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1,2,3-Triazolyl amino acids as AMPA receptor ligands

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ABSTRACT

The central nervous system glutamate receptors are an important target for drug discovery. Herein we report initial investigations into the synthesis and glutamate receptor activity of 1,2,3-triazolyl amino acids. Two compounds were found to be selective AMPA receptor ligands, which warrant further investigation.

(*S*)-Glutamate (**1**) is the primary excitatory neurotransmitter in the central nervous system (CNS) and its receptors are an important target for drug discovery. Dysfunction of glutamatergic systems has been implicated in a wide range of neurological disorders including chronic pain, schizophrenia, Alzheimer's disease, epilepsy, drug dependence and depression.[1–4] There are two distinct types of glutamate receptor: ionotropic and metabotropic. The ionotropic glutamate receptors (iGluRs) are a class of heteromeric ligand-gated ion channels comprising three subtypes: *N*-methyl-D-aspartate (NMDA), (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and 2-carboxy-3-carboxymethyl-4-isopropenyl pyrrolidine (kainate), whereas the metabotropic glutamate receptors (mGluRs) are part of the G-protein coupled receptor (GPCR) super-family comprising eight receptor subtypes.[5] The AMPA receptor is found as a heteromeric tetramer, consisting of a varying combination of four subunits, labelled GluR1–4, which exist in two distinct, alternatively spliced isoforms, flip and flop.[6]

There has been an abundance of research into the development of new compounds targeting AMPA receptors, which has resulted in many potent competitive ligands. However, compounds possessing both potency and receptor subunit specificity remain relatively few.[7] Figure 1 depicts several known AMPA receptor ligands, with the structure of (*S*)-Glutamate given for reference.

All the examples in Figure 1 contain a key substituted π-excessive heterocyclic core. The agonists HIBO (**2**), AMPA (**3**), TDPA (**4**), APPA (**9**) and 2-Py-AMPA (**10**) have a hydroxyl group appended to the heterocycle, with **9** and **10** having an additional aryl substituent. The agonist ACPA (**5**), and the antagonists AMOA (**6**), ATOA (**7**) and ATPO (**8**) have an appended acid substituent (carboxylic acid or phosphonic acid), which is tethered via an ether linkage for **6**–**8**.[8–12]

One class of heterocycle, that is, yet to be investigated in structures of this type is the triazole. These derivatives are attractive candidates because of their ease of preparation via a copper or ruthenium-catalysed cycloaddition ('Click') reaction of suitably functionalized azides and alkynes. Such a 'Click' synthetic strategy provides the potential to prepare a library of compounds from simple and readily available fragments. Here we report the synthesis and assay of a series of homologues (**11**–**16**, Fig. 2) as a first step toward investigating the potential of this new class.

The π-excessive heterocycles of HIBO (**2**), AMPA (**3**) and TDPA (**4**) are known to make important contacts with key amino acid residues within the binding pocket of the corresponding receptor.[13] We anticipated that the triazole of the new ligands (**11**–**16**) would be able to do likewise. The structures **11**–**16** also contain a carboxylic acid substituent as found in the known ligands **5**–**8**. The analogues **13** and **16** contain an additional tethered phenyl group, as found in APPA (**9**) and 2-Py-AMPA (**10**). This series provides some insight into possible binding interactions as a first step toward establishing some preliminary SAR.[14,15]

Given the structural similarity between the proposed triazoles and known competitive ionotropic glutamate receptor ligands **3**–**10**, we anticipated that they may also show affinity for glutamate receptors. To test this hypothesis, compounds **11**–**16** were screened in vitro against both native ionotropic (NMDA, AMPA and kainate) and recombinant metabotropic (mGluR1, mGluR2 and mGluR4) glutamate receptors. Finally, in silico molecular docking was carried out in an attempt to rationalise the in vitro receptor binding results.

