DESIGN AND CHARACTERIZATION OF NEW mGluR5 ANTAGONISTS

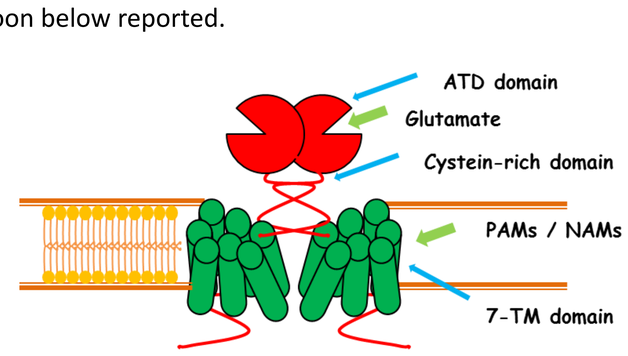
Fabrizio Micheli[1,2], Marco Pieroni[1], Elisa Callegari[3], Davide Graziani[3], Lorenza Pirona[3], Simone Braggio[2], Gabriele Costantino[1]

[1], *Universita' degli Studi di Parma, Dipartimento di Farmacia, Via Area delle Scienze, 23/A 43100 Parma (Italy)*
[2], *Aptuit Verona, s.r.l, Via Fleeming,4. 37135 Verona (Italy)*
[3] *Recordati, S.p.A. Via M. Civitali 1, 20148 Milano (Italy)*

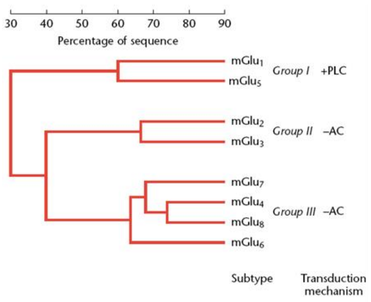
INTRODUCTION

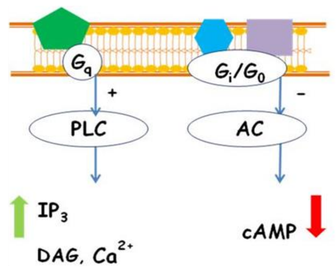
Glutamate is the main excitatory neurotransmitter in CNS and works through both ionotropic (AMPA, NMDA, Kainate) and metabotropic receptors (mgluRs).

mGluRs belong to the C family of G-Protein Coupled Receptors (GPCRs) and are endowed with a peculiar amino terminal domain (ATD) which contains the Glutamate binding site as for the cartoon below reported.



To date, 8 mGluRs have been identified and divided in 3 main groups based on sequence homology and second messenger transduction pathways.





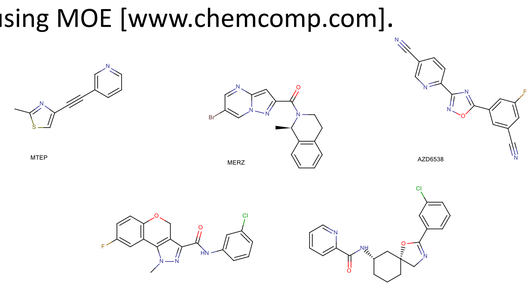
Given the physiological distribution of mGluR5 receptors and according to preclinical studies, it is possible to hypothesize the use of mGluR5 antagonists or negative allosteric modulators (NAMs) to treat pathological conditions such as schizophrenia, depression, Parkinson's disease, pain, drug dependence and Fragile X syndrome.

AIM OF THE STUDY

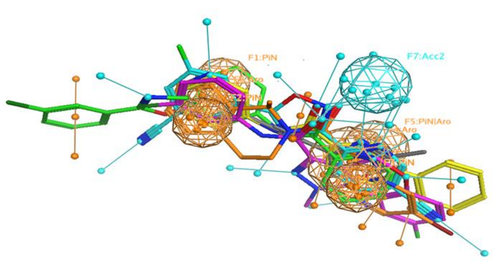
- ✓ To develop an operational pharmacophore scheme for mGluR5 NAMs
- ✓ To use the so obtained pharmacophore to screen compound libraries to identify structurally novel scaffolds.

PHARMACOPHORE IDENTIFICATION AND LIBRARY SCREENING

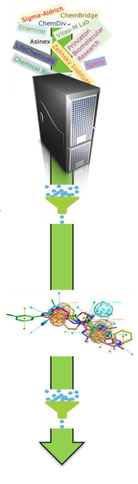
The structures of the five below reported NAMs have been used to prepare a pharmacophoric model using MOE [www.chemcomp.com].



A predefined routine was used for confomational searches (max 250 conformations, energy window of 15 Kcal); moreover, for the «automated» construction, the standard MOE «pharmacophore features» were applied. (e.g. H-bond acceptor, positive ionisable, aromatic rings, hydrophobic groups); unfortunately the «automated model» was not able to take into account all the known SAR of mGluR5 NAMs. Accordingly, using the «query builder» functionality, a second model was built and depicted below.

