195 (**37**) has been crystallized together with GluK1 and shows the typical amino acid-clamp binding and interaction with helix F in the largely open clamshell (pdb 2QS4).

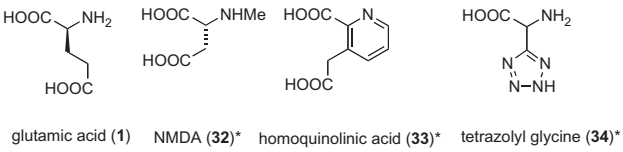


**Figure 8.** Agonists targeting the glutamate site of NMDA receptors (GluN2). Compounds marked with an asterisk have not yet been crystallized with their receptors.

(b) Quinoxalines (Fig. 9; **40**–**46**). These are classical antagonists of AMPA/Kainate receptors, represented by DNQX (**40**) and it congeners CNQX (**41**), NBQX (**42**), YM-90K (**43**) and ZK-200775 (**44**). Quinoxaline antagonists interact with the canonical D1-arginine but do not appear to make much contact with the conserved D2-glutamate residue. The 'aromatic lid' on the S1 lobe of the receptor (usually a tyrosine) stacks tightly against the flat and conformationally rigid aromatic core of these antagonists (e.g., pdb 1LB9). With the exception of the high affinity antagonist ZK-200775 (**44**), which contains an additional phosphonate moiety, the quinoxalines do not appear to interact strongly with the top of helix F or any other residue in D2 but they do form hydrogen bonds to

