3,4-DIHYDROQUINAZOLIN-4-ONES AS NEW LIGANDS OF MGLUR7 RECEPTOR

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Introduction

The metabotropic glutamate receptor 7 (mGluR7) is a member of group III family of mGluRs that bind to G protein and inhibit the adenylate cyclase [1]. Anatomic evidence demonstrates that mGluR7 has the highest CNS density of all group III mGluR subtypes due to widely distribution and presence at broad range of synapses [2]. Recent studies revealed that mGlu7 receptor could play an important role in treatment of anxiety, post-traumatic stress disorder, depression, autism, drug abuse, and schizophrenia [1-4]. In terms of discovery of new selective ligands the mGluR7 receptor is the most challenging of the all mGluR subtypes [4]. AMN082 is the first discovered mGluR7 agonist [5]. The mGluR7 antagonists activity of MDIP and MMPIP was reported by Suzuki *et al.* in 2007 [1], while negative allosteric modulation for ADX71743 was described by Kalinichev *et al.* Among the known mGluR7 ligands ADX71743 and MMPIP were selected as references for new ligands development as well as standards for *in vitro* studies. Variety of chemotypes were synthesized and examined followed by the primary *in vitro* evaluations which led to the selection of new quinazolinone derivatives as a potent mGluR7 negative allosteric modulators.

Chemistry project overwiev

Stege 1	• synthesis of ADX71743 standard • design of compounds libraries • homology modeling, Cresset screening
Stege 2	• >200 compounds from 28 different chemotypes have been synthsized • structure of all compounds was confirmed by LC/MS and NMR • all synthesized compounds were tested *in-vitro* • hit compounds: ALX-063 and ALX-065 were identified
Stege 3	• hit optimization towards improving of kinetic solubility and metabolic stability – 90 derivatives of ALX-065 were obtained • lead compound ALX-186 was identified, further optimization is on-going

Molecular modeling

As there is no crystallographic data available regarding mGluR7 receptor, the **homology modeling** was applied (**Fig 1.**). mGlur5 tempalate was used and induced fit docking for ADX71743 and MMPIP (**Fig 2.**) was performed. This model was used to screening of virtual combinatorial library.

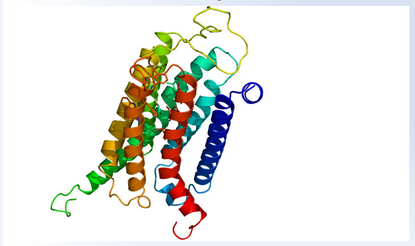


Fig 1. Homology model of metabotropic glutamate receptor 7.

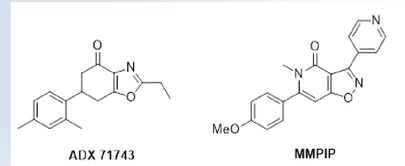


Fig 2. Structure of reference ligands.
ADX71743, IC_{50} (literature data) = 8 nM
MMPIP, IC_{50} (literature data) = 19 nM

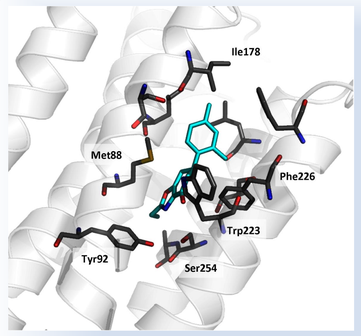


Fig 3. Binding mode of the most potent ADEX 71743 cmpd.

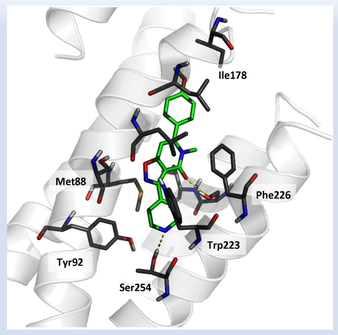


Fig 4. Binding mode of MMPIP cmpd.

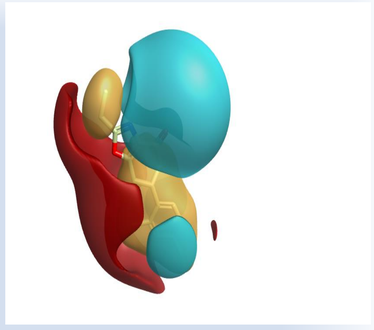
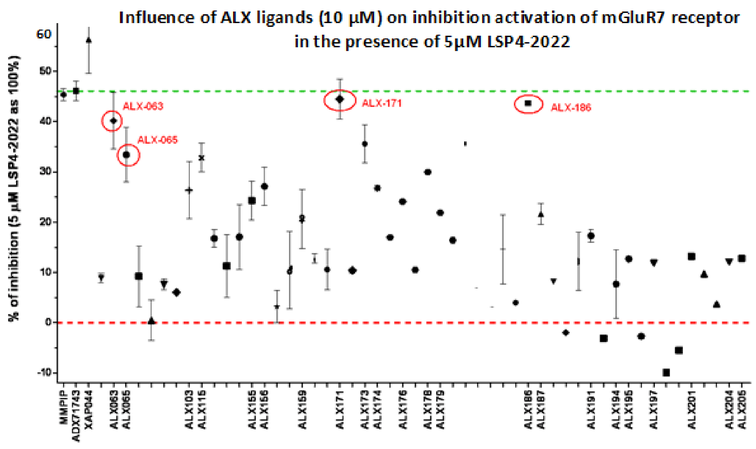


Fig 5. ADX 71743 cresset field view.

