THE DEVELOPMENT OF mGluR$_8$ PAM AGONISTS

A. S. Hogendorf,[1,2] P. Brański,[3] G. Burnat,[3] R. Bugno,[1] A. Hogendorf,[1] B. Chruścicka,[3] A. Stankiewicz,[1] M. Trela[1], A. J. Bojarski[1]

1) Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Cracow, Poland
2) Department of Organic Chemistry, Jagiellonian University, 3 Ingardena Street, Cracow, Poland
3) Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Cracow, Poland

INTRODUCTION

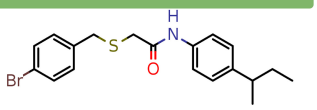
Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). It is an essential molecule, e.g. for cognitive functions such as memory formation and learning.[1]

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 targeting orthosteric site, 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 mGluR$_8$ 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]

CHEMISTRY

Compound libraries (Group I and II) were synthesized, purity examined by LC-MS, structure confirmed by ^{1}H NMR and ^{13}C NMR. Elemental analysis is consistent with calculated values.

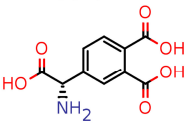


AZ 12216052

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 - mGluR8 Positive Allosteric Modulator has been used in the experiments as a reference.

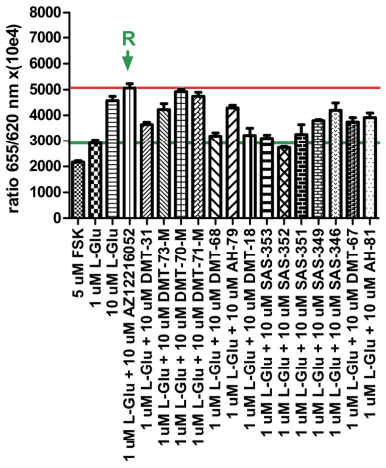
mGluR8 PAM



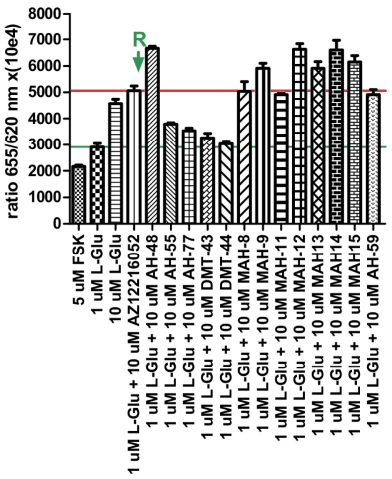
(*S*)-3,4-DPCG

mGluR8 agonist

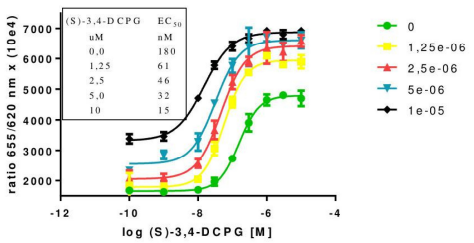
mGluR8, 10 uM Group II Cmd. + EC20 L-Glu, potential PAM activity, column, ratio, cAMP, 05-08-2015



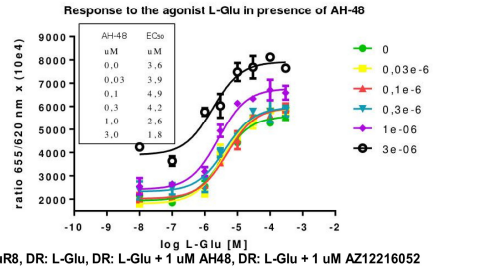
mGluR8, 10 uM Group I Cmd. + EC20 L-Glu, potential PAM activity, column, ratio, cAMP, 05-08-2015



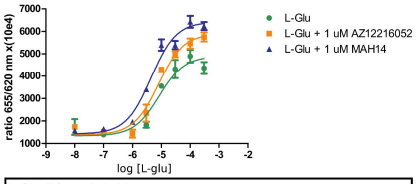
Response to the agonist (S)-3,4-DCPG in presence of AH-48



