A NOVEL mGluR7 NEGATIVE ALLOSTERIC MODULATORS

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Introduction

The metabotropic glutamate receptors (mGluR) represent class C GPCRs and play significant neuromodulatory roles throughout the brain .The metabotropic glutamate receptor 7 (mGluR7) is a member of group III family that binds to protein G and inhibits the adenylate cyclase [1]. The mGluR7 has the highest CNS dentisity of all group III mGluR subtypes due to widely distribution and presence at broad range of synapses [2,3]. Many studies have shown that mGluR7 is an important target for therapeutic intervention in a number of neurological and psychiatric disorders including anxiety, post-traumatic stress disorder, depression, autism, drug abuse, and schizophrenia [1-4]. In terms of discovery of new selective ligands the mGlu7 receptor is one of the most challenging of the all mGluR subtypes [4]. So far only few compounds which modulate the mGlu7 receptor are known: positive modulator - AMN082 [5], negative allosteric modulators - MDIP, MMPIP [1] and ADX71743 [6]. Therefore the discovery of highly selective mGluR7 ligands which can be used in clinical trials seems to be still the most significant challenge. The development of novel chemical scaffold possessing activity towards mGlu7 receptor is the aim of current studies. So far a variety of chemotypes were synthesized and examined *in vitro*, followed by the primary *in vivo* evaluations. The studies have shown new quinazolinone derivatives as promising mGluR7 negative allosteric modulators. Primary pharmacokinetics results demonstrated that concentration of ALX-171 in plasma as well as in the brain is higher than for the reference ADX-71743 compound

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.

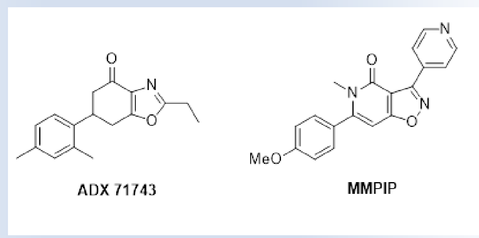


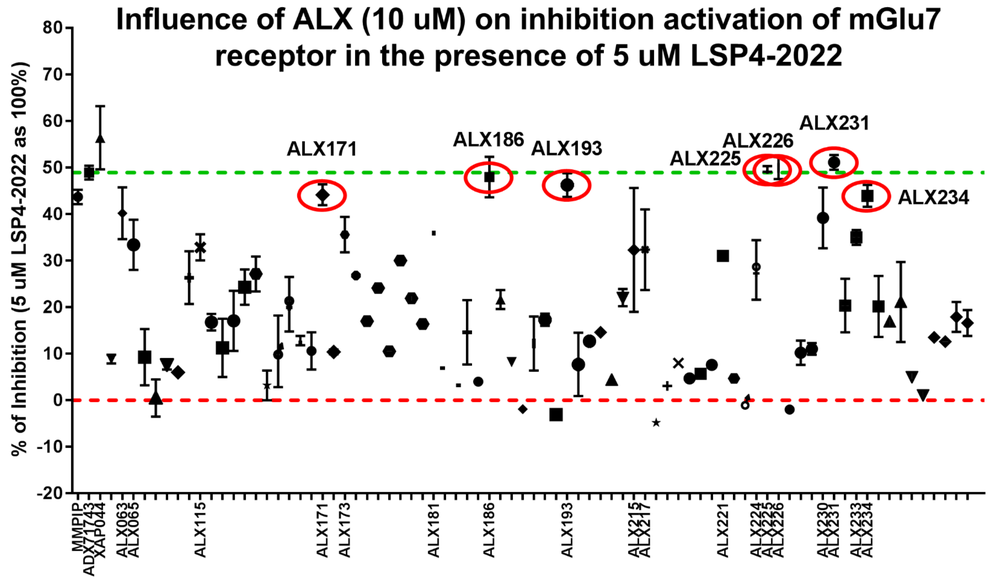
Fig 2. Structure of reference ligands.
ADX71743, IC_{50} (literature data) = 8 nM
MMPIP, IC_{50} (literature data) = 19 nM