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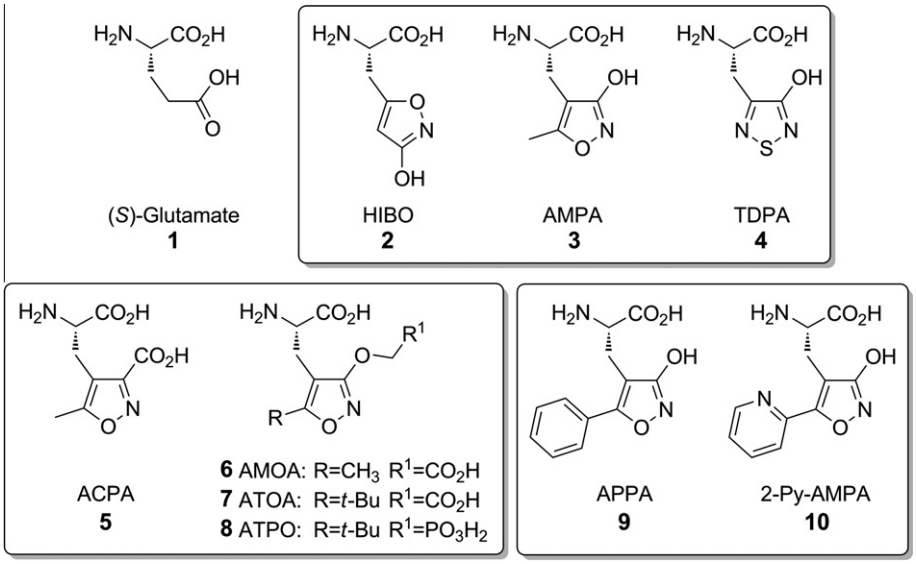


Figure 1. Representative structures of the endogenous ligand, glutamate (**1**) and other known AMPA receptor ligands.

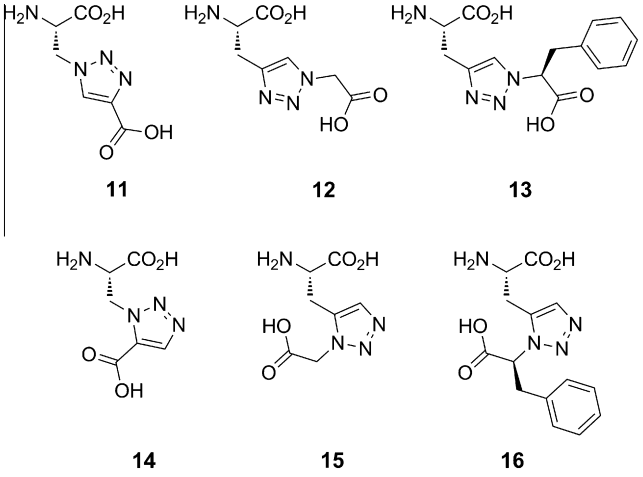


Figure 2. Series of potential glutamate receptor ligands.

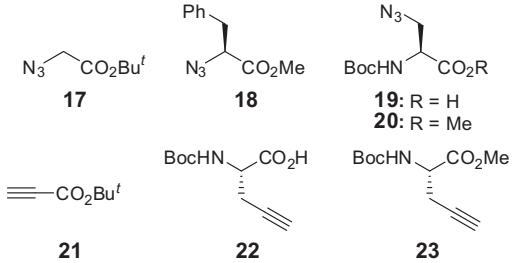
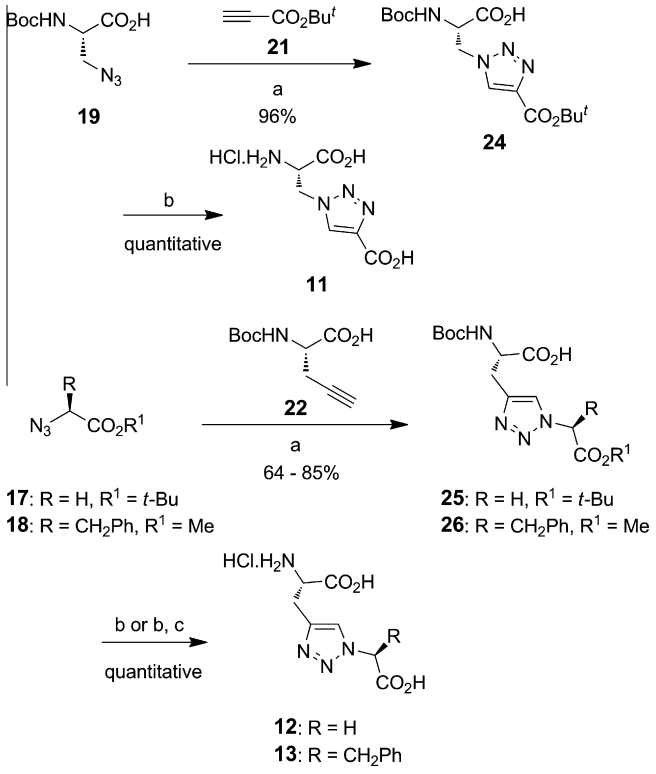