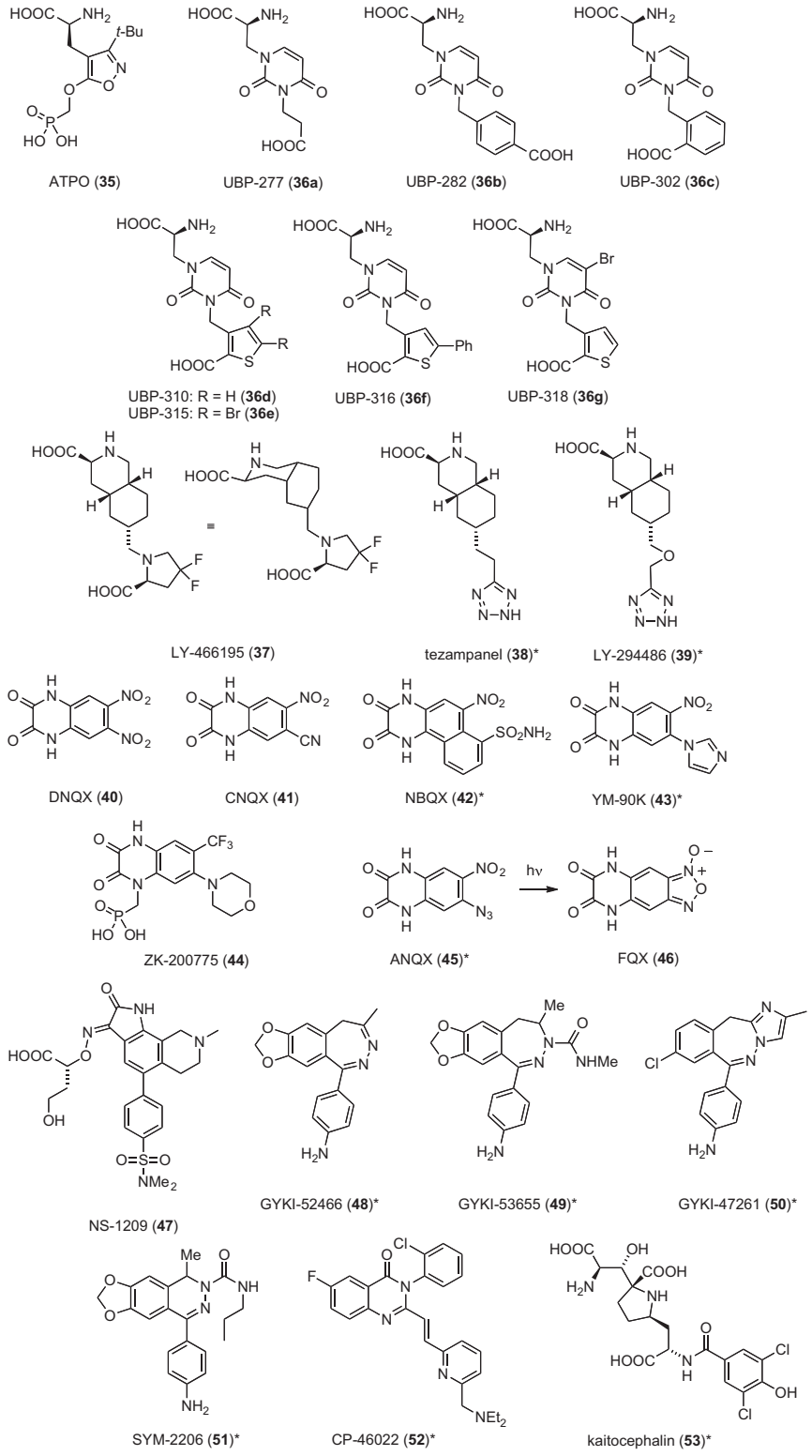


**Figure 9.** Chemical structures of AMPA/kainate receptor antagonists. Compounds marked with an asterisk have not yet been crystallized with their receptors.

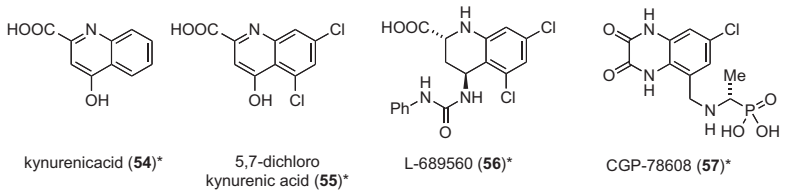


**Figure 10.** Antagonists targeting the glycine site of NMDA receptors (GluN1 and GluN3). Compounds marked with an asterisk have not yet been crystallized with their receptors.

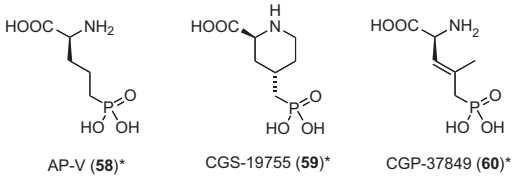


**Figure 11.** Antagonists targeting the glutamate site of NMDA receptors (GluN2). None of these have been crystallized with their receptors.

the backbone carbonyl of the highly conserved proline residue on D1. A special case is the quinoxaline FQX (**46**), which is one of the photoproducts of the azide ANQX (**45**). ANQX was developed as a photo affinity-label and used to study AMPA-receptor trafficking.[91,92]

In addition to this, there is one structural outlier, NS-1209 (**47**), which has been crystallized together with glutamate in a GluA2 clamshell dimer (pdb 2CMO). The carboxylate and the oxime ether nitrogen of this clinically interesting antagonist interact with the D1-arginine, whereas the central aromatic ring stacks tightly against the lid-tyrosine.

To date, there are no crystal structures available for 2,3-benzodiazepines, such as GYKI-52466 (**48**), GYKI-53655 (**49**), or GKYI-47261 (**50**), which are highly selective antagonists of AMPA/kainate receptors. These compounds, as well their congeners SYM-2206 (**51**) and CP-46022 (**52**), are non-competitive antagonists and presumably bind to an allosteric site on the receptor. Unfortunately,

