SAR DETERMINATION AND PRELIMINARY MODELING STUDIES FOR A NEW MGLUR4 POSITIVE ALLOSTERIC MODULATORS

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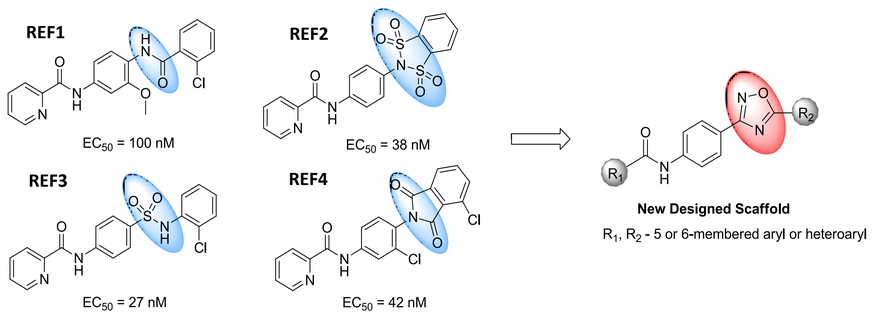
INTRODUCTION

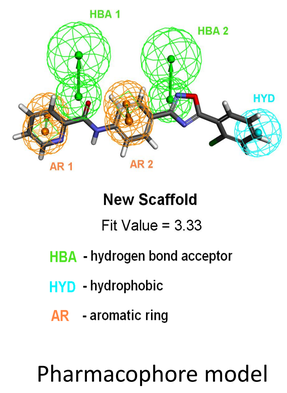
Metabotropic glutamate receptors (mGluRs) are members of the group C family of GPCRs and play important roles in a broad range of central nervous system functions having therapeutic potential in a variety of neurological and psychiatric disorders [1]. Due to the lack of receptor subtype selectivity and physiochemical properties of mGluR orthosteric ligands (poor bioavailability and low potential of blood-brain barrier penetration) a significant effort has been made to identify compounds that can act as allosteric modulators which potentiate the response of endogenous agonists [2]. Number of reviews are available summarizing recent progress in developing new allosteric ligands of mGluRs [3]. Among all, the group III subtypes: mGluR4, mGluR7 and mGluR8 still remains the least explored but with mighty potential for future development of clinical drugs [4].

DESIGN OF NEW SCAFFOLD

A new series of potential mGluR4 PAM's were designed based on structure of known mGluR4 modulators by bioisosteric substitution of mutual parts of molecules with 1,2,4-oxadiazole ring.



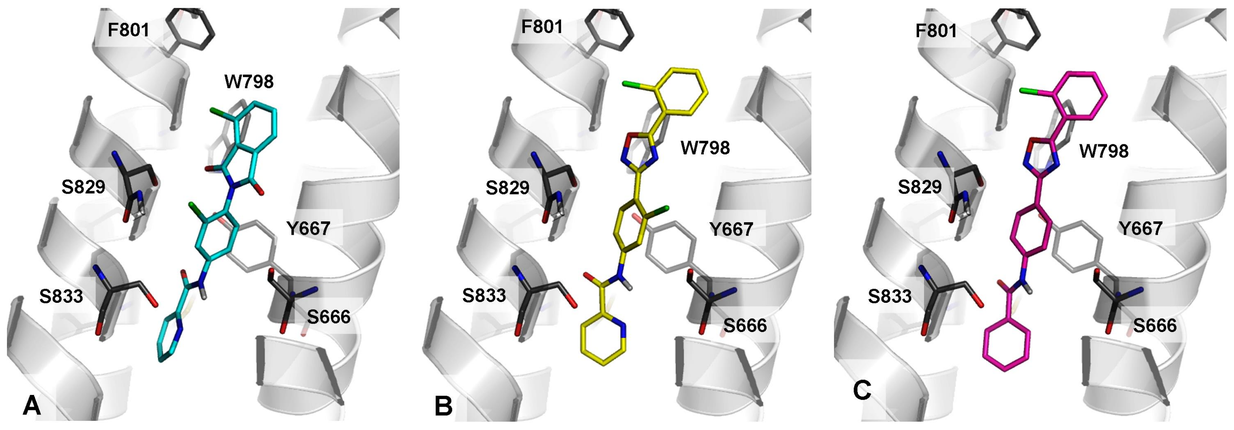
New scaffold based on bioisosterical substitution in selected mGluR4 PAMs



Pharmacophore model

MOLECULAR MODELING

Since no crystallographic data is available for III group of mGluRs, a homology model of mGluR4 was developed using mGluR5 crystal structure (PDB ID:4OO9) and applied for molecular studies. The library of synthetized and reference compounds were docked and their binding modes were analyzed with respect to obtained SAR data.