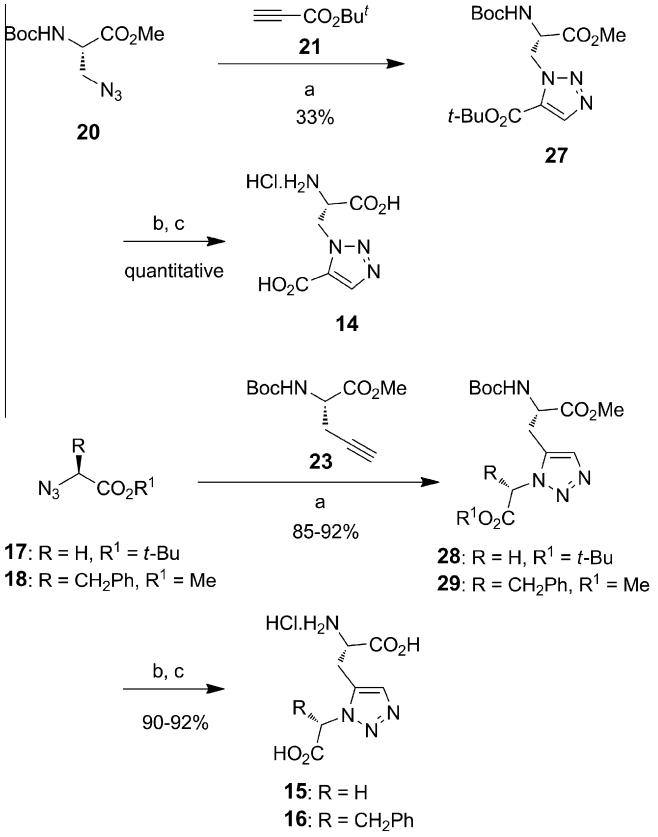
Figure 3. Azide and alkyne building blocks.



Scheme 1. Synthesis of 1,4-disubstituted triazolyl amino acids by copper-catalysed cycloaddition. Reagents and conditions: (a) $CuSO_4{\cdot}5H_2O$ (5 mol %), sodium ascorbate (20 mol %), *t*-BuOH/H_2O (2:1); (b) 6 M HCl, 1,4-dioxane; (c) 1 M $LiOH_{(aq)}$, THF.

Synthesis: The key step in the synthesis of the 1,2,3-triazolyl amino acids involved the copper (I) and ruthenium (II) catalysed cycloaddition of azides (**17**–**20**) with alkynes (**21**–**23**) (Fig. 3). In particular, azide **19** was reacted with alkyne **21** in the presence of copper sulfate and sodium ascorbate to give the protected triazolyl amino acid **24** in 96% yield (Scheme 1). The azides **17** and **18** were separately reacted with alkyne **22** under copper cycloaddition conditions to afford protected triazolyl amino acids **25** and **26** in 85% and 64% yield, respectively. The *N*-Boc and ester protecting groups of **24**, **25**, and **26** were removed as shown in Scheme 1 to give the desired 1,2,3-triazolyl amino acids **11**–**13** in quantitative yield.

