1,2,4-OXADIAZOLE DERIVATIVES AS NEW POSITIVE ALLOSTERIC MODULATORS OF MGLU4 RECEPTOR

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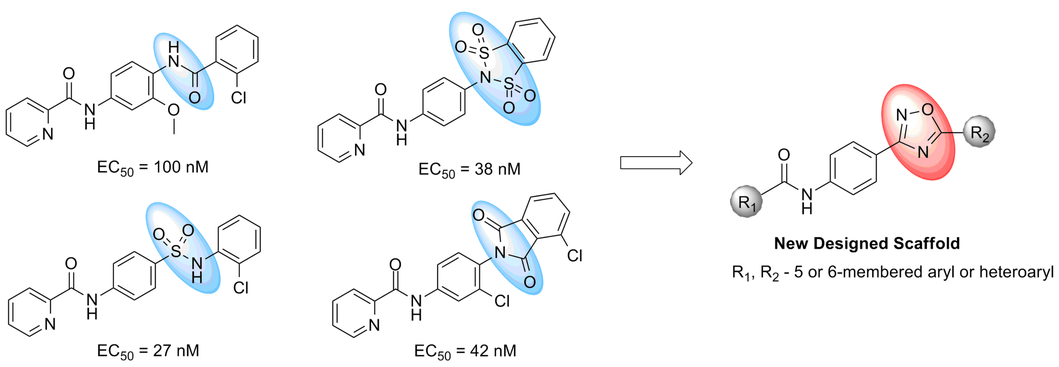
INTRODUCTION

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

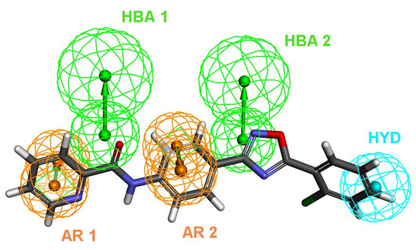
A new series of potential mGluR4 PAM's were designed based on structure of known mGluR4 modulators by bioisosteric substitution of mutual parts of molecules with 1,2,4-oxadiazole ring.



New scaffold based on bioisosterical substitution in selected mGluR4 PAMs

PHARMACOPHORE MODEL

Among known mGluR4 potentiators ten representative compounds with diverse cores were chosen as a training set for pharmacophore model generation. Selection was made based on their structure similarity in functional group alignment combined with high declared activity. After geometry optimization a five-point pharmacophore model was calculated in Common Feature Pharmacophore Generation protocol (Accelrys Discovery Studio). Representative of new designed scaffold (R_1=2-pirydyl, R_2=2-chlorophenyl) was examined in Ligand Pharmacophore Mapping protocol.



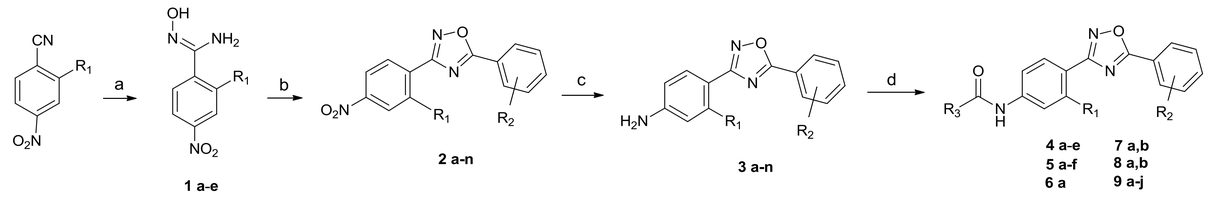
New Scaffold
Fit Value = 3.33

Superposition of compounds representing New Scaffold with generated pharmacophore model.