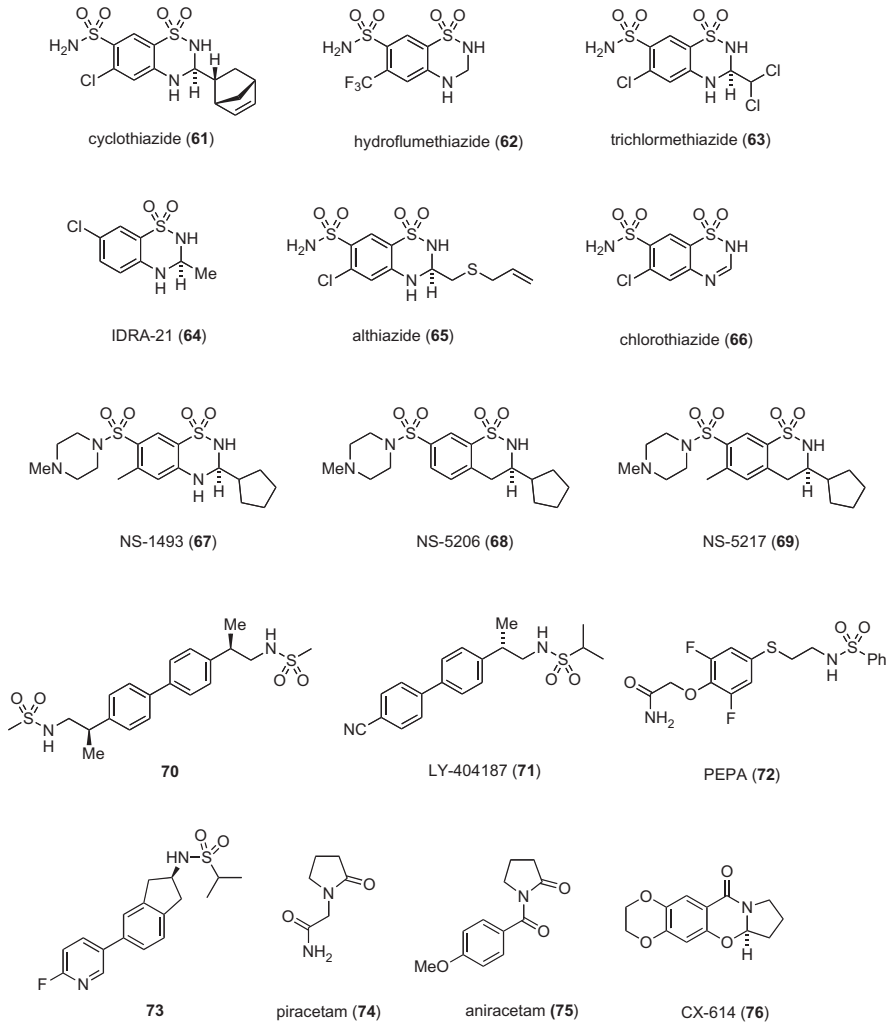


**Figure 12.** Modulators of AMPA receptors.

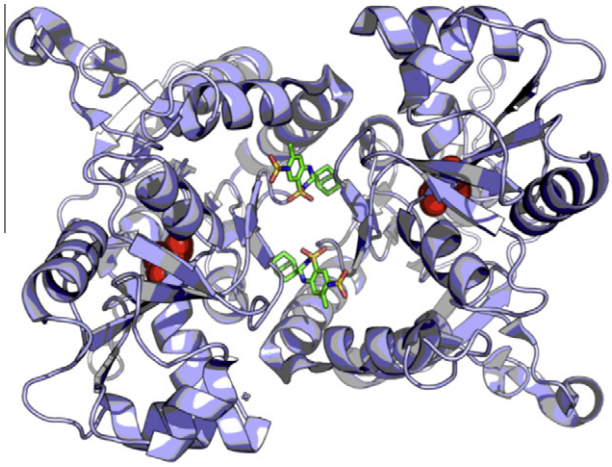

**Figure 13.** Structure of cyclothiazide (**61**) bound to an LBD dimer (pdb 3H6T). The clamshell dimer is seen from the intracellular side and bound glutamate is displayed as red spheres.

there is also currently no structure featuring the natural product kaitocephalin (**53**), which has been characterized as an AMPA receptor antagonist but is also a potent inhibitor of NMDA receptors.[93,94] As a twofold D-amino acid and an L-amino acid with a quaternary stereocenter and a benzoyl residue it would be very interesting to see how (or if) this compound binds to the clamshell.

## 9. Antagonists for NMDA receptors

A large number of antagonists targeting NMDA receptors have been developed in academia and industry but only one of these has been crystallized together with its receptor: 1,3-dichlorokynurenic acid (**55**) binds to the GluN1 clamshell and interacts with the conserved arginine and the proline carbonyl, but makes few polar contacts otherwise (Fig. 10; pdb 1PBQ). It stacks against the aromatic lid (a phenylalanine) and appears to be stabilized by the helix-dipole of helix F. The compound is a halogenated derivative of the tryptophan metabolite kynurenic acid (**54**), which was initially isolated from the urine of dogs by Justus von Liebig.[95] There are no structures featuring the very potent antagonist L-689560 (**56**) or the quinoxaline derivative CGP-78608 (**57**). It should be noted that quinoxalines more commonly associated with AMPA receptors, such as CNQX (**41**), also target the glycine site of NMDA receptors.[96]

Unfortunately, no structures are available for antagonists bound to the glutamate binding-site of NMDA receptors, in particular the widely used antagonist AP-V (**58**) and its congeners **59** and **60** (Fig. 11). The NMDA receptor selectivity of these aminophosphonates is presently difficult to understand.

