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Structural basis for 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptor activation*

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Abstract: (*S*)-Glutamic acid (Glu), the major excitatory neurotransmitter in the central nervous system, operates through ionotropic as well as metabotropic receptors and is considered to be involved in a number of degenerative brain diseases that are currently without any satisfactory therapeutic treatment. Until recently, development of selective Glu receptor agonists had mainly been based on structural optimization of naturally occurring lead compounds structurally related to Glu. Crystallization of the agonist binding domain of the GluR2 subunit of the 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptor subtype of ionotropic Glu receptors (iGluRs) in the presence or absence of an agonist has provided important information about ligand-receptor interaction mechanisms. The availability of such binding domain crystal structures has formed the basis for rational design of ligands, especially for the AMPA subtypes of iGluRs.

INTRODUCTION

(*S*)-Glutamate (Glu) receptors are divided into two main classes, ionotropic (iGlu) and metabotropic (mGlu) receptors. The iGluRs consist of three heterogeneous groups of receptor subtypes named after the selective agonists *N*-methyl-D-aspartate (NMDA, **1**), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate [(*S*)-AMPA, **2**] and kainate (KA, **3**) (Fig. 1), whereas the mGlus are divided into Groups I, II, and III [1–3]. All Glu receptors are operated by the endogenous ligand Glu and are potentially interesting in relation to the treatment of a number of neurological disorders [3,4].

Cloning of the different subunits of the GluRs represents a major breakthrough toward structural and pharmacological characterization of these receptors. In total, 16 different ionotropic receptor subunits have been identified together with 8 different metabotropic receptors (Fig. 1). A comparison of the overall sequence identity of the receptor proteins discloses a relative close relationship between the individual subunits [3]. However, when comparing the ligand-binding domain of NMDA, AMPA, and KA receptors, the amino acid identity is much higher, as many of the essential amino acid residues are conserved in the ligand-binding site of all iGluR subunits [5]. Selective competitive NMDA receptor antagonists as exemplified by (*R*)-2-amino-5-phosphonovaleric acid [(*R*)-AP5, **4**] have been known for decades, whereas the selective competitive AMPA receptor antagonist, NBQX (**5**), and in particular, the GluR5 selective KA receptor antagonist, compound **6** have been discovered more recently [3,6].

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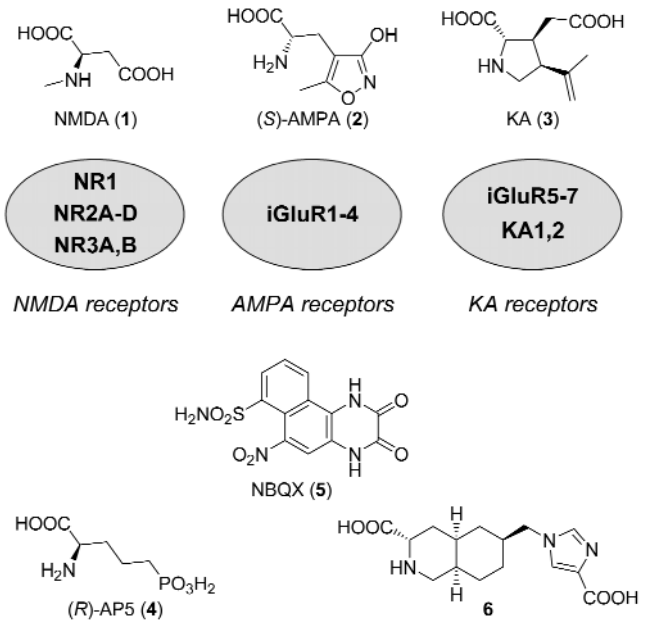

Fig. 1 Schematic illustration of subtypes and multiplicity of iGlu receptors including early examples of subtype-selective agonists (top) and antagonist (bottom).

This review will focus on AMPA receptors and the present and future use of structure-based design in relation to compounds interacting with the ligand-binding site. Special interest will be devoted to stereochemical aspects. Structure-based ligand design has recently become possible in connection with iGluRs, as a number of X-ray structures of the ligand-binding site of GluR2 in complex with agonists as well as competitive antagonists have provided much structural information on this particular topic.