The synthesis of the alternative 1,5-regioisomers **14**–**16**, was achieved using the ruthenium catalyst, Cp*Ru(PPh_3)$_2$Cl.[16,17] The protected azide **20** underwent ruthenium-catalysed cycloaddition with alkyne **21** to afford the fully protected 1,5-triazole **27** in 33% yield. The protected azides **17** and **18** were similarly and



Scheme 2. Synthesis of 1,5-disubstituted triazolyl amino acids by ruthenium-catalysed cycloaddition. Reagents and conditions: (a) Cp*Ru(PPh$_3$)$_2$Cl (2 mol %), THF, N_2, reflux; (b) 1 M LiOH$_{(aq)}$, THF; (c) 6 M HCl, 1,4-dioxane.

separately reacted with alkyne **23** to give the triazoles **28** and **29** in 92% and 33% yield, respectively (Scheme 2). Attempted ruthenium-catalysed reaction using the free acids **19** or **22** gave poor yields of the corresponding triazole, with considerable product decomposition. The *N*-Boc and ester protecting groups of **27**–**29** were removed as detailed is Scheme 2 to give the amino acids **14**–**16** in high yield.

Pharmacology: The in vitro receptor binding potency of compounds **11**–**16** was determined for native ionotropic glutamate receptors (NMDA, AMPA, kainate) and in functional assays for recombinant metabotropic glutamate receptors. The data obtained from the in vitro competitive binding assays at the ionotropic glutamate receptors indicates that compounds **11** and **14** exhibited significant competitive binding with IC_{50} values of 63 μM and 49 μM, respectively (Table 1). Compared to AMPA (**3**), with an IC_{50} value of 0.04 μM, these compounds show weak binding affinity, however, they are comparable to the binding affinity for the antagonists AMOA (**6**), ATOA (**7**) and ATPO (**8**).[11,18] It must be noted that these assays only indicate binding affinity. Further work is required to characterise the mode of receptor activation, that is, agonist or antagonist, of the active compounds **11** and **14**.

The functional assays at the metabotropic glutamate receptors 1, 2 and 4 reveal that compounds **11–13**, **15** and **16** (**14** not tested) were inactive, with an EC_{50} >100 μM. This result is not altogether unexpected, considering ligands **11**–**16** share a related amino acid-substituted π-excessive heterocylic core to the known AMPA ligands shown in Figure 1. Of these isoxazole ligands only HIBO shows any metabotropic receptor activity. This is possibly due to the separation of the distal acid and amino acid groups.

Molecular Docking: In silico molecular docking of the GluR2$_{flop}$ ligand binding subunit (PDB: 1ftm)[19] with glutamate, AMPA and

Table 1
In vitro ionotropic glutamate receptor binding data

Compounds	NMDA K_i (μM)	AMPA IC_{50} (μM)	Kainate IC_{50} (μM)
AMPA (**3**)	>100	0.04[a]	>100
11	>100	63 [54;74][b]	>100
12	>100	>100	>100
13	>100	>100	>100
14	>100	49 [44;55][b]	>100
15	>100	>100	>100
16	>100	>100	>100

Values are expressed as the antilog to the log mean of three-four individual experiments.
[a] Data from Vogensen et al. (2004).[18]
[b] Numbers in parentheses indicate maximum and minimum SEM.

11–**16** was carried out using Autodock 4 to gain some insight into their possible mode of binding. The compounds were automatically docked into the rigid structure of the GluR2$_{flop}$ ligand binding domain. A representative docked structure for the most active ligand **14** is shown in Figure 4B, along with an overlay with docked glutamate and the ligand bound X-ray structure of AMPA[19] for comparison (Fig. 4A). All numbering is taken directly from the Protein Data Bank file.

