FINDING ALLOSTERIC MODULATORS OF METABOTROPIC GLUTAMATE RECEPTORS AS POTENTIAL CNS DRUGS

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Motivation

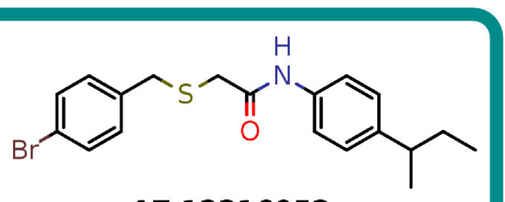
Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). It is an essential molecule, e.g. for cognitive functions such as memory formation and learning.[1]

Group III metabotropic glutamate receptors ($mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$) are considered promising drug targets for treatment of neurological disorders e.g. Parkinson's disease, schizophrenia, major depressive disorder and pain.[2] Apart from the traditional concept of finding orthosteric ligands, mGluR allosteric modulation is considered a very promising approach.[3] Due to little differences in the aminoacid sequences of orthosteric binding sites of $mGluR_4$, $mGluR_7$ and $mGluR_8$, finding selective ligands is notoriously difficult.

$MGlu_8$ receptor, which is positively coupled to $G_{\alpha(i/o)}$, functions as a presynaptic autoreceptor. GRM8 polymorphism may be involved in pathogenesis of schizophrenia.[4] Activation of $mGluR_8$ can elicit both hyperalgesic and analgesic effects. Behavioural experiments suggest that mGluR8 plays role in regulation of anxiety. $mGluR_8$ knockout (KO) mice exhibit an anxiety phenotype further implying involvement of this receptor in mood regulation.[5]

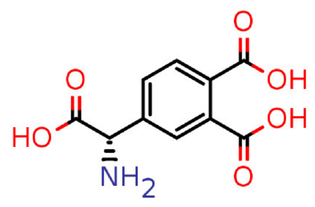
Background and methodology

Chemistry:
Compounds **AH-48**, **MAH-14** and **MAH-15** were synthesized, purity examined by LC-MS, structure confirmed by ^1H NMR and ^{13}C NMR. Elemental analysis is consistent with calculated values.



AZ 12216052

mGluR8 PAM



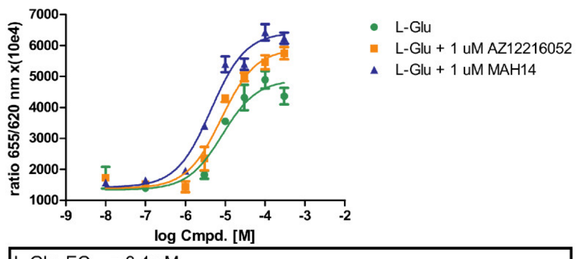
(*S*)-3,4-DPCG

mGluR8 agonist

Biological assays:
Cell line: Host: T-RexTM-293 cell line
Final cell lines: HEK293_T-REX-293_hmGluR8
Direct quantitative determination level of cAMP by Homogenous Time-Resolved Fluorescence
AZ 12216052 - $mGluR_8$ Positive Allosteric Modulator has been used in the experiments as a reference.

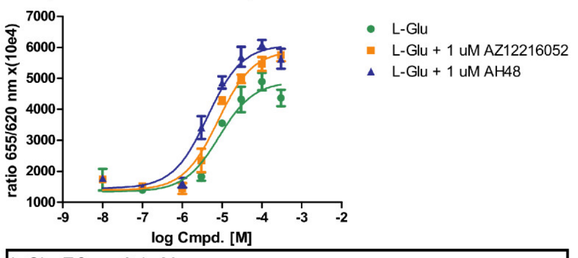
Outcome

mGluR8, DR: L-Glu, DR: L-Glu + 1 uM MAH14, DR: L-Glu + 1 uM AZ12216052



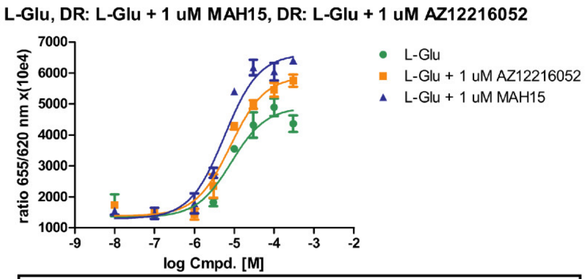
L-Glu, EC_{50} = 8,4 uM
L-Glu + 1 uM AZ12216052, EC_{50} = 8,0 uM, shift = 1,1; Emax = 120,5%
L-Glu + 1 uM MAH14, EC_{50} = 4,4 uM, shift = 1,9; Emax = 131%

mGluR8, DR: L-Glu, DR: L-Glu + 1 uM AH48, DR: L-Glu + 1 uM AZ12216052



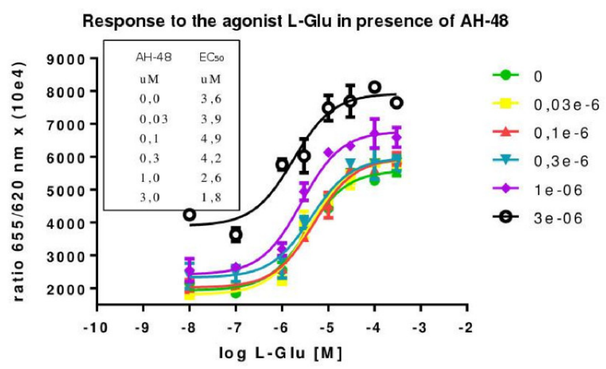
L-Glu, EC_{50} = 8,4 uM
L-Glu + 1 uM AZ12216052, EC_{50} = 8,0 uM, shift = 1,1; Emax = 120,5%
L-Glu + 1 uM AH48, EC_{50} = 4,3 uM, shift = 2,0; Emax = 124%