AMPA RECEPTOR SITES AND DESENSITIZATION

Activation of AMPA and KA receptors mediate fast excitatory transmission via influx of Na^+ and for some receptors also Ca^{2+}, and efflux of K^+ ions through opening of the integral ion channel leading to depolarization of the postsynaptic membrane. After activation, the receptor enters a desensitized state in which the agonist remains bound, but with the ion channel closed (Fig. 2). Recent evidence based on the X-ray structure of the ligand-binding site of GluR2 in complex with the AMPA receptor potentiator cyclothiazide (**7**) suggests that receptor desensitization at the molecular level is associated with the interfacial contact between adjacent receptor subunits, and that stabilization of the protein interface inhibits desensitization [7].

In addition to the agonist binding site, which is also the target of competitive antagonists such as the quinoxalinedione NBQX (**5**) [8], AMPA receptors possess modulatory sites capable of affecting receptor desensitization [7,9]. These modulatory sites are potentially important therapeutic targets and have received much attention in recent years. The agonist-induced desensitization of AMPA receptors can be markedly inhibited by cyclothiazide (**7**) (Fig. 2), which positively modulates ion flux via stabilization of the receptor subunit interface and a subsequent reduction in the degree of desensitization induced by agonists [7]. Furthermore, negative modulators possessing a 2,3-benzodiazepine ring system, as exemplified by talampanel (**8**), have been identified. These AMPA receptor-selective allosteric antagonists may turn out to be effective for the treatment for multiple sclerosis, epilepsy, and Parkinson's disease [10].

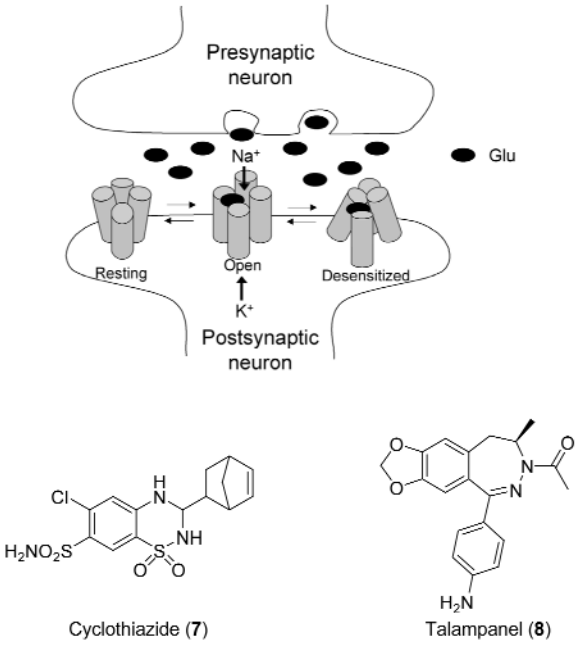

Fig. 2 A schematic representation of postsynaptic AMPA receptors in the closed form in the absence of agonist, in the open agonist-bound form, and in the desensitized form. The chemical structures of some AMPA receptor modulators are shown.

Structure of AMPA receptors

AMPA receptor subunits may be considered as modular constructions of three distinct domains: (1) a transmembrane segment consisting of three transmembrane helices and a re-entrant loop; (2) an extracellular ligand-binding domain (S1S2) composed of two lobes connected by a hinge ("venus flytrap"), distantly related to glutamine binding protein (QBP); and (3) an *N*-terminal domain structurally related to periplasmic binding proteins such as leucine-isoleucine-valine binding protein (LIVBP). It is currently believed that AMPA receptors assemble as dimers-of-dimers. Numerous studies, including X-ray crystallographic approaches, now support a tetrameric structure of the AMPA receptor that resembles some K^+ channels (Fig. 3, page 927) [5,7,11].