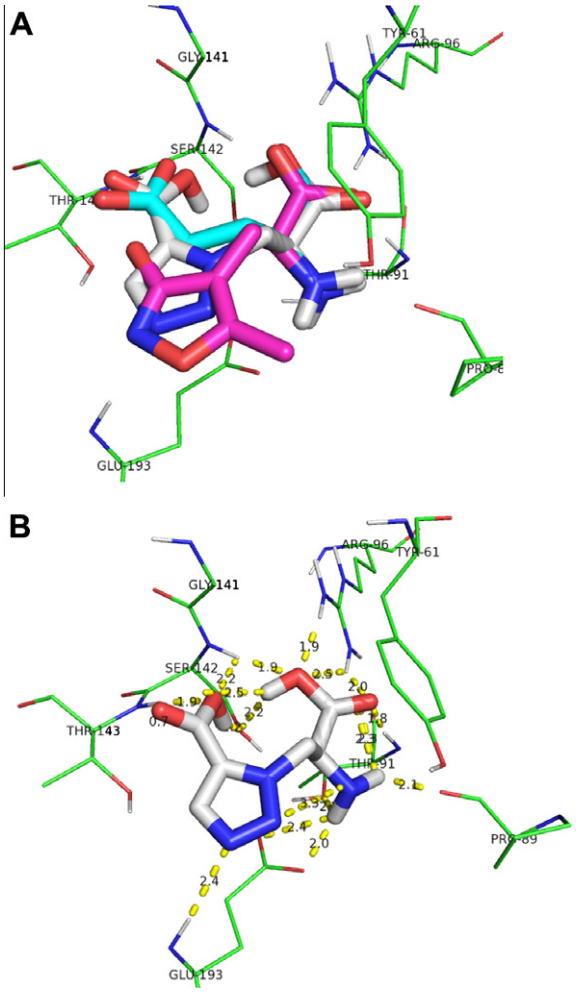


Figure 4. (A) Overlap of triazole **14** (white) with (*S*)-AMPA (**3**) (magenta) and (*S*)-glutamate (**1**) (cyan), as derived from in silico docking against the GluR2$_{flop}$ AMPA receptor subunit (PDB: 1ftm); (B) docked triazolyl amino acid **14** (white) showing possible hydrogen bonding interactions with selected amino acid residues. Only selected amino acid residues are shown. Figure produced in PyMOL.

Figure 4(A) reveals a good overlap of **14** with docked glutamate and the AMPA X-ray crystal structure. Hydrogen bonding interactions of **14** are evident with key amino acid residues Pro89, Thr91, Arg96, Gly141, Ser142 and Glu193 (Fig. 4B). These results suggest a good computational model for the 3D orientation of bound **14**. AMPA was also docked into the $GluR2_{flop}$ ligand binding subunit (results not shown) and this complex was calculated to have a mean docking energy of −9.92 kcal/mol, compared to −9.35 kcal/mol for triazole **11** and −9.14 kcal/mol for triazole **14**. These values are in agreement with the observed potency of these three derivatives (see in vitro data in Table 1). However, unlike AMPA, neither compound **11** nor **14** make polar contacts with the hydroxyl group of Thr143 (left-hand edge, Fig. 4) and compound **11** also lacks a contact with Glu193 (bottom edge, Fig. 4).[13] This may contribute to the lower potency of **11** and **14** compared to AMPA. The distal carboxylic acid groups in docked **12** and **15** (not shown) occupy a region of the receptor not occupied by AMPA. Triazoles **13** and **16** docked with high docking energies, possibly due to the bulky phenyl rings interacting in a sterically or electrostatically disfavoured manner. This is consistent with inactivity of these derivatives. (See Table 1).

Summary: This work highlights a convenient and versatile synthesis of the 1,4- and 1,5-disubstituted 1,2,3-triazolyl amino acid glutamate homologues **11**–**16**. In vitro screening indicated selective binding for **11** and **14** at AMPA receptors, albeit with low potency. Further structural elaboration and pharmacological investigation is needed in order to establish the full potential of the 1,2,3-triazolyl amino acid core structure.

Acknowledgements

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Supplementary data

Supplementary data (full compound characterisation data and in vitro assay details) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.139.

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