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Functional Screening of Allosteric Modulators for G Protein-Coupled Receptors

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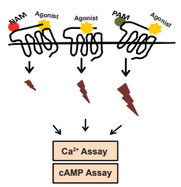
ABSTRACT

G protein-coupled receptors (GPCRs) constitute the largest superfamily of cell surface receptors. A wide range of relatively small and structurally diverse chemicals such as biogenic amines, peptides and hormones bind to GPCRs and mediate the majority of transmembrane signals in living cells. GPCRs undergo conformational changes between two topographically distinct binding sites, one for agonists and the other for the G protein. It is also known that agonist-bound GPCR can also form ternary complexes with other ligands or accessory proteins and display altered signaling properties. These other compounds interact with binding sites called "allosteric sites" that are topographically distinct from the orthosteric site recognized by the agonists for the receptors. Allosteric ligands that enhance the agonist activities are referred as positive allosteric modulators (PAM) while those that inhibit or decrease the activities are called negative allosteric modulators (NAM). There are a number of therapeutic advantages of using allosteric modulators over orthosteric ligands, such as greater GPCR selectivity, decreased toxicity or side effects, enhanced physiological specificity of action, and fine tuned pharmacological responses. However, allosteric modulators have only been described for a few GPCRs. Multispan has been providing the pharmaceutical industry with stable cell lines and screening services to identify new allosteric modulators. In this study, we show some examples of modulator studies in both positive and negative modes in cell lines stably expressing GPCRs such as metabotropic glutamate receptors and chemokine receptors.

INTRODUCTION



The superfamily of G protein-coupled receptors (GPCRs) has more than 1000 members and is the largest family of proteins in the body. GPCRs mediate signaling of stimuli as diverse as light, ions, small molecules, peptides and proteins and are the targets for many pharmaceutical drugs. GPCR ligands bind to receptors at orthosteric sites and activate or inhibit the signaling pathway as agonists or antagonists.

Despite GPCRs being the most fruitful targets for marketed drugs, many orthosteric compounds could not provide selectivity and specificity. In recent years, several new perspectives have emerged in the regulation of GPCR signaling pathways and one of them is allosteric modulation. In contrast to orthosteric ligands, allosteric ligands bind to different regions of the receptor and often provide higher selectivity and specificity. The allosteric ligands can potentiate (PAM) or inhibit (NAM) the agonist activity of orthosteric ligands. The advent of cell-based functional assays as the screening method of choice is leading to an increase in the number of allosteric modulators identified. Multispan has optimized functional assays for allosteric modulation and provided stable cells lines for screening services identifying new modulators for GPCRs. In this report, we present some of the modulator studies on metabotropic glutamate receptors and chemokine receptors using calcium mobilization and cyclic AMP assays.

MATERIALS AND METHODS

Cells: Human embryonic kidney HEK293T cells expressing functional GPCRs.

Compounds: Compounds were purchased from either R&D systems or Sigma, or provided by Multispan customers.

Calcium Mobilization Assay: Cells cultured overnight in Poly-D-Lysine-coated plates were incubated for one hour with buffer containing calcium indicator dye FLIPR® Calcium 4 (Molecular Devices, R8142). Calcium flux was monitored upon addition of compounds using FlexStation III (Molecular Devices).

cAMP Assay: Cells treated with compounds were subjected to cAMP assay using cAMP Hi-Range Kit (Cisbio, 62AM6PEC).

Data analysis: Data were analyzed using Prism 4.03 (GraphPad). Two-tailed Student's t-test was used for treatment comparisons and statistic significance was defined as p<0.05.

Assay Validation Using Known Allosteric Modulators

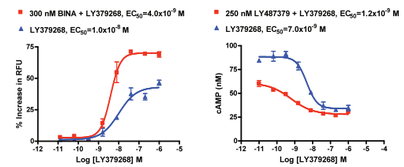


Figure 1. Positive allosteric modulation of the human mGlu2 receptor by compounds BINA and LY487379 in calcium mobilization assay (left) and cAMP assay (right), respectively. BINA increased the maximum Ca^{2+} flux induced by the full agonist LY379268, and LY487379 further enhanced the inhibition of cAMP production by LY379268. Both compounds shifted the agonist dose-response curves towards the left, reducing the EC_{50} values of the agonist.