mGluR8, DR: L-Glu, DR: L-Glu + 1 uM MAH15, DR: L-Glu + 1 uM AZ12216052

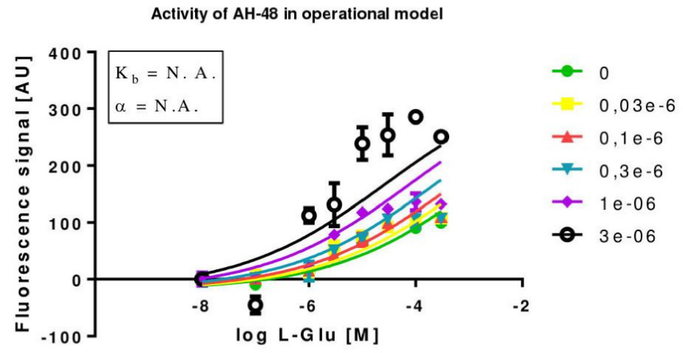


L-Glu, EC_{50} = 8,4 uM
L-Glu + 1 uM AZ12216052, EC_{50} = 8,0 uM, shift = 1,1; Emax = 120,5%
L-Glu + 1 uM MAH15, EC_{50} = 5,7 uM, shift = 1,5; Emax = 135%

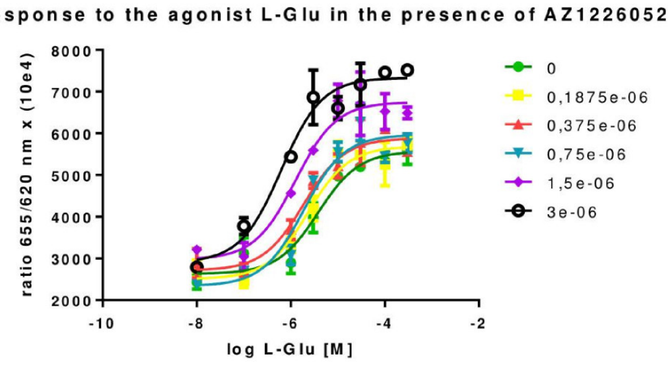
Response to the agonist L-Glu in presence of AH-48



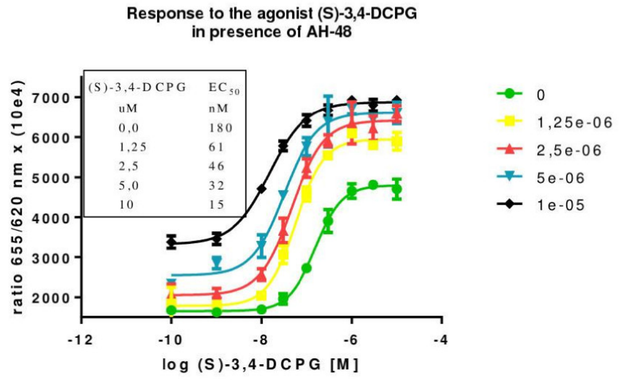
Activity of AH-48 in operational model



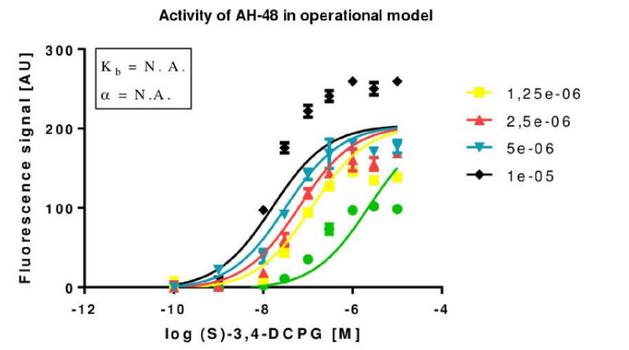
Response to the agonist L-Glu in the presence of AZ1226052



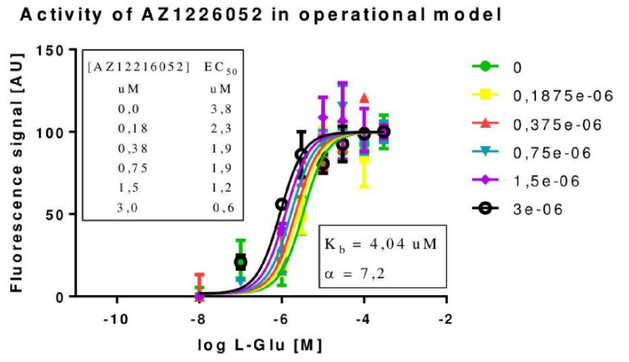
Response to the agonist (S)-3,4-DCPG in presence of AH-48



Activity of AH-48 in operational model



Activity of AZ1226052 in operational model



Summary and outlook

We have synthesized and evaluated chemical scaffold exhibiting potential $mGluR_8$ Positive Allosteric Modulator activity along with a strong agonistic component.
AH-48 has the following characteristics:
- activates $mGluR_8$ as an agonist (EC_{50} = 2.6 uM),
- acts as a Positive Allosteric Modulator (EC_{50} =4.3 uM in the presence of 1 uM L-Glu),
- Activity of **AH-48** with or without presence of L-Glu is completely abolished by 10uM of LY341495
- **AH-48** activates $mGluR_4$ and $mGluR_7$,

MAH-14 acts as a $mGluR_8$ PAM (EC50 = 4.4uM)
MAH-15 acts as a $mGluR_8$ PAM (EC50 = 5.7uM)
We plan further tests (metabolic stability, genotoxicity, anti-target assays) which will help us establish lead structure in the study.

References

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Acknowledgements

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Databases in this study were created using ChemAxon JChem software