Recent Advances in Drug Discovery of GPCR Allosteric Modulators

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Introduction

The importance of the allosteric regulation of cellular functions has been known for decades and even the word “allosterone,” which describes the endogenous allosteric regulator molecules of a cell, has been proposed. Although best described as modulators of enzymes, advances in molecular biology and robotic HTS technologies recently allowed the discovery of small molecule allosteric modulators of various biological systems, including GPCR and non-GPCR targets. Today, allosteric modulators appear to be an emerging class of orally available therapeutic agents that can offer a competitive advantage over classical “orthosteric” drugs. This potential stems from their ability to offer greater selectivity and differentiated control over disease mediating receptors. Most marketed drugs bind to receptors where the body’s own natural molecular activators (i.e. endogenous ligands) bind specifically to a key part of each receptor’s anatomy called the “active site”. Orthosteric ligands are natural or therapeutic molecules — including peptides and proteins — which bind to the active site of receptors. By contrast, allosteric modulators bind to receptors at a different site and modify receptor function even if the endogenous ligand also binds to the active site at the same time. As a result, allosteric modulators are non-competitive, which results in a number of differentiating factors. In fact, most allosteric modulators have little or no effect on receptor function until the active site is bound by an orthosteric ligand. Allosteric modulators therefore have multiple potential advantages compared to small molecule and biologic orthosteric drugs. In particular, they offer new chemistry possibilities allowing access to well known targets that have been considered intractable to historical small molecule approaches. For example, allosteric modulators may soon be developed for targets which heretofore have been only successfully targeted with proteins and peptides. In other words, allosteric drugs with all the advantages of small molecules — brain penetration, easier manufacturing, distribution and oral administration — may soon be viewed as the best life cycle management strategy for protein therapeutics. Another potential benefit of allosteric drugs compared to orthosteric drugs is the greater ease of achieving higher selectivity for the target. The allosteric sites, unlike the orthosteric sites, have been shown to display greater heterogeneity, in all likelihood because they have evolved with less evolutionary pressure compared to the active sites, especially among closely related receptors that may share a common endogenous ligand. In the case of metabotropic receptors for example, the glutamate binding orthosteric site is very well conserved within 8 members of the family, rendering the task of making subtype selective compounds highly
challenging. Allosteric compounds discovered and developed in recent years have been shown to offer exquisite selectivity for one subtype vs other receptors of the same family (as well as other families).

Allosteric drug discovery has been recently the topic of increased interest, addressing various important target classes besides G-protein coupled receptors (GPCRs): proteases, kinases, phosphodiesterases and ligand-gated ion channels.

**Allosteric Modulators of GPCRs: Mechanism of Action and Detection**

GPCRs are the largest family of integral membrane receptors; the ubiquitous distribution of GPCRs and their involvement in virtually all physiological processes make them extremely attractive targets for drug development. Allosteric modulation as a potential solution for the most challenging receptors in this class has been the topic of recent reviews. Allosteric modulators of GPCRs interact with binding sites that are topographically distinct from binding sites of the endogenous ligands (see Figure 1). Furthermore, positive allosteric modulators (PAM) generally do not activate receptors in the absence of an orthosteric ligand. In the presence of orthosteric ligand, PAM enhance the natural physiological activity of the receptor imposing a “ceiling” at the magnitude of their allosteric effect; this property will limit the adverse effects and also the desensitization that might be produced by an orthosteric agonist. Thus, by applying a non-competitive approach that is both more selective and, at the same time, able to preserve the physiological rhythms of endogenous ligand-GPCR signaling, it may be possible to show that therapeutic agents are safer than conventional competitive agonists or antagonists against the same targets.

In addition, allosteric ligands have been identified that bind to an allosteric site with high affinity without affecting the receptor function. These molecules are referred to as neutral allosteric modulators or silent allosteric modulators (SAM).