CHEMSITRY

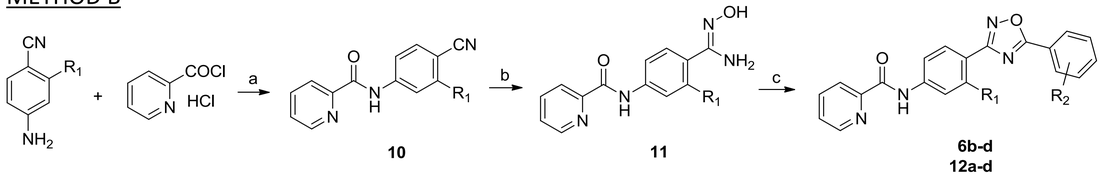
Synthetic pathway for preparation of all reported compounds is presented as Method A or B. Compounds were obtained according to standard procedures described in literature, starting from substituted p-nitrobenzylonitriles. Coupling reaction of benzamide oximes with acid chlorides was carried out in the presence of potassium carbonate in toluene under normal pressure (5–15h) or in the pressure vessel at 150–170^{0}C using microwave irradiation in much shorter period of time (10–15min).

METHOD A



Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, $NaOH_{aq}$, EtOH, reflux, 1-5h or $NH_2OH{\cdot}HCl$, TEA, EtOH, rt, 20 min, reflux, 1-5h; (b) R_2COCl, toluene, K_2CO_3, rt, 30 min than reflux 5-15h; (c) $SnCl_2$, 5N HCl, EtOH, reflux, 2h; (d) R_3COCl, py, rt, overnight or R_3COCl, TEA, THF, rt, overnight; (e) R_3COOH, BOP, TEA, DCM, rt, 1-3 days.

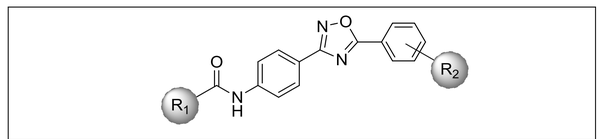
METHOD B



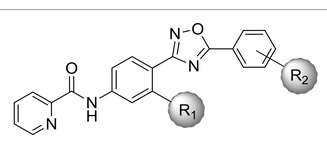
Reagents and conditions: (a) TEA, THF, rt, overnight; (b) $NH_2OH{\cdot}HCl$, $NaOH_{aq}$, EtOH, reflux, 1-5h; (c) R_2COCl, toluene, K_2CO_3, rt, 30 min than reflux 5-10 h.

IN VITRO STUDIES

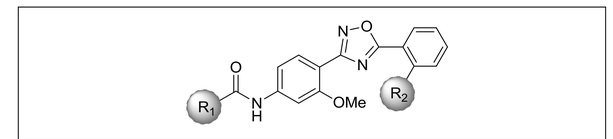
Activity of presented compounds was examined on recombinant human mGlu4 receptor by detecting level of cAMP in the presence of forskolin, adenylyl cyclase activator. Reported PAMs increase the functional activity of glutamate or other orthosteric agonists at mGluR4 receptor. Potentiation of the glutamate 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.



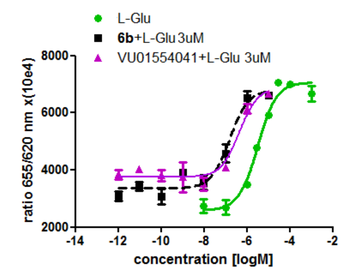
Comp.	R_1	R_2	$EC_{50\ (L\text{-}Glu)}$ [nM]	EC_{50} [nM]
4a	2-pirydyl	2-Cl	3700	neutral
4b	3-pirydyl	2-Cl	inactive	-
4c	4-pirydyl	2-Cl	inactive	-
4d	C_6H_4	2-Cl	inactive	-
4e	2-pirydyl	H	inactive	-