In-vitro screening overview

- The new strategy for identyfication of negative allosteric modulators of mGluR7 receptor was developed.
- mGluR orthosteric ligand LSP4-2022 is used as an agonist for the *in-vitro* tests.
- Selectivity of the ALX ligand library towards other mGlu receptors is also examined.
- Interaction of the ALX-ligands with HEK-293TRexhmGlur_6F line is being verified for identification of off-targets activity.



Summary of *in-vitro* screening:

So far five active ligands: ALX-063, ALX-065, ALX-115, ALX-171, ALX-186 have been identified as new mGluR7 negative allosteric modulators. ALX-186 was identified as the lead compound.

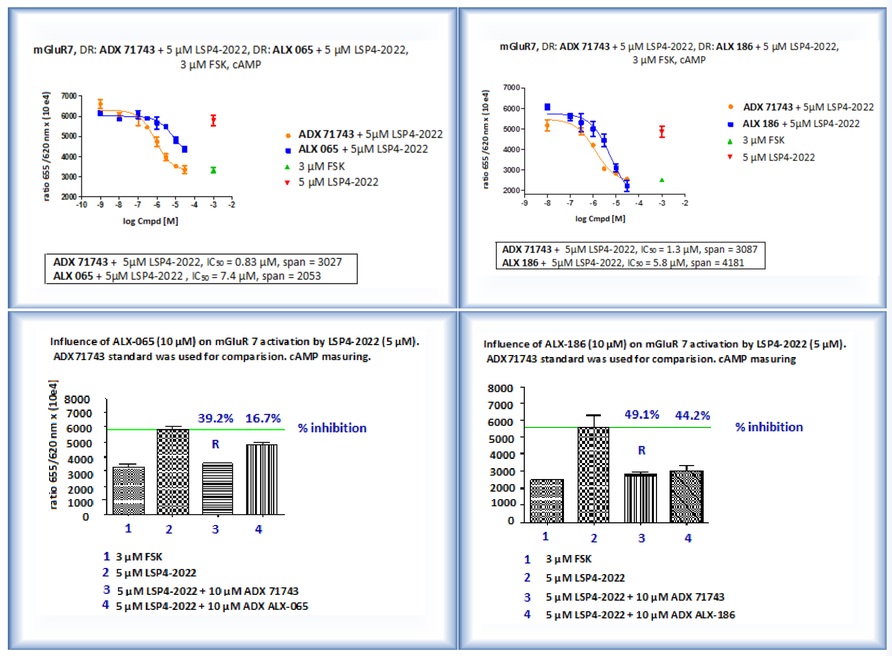
Preliminary ADME results for active ALX-ligands

Compound	Log P	Kinetic solubility in HHB buffer [µM]	Metabolic stability [% at 60 min]
ADX-71743	3.64	509.87	0.01
MMPIP	1.79	5.60	49.79
ALX-063	4.50	18.60	1.52
ALX-065	5.10	6.88	0.81
ALX-115	4.94	2.29	40.37
ALX-171	4.79	15.23	59.26
ALX-186	4.34	60.99	86.65

Summary of ADME tests:

ALX-186 showed the best solubility as well as metabolic stability towards cytochrome P450 enzymes.

Comparision of mGluR7 activity of ALX-065 *vs* ALX-186 to ADX71743



Summary of *in-vitro* tests:

ALX-065 activity: IC_{50} = 7.4 µM
ALX-186 activity: IC_{50} = 5.8 µM

SUMMARY and FUTURE PLANS

- >200 compounds were synthesized,
- hit compounds: ALX-063 and ALX-065 were identified,
- >90 derivatives of the hit molecules were synthesized and analyzed,
- new strategy for *in-vitro* screening was developed,
- ALX-186 was found to be selective for mGluR 7 receptor, while ALX-065 disclosed also week mGluR4 receptor activity,
- all active ALX-compounds were screened for formulation solubility,
- lead molecule ALX-186 indicated good solubility and metabolic stability,
- patent application is under preparation,
- based on preliminary ADME results further improving of activity as well as pharmacokinetic properties is planned,
- optimization of lead compound is on-going.

ON-GOING ACTIVITY: IN-VIVO tests = >

References:

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