In-vitro screening overview

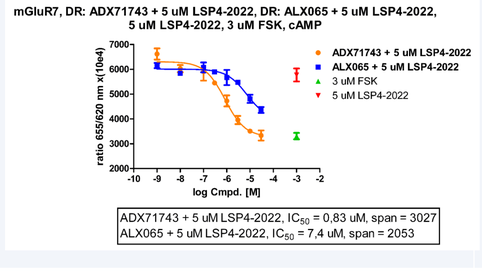
- The new strategy for identyfication of negative allosteric modulators of mGluR7 receptor was developed.
- mGluR orthosteric ligand LSP4-2022 is used as an agonist for the *in-vitro* tests.
- Selectivity of the ALX ligand library towards other mGlu receptors is also examined.
- Interaction of the ALX-ligands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

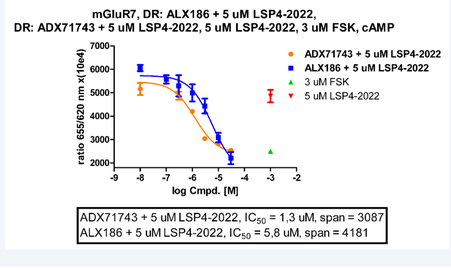
Summary of *in-vitro* screening:

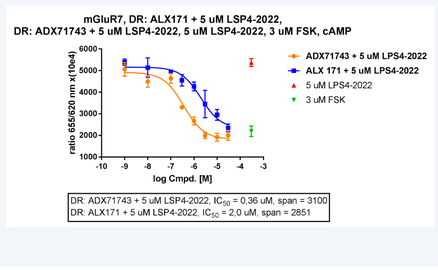
So far few active ligands: ALX-063, ALX-065, ALX-115, ALX-171, ALX-186, ALX-226, ALX-231, ALX-234 have been identified as new mGluR7 negative allosteric modulators.

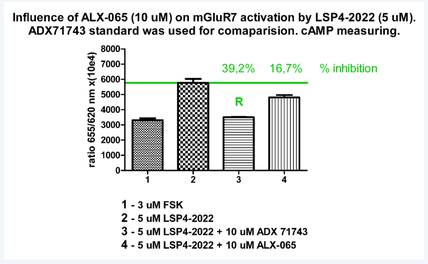


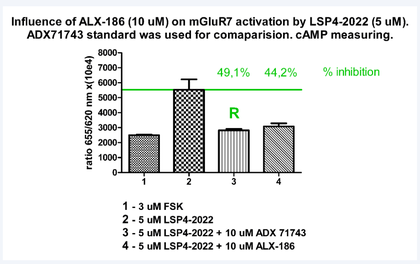
In-vitro results: comparision of mGluR7 activity of ALX-065 *vs* ALX-186, ALX-171 to ADX71743

