

Allosteric Modulation: a Novel Approach to Drug Discovery

Allosteric modulators of GPCRs have emerged as a novel and highly desirable class of compounds, offering a number of distinct advantages over conventional competitive compounds; their increased selectivity, self-limiting activity and structural novelty should translate into promising new drugs for a wide range of diseases



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The advent of data-rich high-throughput functional screening methods has led to the discovery of small molecules with novel modes of action. Application of these screening techniques has enabled the detection of numerous types of molecules including those classified as 'allosteric modulators'. Alone, these molecules can have no intrinsic activity on their target – instead, they modulate the activity of the target when the endogenous ligand for the target is bound concurrently. There is a long history of the study of allosteric modulation of certain classes of proteins, for example, enzymes; however, the development of high-throughput functional assays – that has enabled the discovery of allosteric modulators for other classes of protein targets of high interest to the pharmaceutical industry – is a more recent phenomenon.

At Addex Pharmaceuticals, we have applied innovative screening techniques to discover and develop both positive and negative allosteric modulators for G protein-coupled receptors (GPCRs). Small molecule allosteric modulators offer several advantages over classical competitive compounds in terms of 'developability' and drug-likeness as well as the

potential to address a wider range of targets than previously thought by exploiting novel modes of action. Here, we present these advantages along with an example of a novel positive allosteric modulator discovered at Addex.

GPCRS AS DRUG TARGETS

GPCRs are the largest family of integral membrane receptors, and account for 3-4% of the human genome (1). They have evolved to recognise a wide range of endogenous stimuli, and function by transmitting messages from the exterior to the interior of the cell. Activation of GPCRs by their endogenous ligand shifts the conformation of the receptor from an inactive to an activated state, activating the G-protein associated with the receptor and initiating intracellular signaling cascades that mediate cellular responses. The ubiquitous distribution of GPCRs and their involvement in virtually all physiological processes make them extremely attractive targets for drug development. In fact, the greatest proportion of currently marketed drugs acts on GPCRs (2), emphasising their importance to the pharmaceutical industry.

CONVENTIONAL APPROACHES TO GPCR DRUG DISCOVERY

The drug discovery process involves the design of molecules that interact with a target with high specificity and efficacy. Traditional approaches to drug discovery focus on mimicking or inhibiting the actions of the endogenous ligand for a target receptor. Conventionally, this has been done by the design and synthesis of small molecule agonists or antagonists that act in a competitive manner through interaction with the same binding site as the endogenous ligand, termed the 'orthosteric' binding site.

Competitive agonists and antagonists must have a sufficiently high affinity for the target receptor to displace the endogenous ligand, and must be maintained at a sufficiently high concentration in the region of the receptor in order to exert an effect. Under these conditions, agonists will induce an activated state and antagonists will induce an inactivated state. In both states, receptors will not be responsive to the natural fluctuations in the levels of endogenous ligand, such as the normal rise and fall of neurotransmitters during synaptic transmission, thereby interfering with normal physiological signalling.

Although the competitive compound approach has yielded drugs of great therapeutic value, significant challenges remain with respect to the continued

development of therapeutically-useful GPCR competitive agonists or antagonists, due to the complexity of the interaction of endogenous ligands with their receptors, the lack of receptor selectivity or undesirable side effects.

ALLOSTERIC MODULATORS AS GPCR DRUGS

The word 'allosteric' literally translated from its Greek roots means 'other site.' In contrast to competitive compounds, allosteric modulators of GPCRs interact with binding sites that are topographically distinct from the binding site of the endogenous ligand (see Figure 1). Furthermore, positive allosteric modulators do not activate receptors on their own. In the presence of the endogenous ligand, allosteric modulators enhance (positively modulate) the natural physiological activity of the receptor. Consequently, allosteric modulators can exert their effects while preserving normal physiological signaling patterns.

By applying a non-competitive approach that preserves the physiological nature of endogenous ligand-GPCR signaling, it may be possible to produce therapeutic agents that are safer than conventional competitive agonists or antagonists drugs.

Allosteric modulators offer several potential advantages over classical approaches:

A Novel Drug Class

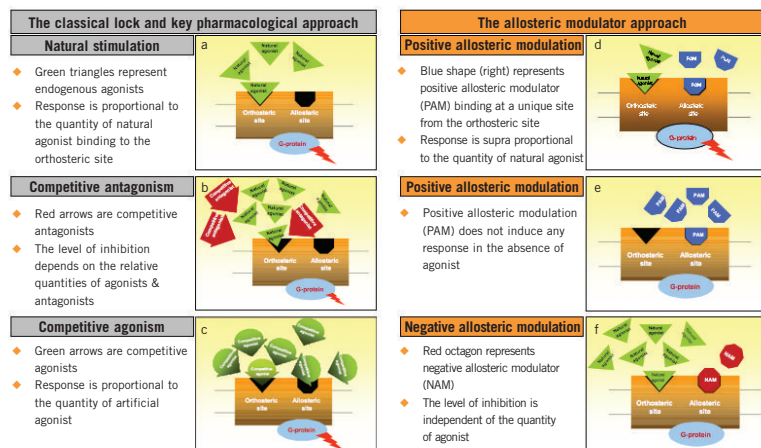
Allosteric modulators are a novel class of small molecule drug candidates with a chemical structure unrelated to that of competitive agonist or antagonist drugs, and – as such – represent first-in-class drug candidates with a high potential for composition-of-matter patent protection. These compounds are typically drug-like and amenable to chemical optimisation for oral bioavailability and favourable pharmacokinetic properties.

Superior Receptor Subtype Selectivity

The orthosteric binding site is, in general, highly conserved within a GPCR family due to the evolutionary pressure to retain amino acid sequences necessary for binding of the endogenous ligand. Thus, achieving receptor subtype selectivity within a GPCR family has not always been possible for competitive compounds. The best examples of this are the muscarinic acetylcholine and the metabotropic glutamate receptor families, for which development of competitive, sub-type selective agonists has not been successful thus far. In contrast, allosteric modulator binding sites – being independent of endogenous stimuli – have evolved with much greater structural diversity than endogenous ligand binding sites, and

Figure 1: Allosteric modulation – a novel approach

A side-by-side comparison of the conventional competitive compound pharmacological model and the allosteric modulator pharmacological model. Figure 1a) Natural agonists bind to the orthosteric binding site of a GPCR to induce a cellular response. Figure 1b) Competitive orthosteric antagonists compete with the natural agonist for binding to the orthosteric binding site. Successful competitive binding depends on relative concentrations of agonist and antagonist. Figure 1c) For competitive agonists, the magnitude of the receptor response is proportional to the concentration at the receptor. Figure 1d) For positive allosteric modulators (PAMs), the receptor response is supra-proportional to the concentration of natural agonist. Figure 1e) PAMs do not induce any receptor response alone. Figure 1f) For negative allosteric modulators (NAMs), inhibition is non-competitive and the degree of inhibition is independent of agonist concentration.



PAM: Positive allosteric modulator NAM: Negative allosteric modulator

consequently offer the potential for the development of drugs with much greater receptor subtype selectivity.

The Ability to Discover Small Molecule Drugs for GPCR Targets That Historically have Evaded Small Molecule Drug Developers

Several GPCR targets are currently thought to be beyond the reach of conventional competitive drug discovery approaches due to the complexity of the interaction of the endogenous ligand with the receptor – including, for example, certain peptides, high molecular weight hormones and lipids. For these targets, the allosteric modulator approach may offer a way to develop orally active small molecule modulators.

The Ability to Re-Address Well Characterised and Clinically Validated GPCR Targets Where The Pharmaceutical Industry has Exhausted Competitive Compound Drug Discovery Approaches

For certain targets, the pharmaceutical industry has only been able to discover competitive compounds. These molecules must interact with the highly-conserved endogenous ligand binding site, often resulting in a limited set of pharmacophores all with similar side effect profiles. Allosteric modulators offer a promising way to revisit these targets, providing novel small molecules that modulate well-validated GPCR targets. One example is the potent competitive dopamine D2 receptor antagonism exhibited by all typical and atypical antipsychotic drugs, and their limited selectivity over other dopamine receptor subtypes – for example, D1-like receptors. Currently marketed compounds, except for the competitive D2 partial agonist aripiprazole (Abilify®), are full competitive antagonists at D2 receptors. The extrapyramidal side effects and hyperprolactinaemia induced by D2 receptor blockade are well known and, to date, unavoidable with competitive compounds.

Because allosteric modulators can have activity which ranges from full positive modulation through neutral modulation (occupancy of the allosteric site with no change in orthosteric agonist affinity or efficacy) to full negative modulation, it should be theoretically possible to develop partial negative allosteric modulators of dopamine D2 receptors. Such compounds would exert a noncompetitive permissive antagonism of D2 receptor activity, allowing for some D2 receptor stimulation upon dopamine binding, but might not induce the side effects associated with competitive D2 receptor blockade. Thus, even 100% receptor occupancy by a partial NAM would not result

in full receptor blockade. And, because partial negative allosteric modulators would not need to compete with dopamine for the orthosteric binding site, they also should have improved receptor selectivity, since they would bind to a less conserved site.

Improved Safety Compared to Orthosteric Molecules Binding the Same Target

Competitive agonists and antagonists directly activate or inhibit their targets as long as they are bound, and may induce other effects including receptor desensitisation or internalisation. In contrast, allosteric modulators may only alter receptor responses in the presence of the endogenous ligand; they are more likely to preserve natural physiological signalling. This preservation, together with superior selectivity (see above), gives allosteric modulators the potential for improved safety compared with competitive compounds.