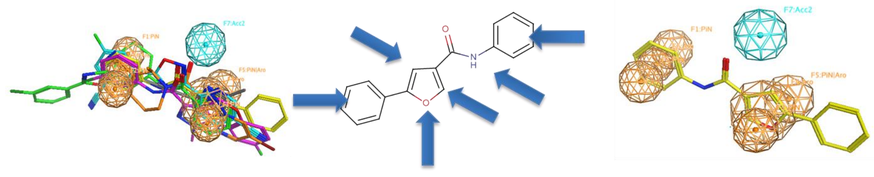


- ✓ Aptuit has a virtual library of about 4M (SMILES) of compounds prepared from 2D and 3D commercial chemical DBs. A series of filters was applied to make the potential hits "developable" (e.g. MW < 600; 50< PSA < 130; no alkylating agents, no known "toxicophores "; HBD < 3; HBA < 7; rot bond < 10).
- ✓ Following this procedure, the library size was reduced to about 2M compounds which were "sieved" using the pharmacophore model above described.
- ✓ The best hits have been further visually analyzed to determine the best starting point for a chemical exploration.



HIT IDENTIFICATION

Among the most interesting hits, the furane below reported was identified



Beyond fitting very well in the pharmacophore model, this structure was considered very interesting as its physico-chemical parameters may guarantee a further wide exploration of the template (MW = 263.2 Da, clogP = 3.77, PSA = 42 Å^2)

A search performed using Scifinder revealed the existance of 1414 analogues and none of them was ever reported as mGluR5 ligand. Among these compounds, 1119 were commercially available. Applying further filters for CNS applications (MW < 500; PSA < 80; clogP < 5; HBA < 5) , a subset of 354 commercially available molecules have been identified. These structures were analyzed using Vortex and clusterized in 19 groups. The centroid of each group was ordered to the vendors.

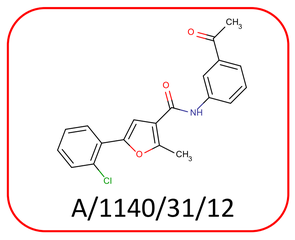
Measure of Intracellular Calcium Levels

Purchased compounds were tested in *in-vitro* calcium mobilization functional assay in a CHO T-REx h-mGluR5 cell line and the results are reported in the following table:

COMPOUND NAME	IC_{50} µM	SD
Amb1223302	8.02	5.27
Amb1559861	12.39	10.47
Amb2740086	5.14	3.69
A/1140/31/1	11.79	2.16
A/1140/31/2	8.19	1.99
A/1140/31/11	N/A	
A/1140/31/12	3.90	2.08
A/1140/31/13	N/A	
A/1140/31/14	13.35	3.21
A/1140/31/16	20.68	2.47
A/1140/31/17	N/A	

Results represent IC_{50} average ± SD of 3 different determinations

Among the compounds, the most potent resulted furane **A/1140/31/12** with a functional activity < 4 µM.



A/1140/31/12

A/1140/31/12 was able to displace ^{3}H-MPEP with an IC_{50}= 13 µM, thus indicating a negative allosteric mechanism.

A second series of close analogues, commercially available, will be ordered to further enrich the existing SAR without the need of starting wet chemistry activities.
Once the results will be available, it will be possible to tailor synthetic activities to further increase potency and/or to modify the scaffold.
Selectivity assays and further characterization are in progress
At the same time, further hits obtained from the initial virtual screening campaign will be tested.

Material and Methods

A **CHO T-REx h-mGluR5** cell line, stably transfected with an inducible expression vector encoding for the human mGlu5 receptor using the Tetracycline-Regulated Expression system (T-REx® Life Technologies), was generated in Recordati laboratories.
Cells were seeded into black-walled, clear-bottom, 96-well plates at a density of 80.000 cell/well, in RPMI supplemented with 10% dialyzed FBS. Following 18-h incubation with 3ng/ml tetracycline, the cells were loaded with 2mM Ca^{2+}- sensitive fluorescent dye Fluo-4/AM (Molecular Probes) in Hanks' balanced saline solution (HBSS) with 20 mM Hepes and 2.5 mM probenecid, for 1h at 37°C. Cells were washed three times with HBSS to remove extracellular dye. Fluorescence signals were measured by using the fluorescence microplate reader Flexstation III (Molecular Devices) at sampling intervals of 1.5 s for 60 s. The antagonist potency was determined using EC_{80} of the quisqualate (agonist). The antagonists were pre-incubated 10 minute before agonist application. IC_{50s} were determined by nonlinear regression analysis using the software Prism 4.0 (Graphpad).