Design of selective AMPA receptor agonists

A number of naturally occurring Glu analogs originating from microorganisms and plants have been identified as AMPA receptor agonists showing varying degrees of selectivity. Some of these compounds—e.g., ibotenic acid (**9**) and willardiine (**10**) (Fig. 4)—have been used as leads for the design of more selective and potent agonists [12,13]. The AMPA receptors were originally identified pharmacologically by using the naturally occurring Glu analog quisqualic acid (**11**). This 1,2,4-oxadiazole-3,5-

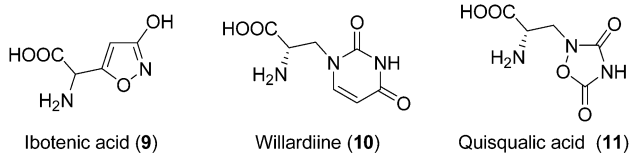

Fig. 4 Chemical structures of some naturally occurring Glu analogs containing different distal acidic functionalities.

dione analog of Glu was later shown also to possess potent mGluR agonist effects, as well as affinity for KA receptors. As a consequence, quisqualic acid was replaced by the highly selective compound (*S*)-AMPA (**2**) as a standard agonist for AMPA receptors [9]. Bioisosteric replacement of the distal carboxyl group of Glu, extension of the Glu backbone, and incorporation of the Glu backbone into more rigid scaffolds have provided a wide range of new potent pharmacological tools with marked receptor selectivities. The successful crystallizations of the ligand-binding domain of GluR2 in complex with agonists [14–16] have provided a wealth of information, which is helpful for an improved understanding of the molecular basis of the ligand-receptor interactions. In addition, these receptor structures have made structure-based design of new AMPA and KA receptor ligands possible.

AMPA analogs

Analogs of (*S*)-AMPA (**2**) having aliphatic, aromatic, or heteroaromatic substituents at the 5-position of the isoxazole ring have been synthesized and pharmacologically characterized [17–19]. Replacement of the methyl group of (*S*)-AMPA (**2**) with small alkyl groups provided compounds with potencies in the same range as that of (*S*)-AMPA (**2**) and showed the same intrinsic activity as (*S*)-AMPA (**2**) in electrophysiological studies on cortical slices [19]. Increase of the size of the 5-alkyl substituent to three or more carbon atoms, such as the *tert*-butyl analog ATPA (**12**) (Fig. 5), markedly reduced AMPA receptor agonist potency (Table 1) [17].

Table 1 AMPA receptor binding affinities and electrophysiological data of AMPA analogs.

Compound	[^{3}H]AMPA[a] ($IC_{50,}$ μM)	Electrophysiology[b] ($EC_{50,}$ μM)
AMPA	0.04	3.5
ATPA (**12**)	3.9	48
APPA (**13**)	35	385
2-Py-AMPA (**14**)	0.57	7.4
3-Py-AMPA (**15**)	>100	>1000
4-Py-AMPA (**16**)	5.5	96
Tet-AMPA (**17**)	72	>1000
1-Me-Tet-AMPA (**18**)	54	>1000
2-Me-Tet-AMPA (**19**)	0.03	0.92

[a]Data from whole rat brain homogenate.
[b]Data from the rat cortical wedge.

The results of these studies gave rise to a hypothesis concerning the topography of the agonist-binding site of AMPA receptors, including the presence of a cavity capable of accommodating lipophilic substituents of a limited size. The presence of such a cavity has recently been confirmed by X-ray crystallographic studies on the GluR2 binding domain co-crystallized with agonists [16]. Whereas all alkyl-substituted AMPA analogs synthesized so far show either full agonism or inactivity at AMPA receptors, the 5-phenyl substituted analog of AMPA [APPA (**13**)] showed the characteristics of a weak partial AMPA receptor agonist with an efficacy of approximately 60 % relative to that of AMPA. Quite surprisingly, (*S*)-APPA turned out to be a full AMPA receptor agonist, while (*R*)-APPA proved to be a weak competitive AMPA receptor antagonist [20].

As a consequence of this enantiopharmacology of APPA, the three possible pyridyl analogs, (**14**–**16**) (Fig. 5), were synthesized [19]. Whereas 2-Py-AMPA (**14**) proved to be a potent and full agonist with considerably higher affinity than APPA, 3- and 4-Py-AMPA (**15**) and (**16**), respectively, possessed very weak agonist effects at AMPA receptors [19]. This clearly demonstrates that positioning of