Self-Limiting Activity

There is a limited ‘ceiling’ level of the effects of allosteric modulators beyond which further increments in modulator dose will not produce additional target-based effects. This ceiling is determined by the nature and magnitude of the cooperative interaction between an allosteric and an orthosteric compound (positive, negative or neutral effect on binding affinity), and the effect of the allosteric compound on the efficacy of the orthosteric ligand (3). This limitation in the degree of effect of some allosteric modulators could result in an improved safety profile, compared with orthosteric compounds binding the same target. Consequently, low-affinity modulators could be given in larger doses while maintaining a favourable overall profile. It is important to note that physiologically meaningful effects are often achieved with relatively small changes in receptor activity. As such, the more moderated effect of allosteric modulators should not mean that they will be less efficacious than orthosteric modulators.

Clinical Use in Combination

Given that allosteric modulators target different binding sites than orthosteric modulators, they may find clinical utility in combination therapies for certain clinical indications. For example, a positive allosteric modulator could be given in combination with an orthosteric agonist to increase the efficacy of the orthosteric compound while allowing for a decrease in the dose administered, and thereby improving the overall side-effect profile.

AN EXAMPLE – ADX47273

ADX47273 is an illustration of the potential for the discovery and development of allosteric modulators (see Figure 2). It is a novel positive allosteric modulator of the metabotropic glutamate receptor subtype 5 (mGluR5). In a high-throughput screening campaign we identified several series of novel, selective mGluR5 positive allosteric modulators, and

performed lead optimisation to develop these molecules as novel treatments for central nervous system diseases, including schizophrenia and cognitive dysfunction.

As can be seen in the traces shown in Figure 2b, ADX47273 exhibits the properties expected for a positive allosteric modulator. That is, the compound alone – at a concentration of 10 μ M – does not induce any functional response (in this case, change in fluorescence induced by intracellular Ca²⁺ release). However, ADX47273 in the presence of an EC₂₅ concentration of glutamate potentiates the functional response up to the maximum observed for glutamate in this measurement system. These results demonstrate that ADX47273 alone is devoid of intrinsic activity on mGluR5, but that it acts to potentiate the effect of glutamate on mGluR5 receptors.

Another property of positive allosteric modulators exhibited by ADX47273 is an increase in the affinity of the endogenous ligand for the target receptor. This increase is manifested in a leftward shift in the EC₅₀ of the endogenous ligand, in this case glutamate. As depicted in Figure 2c, 30 μ M ADX47273 induces a concentration-dependent ~13-fold shift to the left in the glutamate EC₅₀. Unlike orthosteric agonists of mGluRs, ADX47273 is specific for mGluR5 with no activity at any of the other mGluR receptor subtypes.

CONCLUSION

Allosteric modulators of GPCRs have emerged as a novel and highly desirable class of compounds. They offer a number of distinct advantages over conventional competitive compounds, including the potential for fine-tuning of GPCR signalling and the promise to address here-to-fore intractable targets. Their increased selectivity, self-limiting activity and structural novelty should translate into promising new drugs for a wide range of diseases.

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Figure 2

The chemical structure and pharmacological properties of ADX47273, a positive allosteric modulator of mGluR5. Figure 2a) The chemical structure of ADX47273 – (S)-(4-fluorophenyl)-(3-[3-(4-fluorophenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl)-methanone. Figure 2b) The bottom trace shows the response in cells expressing mGluR5 induced by 10 μ M ADX47273 alone; the middle trace shows the response induced by an EC₂₅ concentration of glutamate; and the upper trace shows the response induced by an EC₂₅ concentration of glutamate plus 10 μ M ADX47273. Figure 2c) The shift to the left of the glutamate EC₅₀ in the presence of increasing concentrations of ADX47273. ADX47273 at 30 μ M induces a leftward shift of ~13-fold in the glutamate EC₅₀.

Figure 2a)

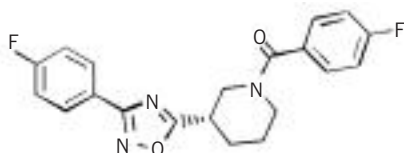


Figure 2b)

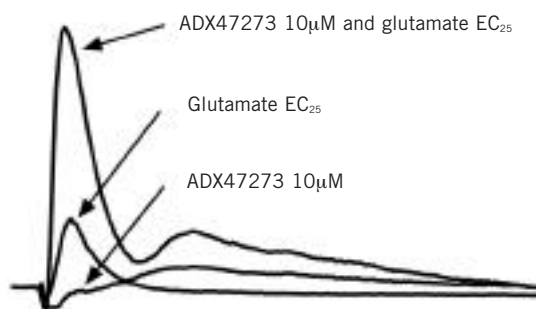


Figure 2c)