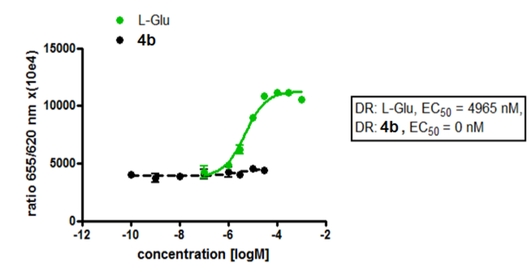


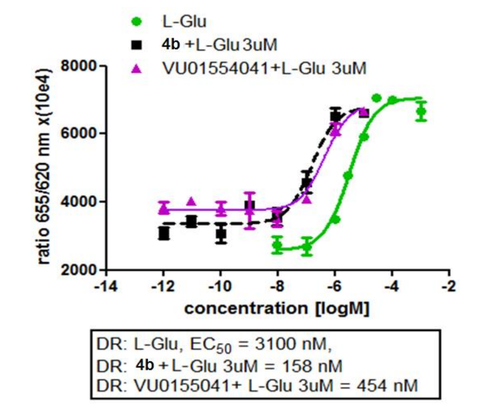
Results showed that reference compound **REF4** (A) and **4b** (B) share similar binding mode, where 2-pirydyl moiety was placed between two serine side chains (S833 and S666), central ring formed aromatic interaction with Y667 and the last ring created aromatic interaction with W798 and F801. Interestingly, compounds **1d** (C) lost activity when interaction with S833/S666 was switched off.

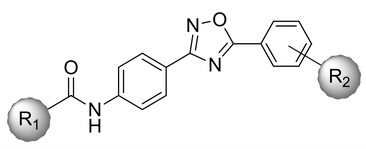
SAR STUDIES – IN VITRO EXPERIMENT

Activity of presented compounds was examined on recombinant human mGlu4 receptor by detecting level of cAMP in the presence of forskolin, adenylyl cyclase activator. Reported PAMs increase the functional activity of glutamate or other orthosteric agonists at mGlu4 receptor.

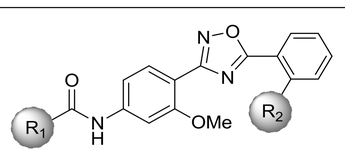
Potentiation of the glutamate response of mGluR4 by the synthesized compounds was observed as a decrease of EC_{50} value for the glutamate in the presence of these molecules comparing to EC_{50} value in the absence of ligands. Summarized results of structure-activity relationships are presented in tables below.



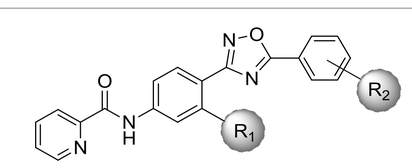




Comp.	R_1	R_2	$EC_{50\ (L\text{-}Glu)}$ [nM]	EC_{50} [nM]
1a	2-pirydyl	2-Cl	3700	neutral
1b	3-pirydyl	2-Cl	inactive	-
1c	4-pirydyl	2-Cl	inactive	-
1d	C_6H_4	2-Cl	inactive	-
1e	2-pirydyl	H	inactive	-



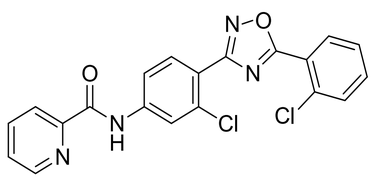
Comp.	R_1	R_2	$EC_{50\ (L\text{-}Glu)}$ [nM]	EC_{50} [nM]
2a	3-F-2-pirydyl	2-Cl	920	neutral
2b	3-Cl-6-F-2-pirydyl	2-Cl	inactive	-
2c	3-Cl-2-pirydyl	2-Cl	1920	500
2d	3-MeO-6-Cl-2-pirydyl	2-Cl	inactive	-
2e	3,5-diF-2-pirydyl	2-Cl	2500	neutral
2f	6-F-2-pirydyl	2-Cl	480	neutral
2g	2-pirymidyl	2-Cl	3100	neutral
2h	2-thienyl	2-Cl	2200	12000
2i	3-F-2-pirydyl	2-F	890	neutral
2j	3,6-diCl-2-pirydyl	2-F	inactive	-