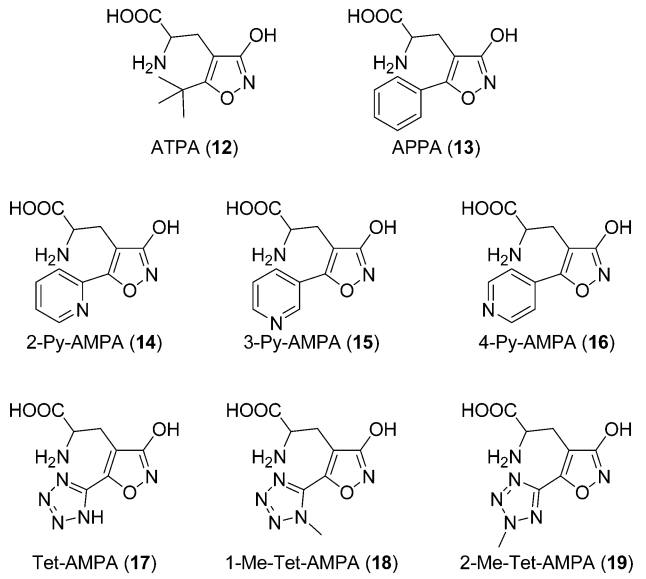

Fig. 5 Chemical structures of selected analogs of AMPA.

the nitrogen atom in the heterocyclic 5-substituent is of decisive importance for the activity of this type of AMPA receptor ligands.

In order to elucidate the structure–activity relationship of these 5-substituted AMPA-analogs further, a number of 5-membered heteroaromatic substituents were introduced, including an unsubstituted 5-tetrazolyl [Tet-AMPA (**17**)], 1-methyl-5-tetrazolyl [1-Me-Tet-AMPA (**18**)], and 2-methyl-5-tetrazolyl [2-Me-Tet-AMPA (**19**)] [18]. Whereas Tet-AMPA (**17**) and 1-Me-Tet-AMPA (**18**) were devoid of agonist activity at AMPA receptors, 2-Me-Tet-AMPA (**19**) proved to be the most potent AMPA receptor agonist synthesized so far, at least 10 times more potent than AMPA [18]. This indicates that steric as well as electronic properties play important roles for the binding of the ligands to the receptor.

Electrophysiological studies of the enantiomers of 2-Me-Tet-AMPA (**19**) showed that the excitatory properties reside exclusively in the *S*-enantiomer similar to those of AMPA (Table 2) [21]. Neither (*R*)-AMPA nor (*R*)-2-Me-Tet-AMPA show detectable receptor affinity. Compared to AMPA, which did

Table 2 AMPA receptor binding affinities and electrophysiological data of stereoisomers of AMPA and related compounds.

Compound	[^{3}H]AMPA[a] ($IC_{50,}$ μM)	Electrophysiology[b] ($EC_{50,}$ μM)
(*R*)-AMPA	76	580
(*S*)-AMPA (**2**)	0.021	3.8
(*R*)-2-Me-Tet-AMPA	15	352
(*S*)-2-Me-Tet-AMPA	0.009	0.11
(*R*)-5-HPCA (**20**)	0.47	nd[c]
(*S*)-5-HPCA (**21**)	>100	nd[c]
(*R*)-TDPA (**22**)	0.27	6.6
(*S*)-TDPA (**23**)	0.065	20

[a]Data from whole rat brain homogenate.
[b]Data from the rat cortical wedge.
[c]Not determined.

not distinguish between the individual cloned AMPA receptors [21, 22], (*S*)-2-Me-Tet-AMPA showed some selectivity in electrophysiological studies preferring homomers of GluR3 and GluR4 over receptors constructed from GluR1 or GluR1/GluR2 expressed in *Xenopus* oocytes.

Based on a crystal structure of the ligand-binding domain of the GluR2 subunit in complex with (*S*)-2-Me-Tet-AMPA [16], the 2-methyl tetrazole heterocyclic unit apparently is not involved in hydrogen-bonding interactions with the protein. The 2-methyl-tetrazole moiety actually fits almost perfectly into the partly hydrophobic and partly polar cavity of the receptor protein, resulting in favorable van der Waals interactions with the receptor [16]. Although a deep insight into the binding mode of 5-heteroaryl substituted AMPA analogs to GluR2 at the molecular level has been provided through the crystal structure of (*S*)-2-Me-Tet-AMPA [16], the precise mechanism underlying the enhanced affinity observed for 2-Py-AMPA (**14**) as compared with 3-Py-AMPA (**15**) remains to be accounted for. However, molecular modeling studies based on the crystal structure of the GluR2 ligand-binding domain in complex with (*S*)-2-Me-Tet-AMPA indicated that the pyridyl nitrogen atom of (*S*)-3-Py-AMPA might be positioned close to and interact with Glu402 or Thr686, thus preventing domain closure and resulting in an unfavorable conformation of the binding position. The pyridyl nitrogen atom of the potent agonist (*S*)-2-Py-AMPA [23], on the other hand, cannot get into close contact with Glu402 or Thr686. Instead, it may make favorable interactions, directly or through a water molecule, to the backbone NH of Glu705, or alternatively form intramolecular interactions with the α-ammonium group in (*S*)-2-Py-AMPA [16].