Compound Profiling Examples

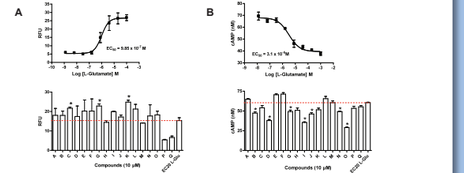


Figure 2. (A) Human mGlu1 receptor: Ca^{2+} flux stimulated by the control agonist L-Glutamate (top) and customer compounds in the presence of EC_{20} of L-Glutamate (bottom). (B) Human mGlu2 receptor: inhibition of forskolin-stimulated cAMP levels by the control agonist L-Glutamate (top) and customer compounds in the presence of EC_{20} of L-Glutamate (bottom). Compounds C, G and K significantly increased the Ca^{2+} flux induced by the agonist at EC_{20}, showing positive modulator activity. Compounds B, D, G, I, J, N and O enhanced the inhibition of cAMP production by the agonist at EC_{20}, showing positive modulator activity. *p<0.05, compared to "EC_{20} L-Glu".

Compound Profiling Examples

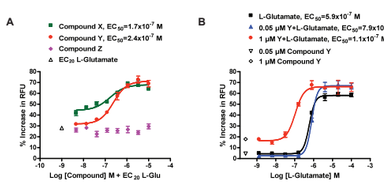


Figure 3. A) Ca^{2+} flux in cells expressing the human mGlu2 receptor by varying concentrations of customer compounds in the presence of L-Glutamate at EC_{20}. Compounds X and Y dose-dependently stimulated Ca^{2+} signals above the level induced by L-Glutamate. B) Further studies of Compound Y revealed that it dose-dependently shifted the dose-response curve of L-Glutamate to the left, reducing its EC_{50} value at 1 μM but not at 0.05 μM.

Compound Profiling Examples

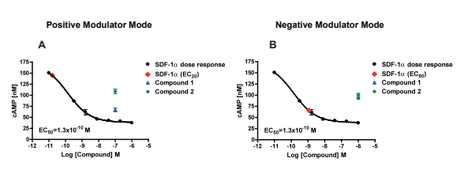


Figure 4. Compound profiling for two compounds at single concentration in positive (A) and negative (B) allosteric modulator modes for the human CXCR4 receptor using cAMP assays measured in the presence of EC_{20} and EC_{80} of SDF1-α, respectively. Both compounds enhanced the effect of SDF1-α at EC_{20} and reduced its effect at EC_{80}. More detailed studies revealed that these compounds were partial agonists (data not shown).

Multispan's Cell-Based Functional Assays for Allosteric Modulation

Receptor Family	GPCRs
Adenosine receptors	A1, A2B
Adrenergic receptors	α1B, α2A, α2B, α2C, ß1, ß2, ß3
Calcium-sensing receptors	CaS
Chemokine receptors	CCR1-10, CXCR1-6, CX3CR1, XCR1, CMKLR1
Dopamine receptors	D1, 2, 5
Endothelin receptors	ETB
Glycoprotein hormone receptors	TSH
Metabotropic glutamate receptors	mGluR1, 2, 3, 4, 5, 6, 7, 8
Muscarinic acetylcholine receptors	M1, 2, 3, 4, 5
Neurokinin receptors	NK1, 2, 3
Opioid receptors	μ, δ, κ,
Purinergic receptors	P2Y1, 2, 4, 6, 11, 12, 13, 14
Serotonin receptors	5-HT1A, 1B, 2A, 2B, 2C, 4, 6, 7

SUMMARY

- Positive allosteric modular assays were established with PAM controls compounds for mGlu2 receptor that showed comparable EC_{50} values to published reports.
- Positive and negative allosteric modulator assays in cells expressing members of the metabotropic glutamate receptor family were used to analyze multiple compounds, and positives hits have been obtained.
- Multispan offers cell-based functional assays for a large panel of GPCRs, many of which have been reported to subject to allosteric modulations, paving the way to discovering new allosteric modulators through compound screening and profiling.