## 10. Modulators and channel blockers of iGluRs

Like most ion channels, iGluRs desensitize following prolonged activation. This event takes place relatively rapidly (within milliseconds) in AMPA/kainate receptors but requires more time (tens to hundreds of milliseconds) in NMDA receptors.[1] Due to its important role in physiology, desensitization has been studied in great detail and many modulators have been developed.[27] There are several crystal structures of GluA2 receptor LBDs that have helped define the mechanism of desensitization (Fig. 12). Modulators generally bind to the LBD interface between the D1 lobes, thus inhibiting or slowing down their separation in the course of desensitization.

Modulators display a wide structural variety but certain chemotypes can be identified (Fig. 12): (a) Benzothiadiazine derivatives, such as cyclothiazide (**61**), and its congeners **62**–**69**; (b) linear sulfonamides, for example, the $C_2$-symmetrical *bis*-sulfonamide **70**, LY-404187 (**71**) and PEPA (**72**); and (c) pyrrolidones and acylpyrroles, for example, piracetam (**74**), aniracetam (**75**) or CX-614 (**76**). The structure of cyclothiazide (**61**) bound between the D1 lobes of two clamshells is shown in Figure 13.

With only one structure of an iGluR featuring a TMD available (pdb 3KG2), it comes as no surprise that the binding-sites of NMDA receptor blockers, such as MK-801 (**77**), phencylidine (**78**), ketamine (**79**) or the recently discovered natural product lophocladine A (**80**),[97] have not yet been structurally characterized. However, since blockers of fourfold-symmetrical potassium channels are amenable to structural studies,[98] it seems possible that we will soon understand how these important blockers operate. Related structural studies could also explain the voltage-dependent block of NMDA receptors by $Mg^{2+}$ and of non-NMDA receptors by spermine (Fig. 14).

Finally, several structures of the ATD of iGluRs have been published (Table 1) but, to date, none of these feature a functionally important ligand, such as $Zn^{2+}$, a polyamine, or the developmental drug ifenprodil (**81**) which interacts with GluN3 and functions as an NMDA receptor antagonist.[99]

## 11. Conclusion

Almost 150 structures of agonists, antagonist and modulators bound to LBDs of iGluRs, as well as several *apo* structures, are now available and many more can be expected in future years (Table 1). Hopefully, crystal structures of GluA1 and GluK3–5 or a full-fledged NMDA receptor will soon emerge in this rapidly moving field. Structural studies of the C-terminal domain of iGluRs could help answer questions regarding receptor localization and modulation. On the ligand side, we are still missing coordinates for several important and interesting agonists and antagonists (e.g., NMDA, AP-V, kaitocephalin, 4-gluazo and GYKI-52466). Nevertheless, we are beginning to understand which molecular features determine subtype-selectivity as far as this is possible using detailed but static crystal structures. After all, one should bear in mind that affinities, let alone efficacies, cannot be reliably calculated with our current techniques, even when multiple crystal structures are available for calibration and comparison. However, the existing crystal structures provide guidance where to substitute known ligands (e.g., glutamate itself, AMPA, or kainate) to achieve the desired effect. They certainly offer a rationale for the development

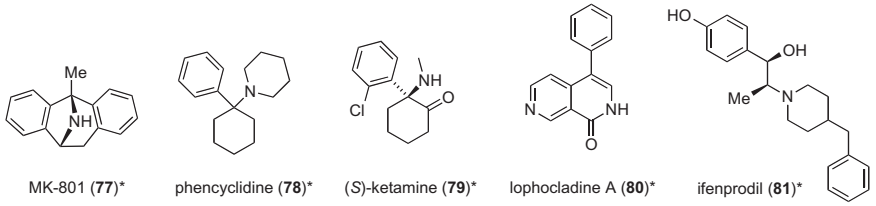


**Figure 14.** Compounds that block the TMD of the NMDA receptors or target the ATD of iGluRs. None of these have been crystallized with the TMD or ATD.

of new and possibly more selective agonists and antagonists, exploiting spaces in the clamshell and its cleft that have not been occupied by existing types and interactions with non-conserved amino acid residues that have not been addressed. The design of ligands with new functional properties, for example, responsiveness to light, will also benefit from continued structural studies. Finally, this wealth of structural information could allow for the design of orthogonal receptors that cease to respond to the natural agonists but can be activated by unnatural, orthogonal ligands.

## Acknowledgments

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