CONFORMATIONALLY RESTRICTED AMPA ANALOGS

Conformational restriction of the skeleton of Glu has been used in the design of selective iGluR ligands. However, only a very few AMPA receptor selective semi-rigid Glu analogs have been reported. One such example is the cyclized analog of AMPA, 5-HPCA [24] (Fig. 6), which has recently been resolved [25]. 5-HPCA was resolved using chiral chromatography, and the absolute configuration was based on an X-ray analysis in combination with a stereoconservative synthesis of (*S*)-5-HPCA (**21**) from (*S*)-AMPA and a comparison of ab initio-calculated circular dichroism spectra with the experimentally determined spectra. Interestingly, the pharmacological effect of 5-HPCA was shown to reside exclusively with the *R*-enantiomer (**20**), in striking contrast to the usual stereoselectivity trend among AMPA receptor agonists (Table 2).

To examine the structural basis of this observed enantiopharmacology, (*R*)- and (*S*)-5-HPCA were docked into the GluR2 ligand-binding site (Fig. 6). These studies disclosed that (*R*)-5-HPCA (**20**) (Fig. 6B, dark brown carbons) could adopt a conformation similar to that of (*S*)-AMPA [15] and present almost all of the required pharmacophore elements to the receptor [25]. Thus, conformational restriction forces the major pharmacophore elements of (*R*)-5-HPCA (**20**) into a spatial orientation similar to those of more flexible (*S*)-AMPA analogs when bound to AMPA receptors.

(*S*)-5-HPCA (**21**) presents a markedly different picture (Fig. 6C) and is clearly less well accommodated by the receptor due to the rigid nature of this compound. The best-scored pose (Fig. 6C, blue carbons) presents an unlikely inverted binding mode, with the anionic 3-isoxazolol ring bound to R485, and the NH_2^+ interacting with E705 at an unfavorable angle. While it is possible to dock (*S*)-5-HPCA (**21**) in a more (*S*)-AMPA-like binding mode (Fig. 6C, light brown carbons), the score is poorer and the axial ammonium proton is directed toward the face of Y450. Most critically, conformational restriction of the *S*-α-amino acid in this way has abolished the ability of the ammonium group to donate a hydrogen bond to E705, which according to all structural evidence to date is a prerequisite for agonism at iGluRs [15, 16]. Neither pose of (*S*)-5-HPCA (**21**) is able to fulfill α-amino acid recognition by hydrogen bond donation to T480 and E705 (missing hydrogen bonds and poor interactions are shown in red in Fig. 6C).

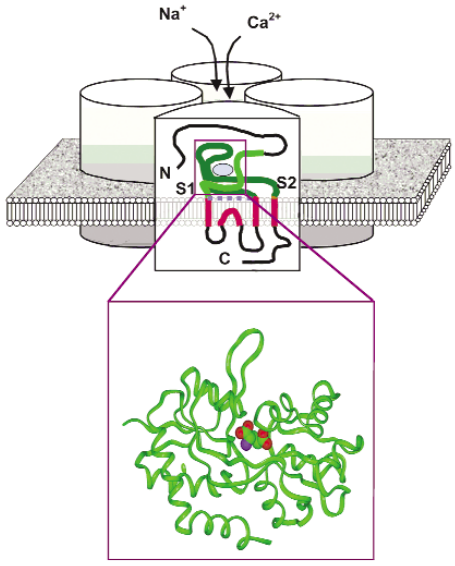

Fig. 3 Model of the topology of AMPA receptors with Glu bound to one of the subunits.

