

Functional & Radioligand Binding Assays for Metabotropic Glutamate Receptors

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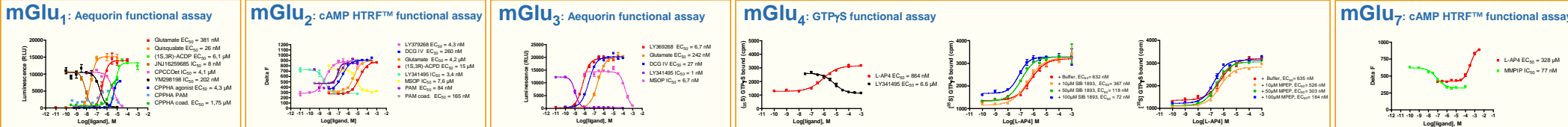
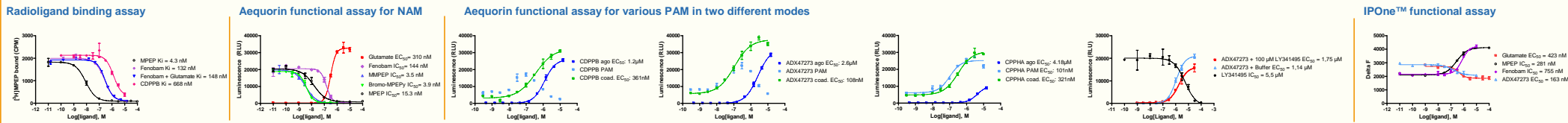
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Introduction Glutamate is considered as the major excitatory neurotransmitter in the mammalian central nervous system. Glutamate transmission is mediated by ion channel receptors, called ionotropic glutamate receptors, which include NMDA, AMPA and kainate receptors but also by G protein coupled receptors, called metabotropic glutamate receptors (mGlu). These receptors bind glutamate and function to modulate or 'fine-tune' excitatory and inhibitory transmission by presynaptic, postsynaptic, and glial mechanisms. Metabotropic glutamate receptors play important roles in neurotransmission, neuronal development, synaptic plasticity and neurological disorders. In spite of the potential of these receptors for therapeutic applications, advances in drug discovery have been limited by availability of robust assays for primary screening and compound characterization. Here we present the validation of functional and radioligand binding assays for several metabotropic glutamate receptors. Such assays were used to characterize orthosteric agonists and antagonists, allosteric agonists, neutral allosteric modulators, positive allosteric modulators (PAM) and negative allosteric modulators (NAM). (Methods and further information available upon request).

mGlu₅ Different assays were developed for mGlu₅ pharmacological characterization in 96 and 384 well formats. Radioligand binding assay was developed with both orthosteric and allosteric radioligands and were used to characterize different categories of mGlu₅ ligands. Ki were similar to those described previously (Malherbe *et al.*, 2003; Porter *et al.*, 2005; Chen *et al.*, 2007). Glutamate addition in the binding buffer did not result in a receptor conformation with higher affinity for Fenobam. Aequorin functional assay resulted in a robust test suitable for both primary functional screening and hit to lead follow up. Testing of NAM was very reproducible and allowed to obtain potencies similar to those described in the literature (Porter *et al.*, 2005; Chen *et al.*, 2007). For PAM characterization, all tested compounds did show allosteric agonism (activity in absence of orthosteric ligand) in aequorin assay. Such activity was not inhibited by an orthosteric antagonist or by using glutamate free medium or coexpressing EAAC1 glutamate transporter (Zhang *et al.*, 2007). Such activity interfered with the PAM screening when the glutamate was added 3 min after the test compound due to a depletion of the signal; then coaddition of glutamate and test compound was used as a primary test for PAM. IPOne™ assay allowed to characterize PAM and NAM but also inverse agonism activity of MPEP and Fenobam (Porter *et al.* 2005).



Conclusions

- > The stable expression of different metabotropic glutamate receptors in inducible or stable cell lines allowed development of functional and radioligand binding assays suitable for HTS as well as for molecular pharmacology.
- > Aequorin assay is well adapted to functional HTS allowing to identify both PAM and NAM in the same screening. Further use of neutral allosteric modulator like 5-MPEP helped for binding site characterization.
- > Allosteric agonism of PAM for mGlu₅ were observed in all conditions tested and were not competed by orthosteric antagonists. Such activity was reported with mGlu₅ deleted with glutamate binding site.
- > GTP_γS, cAMP™ or IPOne™ functional assays are suitable for characterization of PAM and NAM as well as inverse agonist.
- > Binding assays completed the panel of tests to discriminate the putative binding sites of allosteric modulators.
- > This is essential to determine relevant parameters measured in the various assays to follow the structure activity relationship.
- > mGlu₁, mGlu₂, mGlu₃, mGlu₄ and mGlu₇ are already available for testing and profiling and will be validated for further radioligand and functional assays.
- > mGlu₆ and mGlu₈ cell lines are under current development.

References

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Malherbe P. *et al.* (2003) Mol. Pharmacol. 64, 823-832
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