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

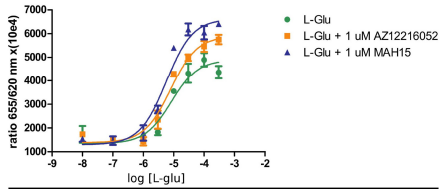


mGluR8, DR: L-Glu, DR: L-Glu + 1 uM MAH14, DR: L-Glu + 1 uM AZ12216052



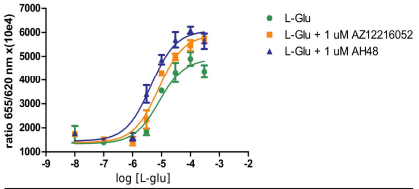
L-Glu, EC_{50} = 8,4 uM
L-Glu + 1 uM AZ12216052, EC_{50} = 8,0 uM, shift = 1,1; Emax = 120,5%
L-Glu + 1 uM MAH14, EC_{50} = 4,4 uM, shift = 1,9; Emax = 131%

mGluR8, DR: L-Glu, DR: L-Glu + 1 uM 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%

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%

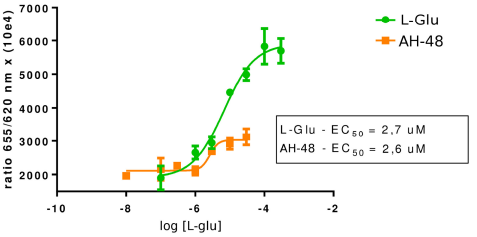
Tab.1 Allosteric binding sites sequences of mGluRs[6]

PIN	mGlu$_1$	mGlu$_5$	mGlu$_2$	mGlu$_3$	mGlu$_4$	mGlu$_6$	mGlu$_7$	mGlu$_8$	mGlu*	Conservation	quality**	Consensus
2.45	624	G	G	G	G	G	G	G	G	11	17	100
2.46	625	I	I	V	V	I	I	I	I	9	16.7	77
2.49	628	G	G	C	S	C	I	C	C	5	7.4	55
3.36	651	I	V	L	L	L	L	L	I	9	16.2	66
3.37	652	G	G	G	G	G	G	G	G	11	17	100
3.39	654	S	S	A	S	G	G	G	G	8	13.9	55
3.40	655	P	S	F	F	M	T	M	M	4	8.4	33
3.43	658	S	C	C	C	S	S	S	S	5	9.4	55
3.44	659	Y	Y	Y	Y	Y	Y	Y	Y	11	17	100
5.43	743	P	P	S	S	L	C	S	S	4	10	55
5.44	744	L	L	L	L	L	L	L	Q	7	13.2	88
5.47	747	N	N	N	D	S	S	S	N	7	13.9	44
5.51	751	I	I	I	V	M	M	M	I	8	15.2	44
6.46	781	T	T	T	T	T	T	T	T	11	17	100
6.49	784	I	I	I	I	V	I	V	I	9	16.4	77
6.50	785	W	W	W	W	W	W	W	W	11	17	100
6.53	788	F	F	F	F	F	F	F	F	11	17	100
7.32	802	M	T	M	M	L	L	L	L	6	14.6	55
7.35	805	S	A	S	S	S	S	S	S	8	16.4	88
7.36	806	V	V	V	V	V	L	M	M	8	15.5	55
7.39	809	S	S	S	S	S	S	S	S	11	17	100
7.40	810	A	V	G	G	A	A	A	A	8	13.4	66
7.43	813	A	A	V	V	S	S	A	A	6	13.1	44

CONCLUSIONS

We have developed two distinctive chemotypes with 22 entities exhibiting PAM properties. Six compounds from group II are more potent than the refference compound **AZ 12216052**, which is the only commercialy aviable mGluR$_8$ ago-PAM till date. Lead compound AH-48 has the following characteristics:

- it activates mGluR$_8$ as an agonist (EC50 = 2.6 uM),
- it acts as a Positive Allosteric Modulator (EC50 = 4.3 uM in the presence of 1 uM L-Glu),
- AH-48 acts as mGluR$_8$ full PAM-agonist in contrast to benchmark compound **AZ 12216052** which activates the receptor only partially.



The transmembrane binding sites sequences between different group III mGluRs differ very slightly (tab.1). Thus obtaining selective ligands is very challenging. Herein presented group I and II ligands are not mGluR$_4$ selective nor is **AZ 12216052**. A further investigation of binding modes in order to exploit binding site differences may potentially yield selective ligands.

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Databases in this study were created using ChemAxon JChem software.