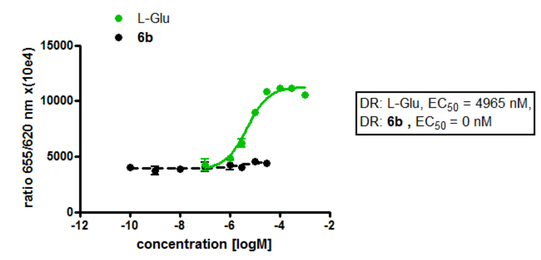


Comp.	R_1	R_2	$EC_{50\ (L\text{-}Glu)}$ [nM]	EC_{50} [nM]
5a	OMe	H	1830	neutral
5b	OMe	2-Cl	200	neutral
5c	OMe	3-Cl	inactive	-
5d	OMe	4-Cl	inactive	-
5e	OMe	2-F	270	6970
5f	OMe	2-Cl,4-F	1980	neutral
6a	Cl	H	190	neutral
6b	Cl	2-Cl	160	neutral
6c	Cl	2-Cl,4-F	1350	neutral
6d	Cl	2-MeO	1100	neutral
12a	F	H	3500	4400
12b	F	2-Cl	2600	neutral
12c	F	2-Cl,4-F	inactive	-
12d	F	2-MeO	inactive	-
7a	CF_3	H	640	neutral
7b	CF_3	2-Cl	240	730
8a	Me	H	1000	neutral
8b	Me	2-Cl	480	neutral



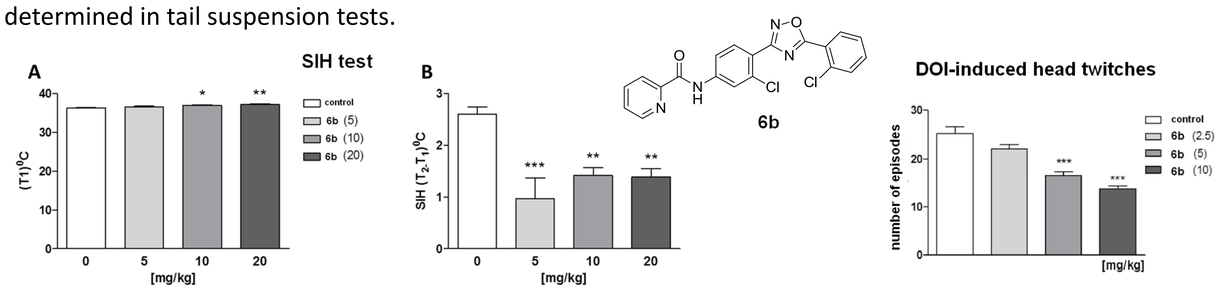
Comp.	R_1	R_2	$EC_{50\ (L\text{-}Glu)}$ [nM]	EC_{50} [nM]
9a	3-F-2-pirydyl	2-Cl	920	neutral
9b	3-Cl-6-F-2-pirydyl	2-Cl	inactive	-
9c	3-Cl-2-pirydyl	2-Cl	1920	500
9d	3-MeO-6-Cl-2-pirydyl	2-Cl	inactive	-
9e	3,5-diF-2-pirydyl	2-Cl	2500	neutral
9f	6-F-2-pirydyl	2-Cl	480	neutral
9g	2-pirymidyl	2-Cl	3100	neutral
9h	2-thienyl	2-Cl	2200	12000
9i	3-F-2-pirydyl	2-F	890	neutral
9j	3,6-diCl-2-pirydyl	2-F	inactive	-





BEHAVIORAL STUDIES

Biological activity of 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.



CONCLUSIONS

A new series of mGluR4 positive allosteric modulators was reported. SAR evaluated for hit **4a** resulted with more potent lead compounds **5b**, **5e**, **6b**, **7b** and **8b** (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 mGluRs a discovery of new PAMs remains an ongoing challenge in this stage of drug discovery.

ACKNOWLEDGMENT

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LITERATURE

[1] Niswender, C.M. et al. Ann. Rev. Pharmacol. Toxicol. **2010**, 50, 295-322; Urwyler, S. Pharmacol. Rev. **2011**, 63, 59-126; [2] Lindsley, C. W. et al. Curr. Top. Med. Chem. **2009**, 9, 949-963; [3] Flor, P. J. et al. Biochem. Pharmacol. **2012**, 84, 414-424; Golubeva, A.V. et al. Curr. Top. Med. Chem. **2015**, 16, 1-80; [4] Lavreysen, H. et al. Curr. Med. Chem. **2008**, 15, 671-684; Goudet, C. et al. FASEB J, **2012**, 26, 1682-1693; Gregory, K. et al. Neuropharmacology, **2011**, 60, 66-81.