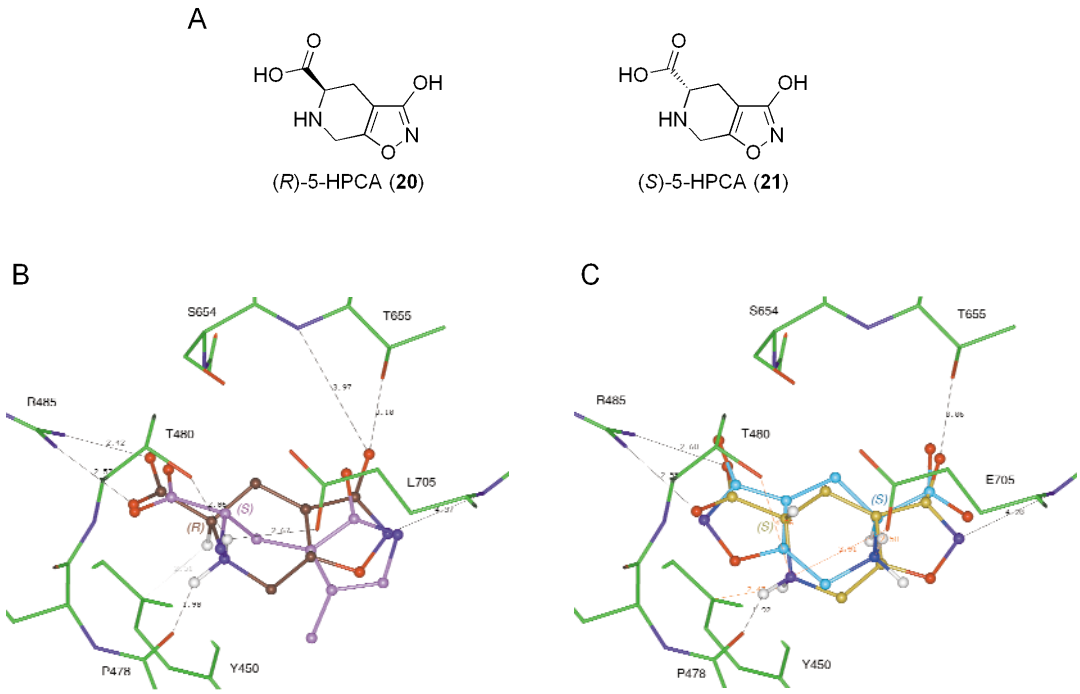

Fig. 6 A: Structure of (*R*)- and (*S*)-5-HPCA (**20** and **21**, respectively). **B** and **C**: Docking of tri-ionized (*R*)- and (*S*)-5-HPCA to the GluR2 agonist binding site. Hydrogens are omitted except for those of the α-carbon and ammonium centers. Atoms color-coded by type except for ligand carbon atoms. Important hydrogen bonds shown as dashed black lines and poor contacts by dashed red lines. **B**: Lowest-energy pose of (*R*)-5-HPCA (**20**) (dark green carbons). Experimental (*S*)-AMPA position (pink carbons) shown for comparison. **C**: (*S*)-5-HPCA (**21**) docked to GluR2. Lowest-energy pose (blue carbons) shows an inverted binding mode (3-isoxazolol on the left) while the more (*S*)-AMPA-like pose (light brown carbons) is higher in energy.

BIOISOSTERIC REPLACEMENT

The 1,2,5-thiadiazol-3-ol analog of Glu (TDPA) in which the distal carboxylate group of Glu has been replaced by a heterocyclic group possesses acidic protolytic properties similar to those of Glu with respect to the distal acidic moiety [26]. TDPA was recently resolved by chromatographic enantioseparation using a Crownpac CR(+) column, and an X-ray crystallographic analysis demonstrated that the first eluting (–)-enantiomer possesses the *R*-configuration [27]. Electrophysiological studies showed that both (*R*)- and (*S*)-TDPA (**22** and **23**, respectively, Fig. 7) are AMPA receptor agonists, (*R*)-TDPA (**22**) being the eutomer, in striking contrast to the 3-isoxazolol-based Glu analogs discussed above (Table 2). However, in [^{3}H]AMPA binding, (*S*)-TDPA (**23**) displayed approximately five times higher affinity than the *R*-enantiomer (**22**). This discrepancy between binding affinity and potency in cortical neurons is most likely due to an interaction, probably as a substrate, with the Glu transporter protein, EAAT2, observed only for the (*S*)-TDPA [28].