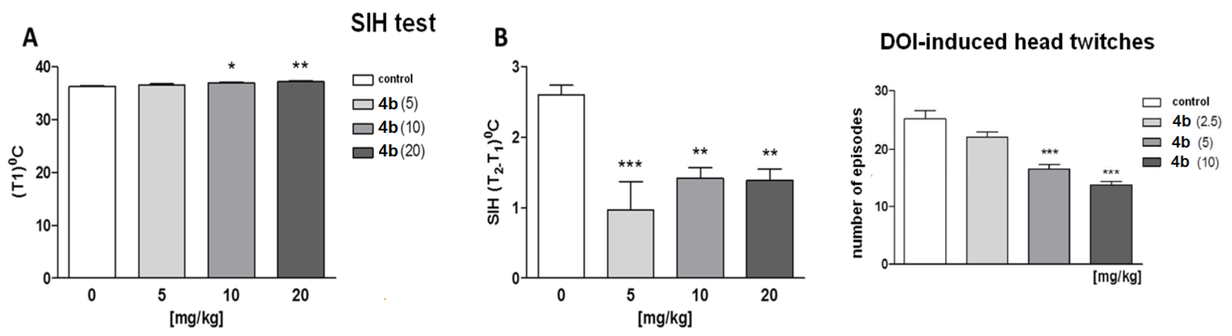
Comp.	R_1	R_2	$EC_{50\ (L\text{-}Glu)}$ [nM]	EC_{50} [nM]
3a	OMe	H	1830	neutral
3b	OMe	2-Cl	200	neutral
3c	OMe	3-Cl	inactive	-
3d	OMe	4-Cl	inactive	-
3e	OMe	2-F	270	6970
3f	OMe	2-Cl,4-F	1980	neutral
4a	Cl	H	190	neutral
4b	Cl	2-Cl	160	neutral
4c	Cl	2-Cl,4-F	1350	neutral
4d	Cl	2-MeO	1100	neutral
5a	F	H	3500	4400
5b	F	2-Cl	2600	neutral
5c	F	2-Cl,4-F	inactive	-
5d	F	2-MeO	inactive	-
6a	CF_3	H	640	neutral
6b	CF_3	2-Cl	240	730
7a	Me	H	1000	neutral
7b	Me	2-Cl	480	neutral

BEHAVIORAL STUDIES

Biological activity of the most potent compounds was confirmed in in vivo studies in behavioral tests after central administration in rats. Potential anxiolytic effects were investigated in stress-induced hyperthermia test, antipsychotic effects were examined In DOI-induced head twitches tests and antidepressant-like effects were determined in tail suspension tests.



4b



ADME-Tox CHARACTERISTICS

- ✓ Solubility: insoluble or hardly soluble in water
- ✓ Partition coefficient octanol/water: in range 2.64 – 3.34
- ✓ Good permeability (PAMPA test)
- ✓ Good blood-brain permeability (in silico)
- ✓ Stable in human plasma
- ✓ Metabolic stability: stable in presence of UDPGA but metabolized in presence of NADPH (except **1a**)
- ✓ No mutagenic activity (Ames test)
- ✓ No cytotoxic activity (MTS test)
- ✓ No cardiotoxic acticivity (hERG, concentration 10 μM)
- ✓ No antyproliferative effect (concentration range 5 – 75 μM)

Pharmacokinetic studies – in progress

CONCLUSIONS

- A new series of mGluR4 positive allosteric modulators was reported. SAR evaluated for hit **1a** resulted with more potent lead compounds **3b**, **3e**, **4b**, **6b** and **7b** (EC_{50} < 500 nM). *In vitro* studies (hmGluR4) showed that 2-pirydyl moiety is essential structural element for mGluR4 potentiation. Substitution of phenyl ring (R_2) in positions other than ortho or with substituents other than Cl or F was unsuccessful. Significant improvement was observed after introduction of substituent R_1 (Me, OMe, Cl, CF_3).
- Lead compounds were examined in *in vivo* studies showing anxiolytic and antidepressant activity with no antipsychotic effect.
- Reported results proved that due to 'shallow' SAR of allosteric modulators a discovery of new PAMs remains an ongoing challenge in this stage of drug discovery.

ACKNOWLEDGMENTS

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