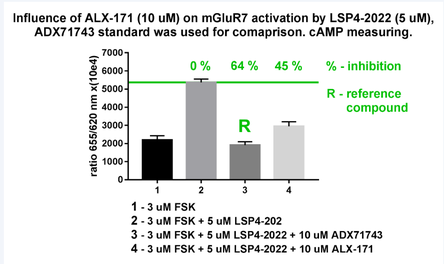






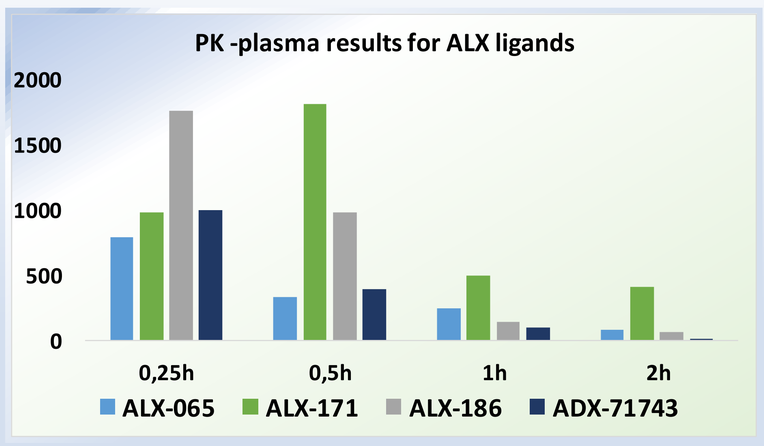


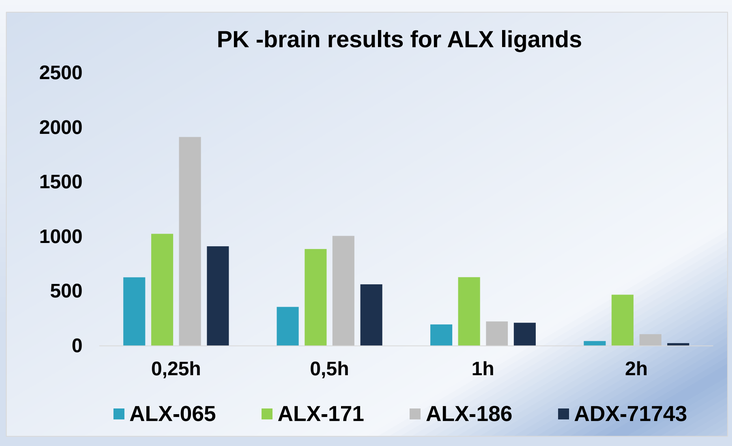




Summary of *in-vitro* tests:

ALX-065 activity: IC_{50} = 7.4 μM, **ALX-186** activity: IC_{50} = 5.8 μM, **ALX-171** activity: IC_{50} = 2.0 μM





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
ALX-226	4.48	4.72	76.04
ALX-231	4.94	≤ 3.9	68.22

Summary of ADME tests:

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

Comparision of pharmacokinetics results for ALX ligands

Brain pharmacokinetics results for ALX ligands

Compound	AUC 0-t [ng/ml*h]	Concentration [ng/ml] **0.25h**	Concentration [ng/ml] **0.5h**	Concentration [ng/ml] **1h**	Concentration [ng/ml] **2h**
ALX-065	459.18	627.75	356.33	195.89	43.40
ALX-171	5633.84	1026.12	886.82	629.17	467.62
ALX-186	1360.56	1912.71	1006.83	223.56	107.38
ADX-71743	610.27	911.46	562.91	211.66	25.14

Plasma pharmacokinetics results for ALX ligants

Compound	AUC 0-t [ng/ml*h]	Concentration [ng/ml] **0.25h**	Concentration [ng/ml] **0.5h**	Concentration [ng/ml] **1h**	Concentration [ng/ml] **2h**
ALX-065	691.77	789.79	334.74	249.90	85.14
ALX-171	5456.12	981.04	1815.65	493.18	408.77
ALX-186	1083.43	1768.10	982.24	143.62	67.84
ADX-71743	512.23	1003.34	398.25	100.50	9.14

Summary of PK results:

Primary pharmacokinetics results demonstrate that concentration of ALX-171 in plasma as well as in brain is higher than for ADX 71743 standard.

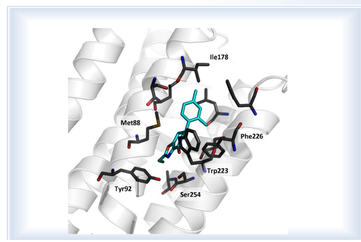


Fig 3. Binding mode of the most potent ADEX 71743 cmpd.

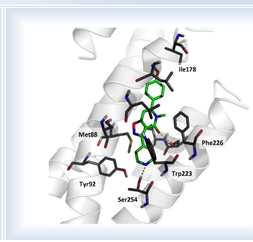


Fig 4. Binding mode of MMPIP cmpd.

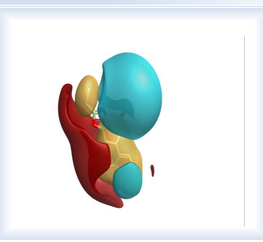


Fig 5. ADX 71743 cresset field view.

SUMMARY:

- >250 compounds were synthesized,
- hit compounds: ALX-063 and ALX-065 were identified,
- >90 derivatives of the hit molecules were synthesized and analyzed,
- preliminarny ADME tests were done for all ALX- compounds,
- ALX-186, ALX-171 were found to be selective for mGluR7 receptor, while ALX-065 demonstrated also week mGluR4 receptor activity,
- all active ALX-compounds were screened for formulation solubility,
- molecule ALX-186 indicated better solubility and metabolic stability than ALX-171 but its concentration in the brain and in plasma is lower,
- concentration of ALX-171 in plasma as well as in the brain is higher than for the reference ADX-71743 compound,
- patent application is under preparation,
- based on the last *in-vitro* and ADME results next pharmacokinetic tests for ALX-226 and ALX-231 are planned,
- optimization of lead compound is still on-going.

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