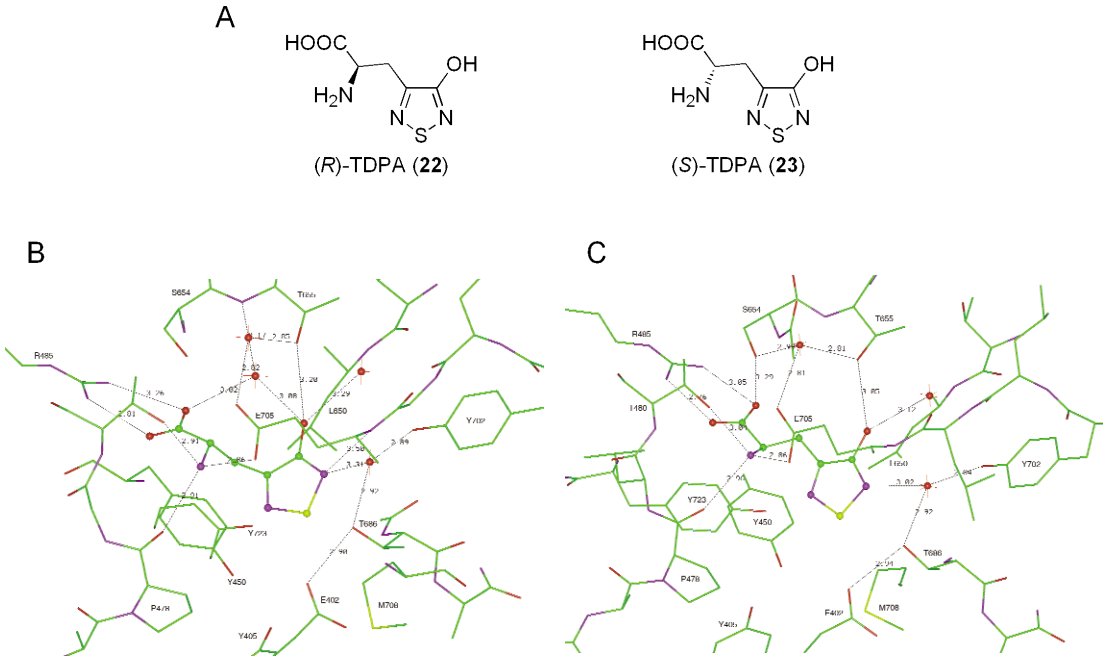

Fig. 7 A: Chemical structures of (*R*)- and (*S*)-TDPA (**22** and **23**, respectively). **B** and **C**: Docking of (*S*)- and (*R*)-TDPA, respectively, to the GluR2 agonist binding site.

In order to better understand the low AMPA receptor stereoselectivity observed for TDPA at the molecular level, (*S*)- and (*R*)-TDPA were docked into the fully domain-closed X-ray structure of the GluR2 binding domain (Fig. 7) [29]. The docking studies indicate that both (*S*)- and (*R*)-TDPA can fold into conformations that present the required pharmacophore elements to the receptor in similar orientations, in the latter case by dint of the unsubstituted sp^2-hybridized 5-position ring nitrogen, which distinguishes this compound from the inactive (*R*)-AMPA (Table 2). (*S*)-TDPA (**23**) is predicted to adopt a binding mode very similar to that of (*S*)-AMPA, while for (*R*)-TDPA (**22**) the precise orientation of the bound heterocycle and induced water architecture are likely to show some differences from those previously observed. Whether (*R*)- and (*S*)-TDPA bind differently to the individual AMPA receptor subtypes remains to be examined.

The compounds included in this review represent examples of AMPA receptor agonists originally designed and developed before detailed knowledge about the AMPA receptor binding domain was ob-

tained. With the crystal structures of GluR2 binding domain now available, it is possible to attain a deeper understanding of the interaction of these compounds with the receptor protein. Furthermore, the crystal structures reported so far open up a new era for medicinal chemists and become valuable tools for future rational design of subtype-selective ligands targeted at the individual iGluRs.

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