Binding of GPCRs by their endogenous ligand triggers a wide range of effects, starting with a change of the receptor conformation from an inactive to an activated state. This leads to activation of the receptor associated with G-protein and initiates intracellular signaling cascades that mediate cellular responses. Allosteric modulators are thought to stabilize or induce changes in the receptor state causing a shift in their responsiveness to endogenous ligands.

![Figure 1. Negative Allosteric Modulators diminish the signal of a membrane-spanning GPCR. Positive Allosteric Modulators boost it.](image)

Biophysical methods such as surface plasmon resonance (SPR) and NMR that directly measure the GPCR-ligand interaction have been investigated in particular for fragment based drug discovery (FBDD). These approaches and the emerging structural biology technologies could be complementary biological methods, which may be useful for obtaining information on molecular interaction.

The tools used to identify allosteric modulators have benefited from modern molecular biology techniques such as complex cell engineering allowing functional expression of targets of interest. Fluorescence-based assays measuring secondary messengers such as calcium (Ca++) or cyclic adenosine monophosphate (cAMP) are widely used in GPCR drug discovery. The miniaturization of the assay format from 96- to 384- and even 1536- to 3072-well plates and technological developments for assay
readouts have made the screening of large corporate chemical libraries in high throughput mode possible. However, conventional high throughput screening (HTS) assays show a number of limitations. Firstly, most of these assays are endpoint assays, and completely ignore dynamic changes linked to receptor activation. Secondly, most assays include a long incubation and/or revelation time, allowing potentially non-specific binding of candidate compounds to cellular components other than the targeted receptor; and, finally, the signal measured is several steps removed in the signaling cascade, allowing potential up- or down-modulation of every step, leading to a loss of linearity between initial level of activation of receptor and the level of output measured. As a result of these factors, the potential for false positive and negative in such assays is unacceptably high for successful allosteric drug discovery.

At Addex we have developed proprietary novel whole-cell assays that address the above issues. For example, the ProxyLite™ assay was designed to detect allosteric modulatory activity with greater sensitivity than conventional assays. It allows real-time dynamic measures of receptor activation by measuring signals that occur during the receptor activation event. ProxyLite therefore bypasses the downstream screening cascade and reduces potential of false positives and, perhaps more importantly, has revealed false negatives that occur during screening campaigns with less sensitive tools. At the same time, ProxyLite helps to generate robust activity data that facilitates medicinal chemistry, and therefore drives greater efficiency during optimization.

In addition to novel biological tools, Addex has assembled a screening library which is biased towards the chemical features of allosteric ligands with the help of computational chemistry.

**Allosteric modulators of GPCRs: a new challenge in medicinal chemistry**

In the allosteric modulator field, the medicinal chemists have to optimize both potency and efficacy which are two independent important parameters contributing to the pharmacological profile of the allosteric molecule. Modulation of the functional activities of allosteric modulators by minor structural changes has been observed within several series, in particular with mGluR allosteric ligands. Figure 2 shows several examples of “PAM-NAM” switches in the mGluR5 ethynyl pyridine series. In this MPEP series, partial and silent allosteric ligands of mGluR5 have been discovered. These molecules are differentiated by the position of the methyl substituent on the pyridine ring. 5-Methyl-6-(phenylethynyl)pyridine (6MPEP) is a neutral allosteric ligand and it displaces [H]3-Methoxy-PEPy from the MPEP binding site with a Ki = 588 nM while exhibiting no functional response on its own. 2-(3-Methoxyphenyl)ethynyl)-5-methylpyridine (M-5MPEP) is a partial agonist and as such is partially inhibiting the functional response of the mGluR5 receptor to the glutamate. Such a profile, exhibiting activities along the continuum between activation and antagonism has only been described for allosteric modulators. Orthosteric antagonists are essentially binary in their functionalities and cannot function as partial agonists. A continuum of efficacy switching in a related series of ethynyl pyridine carboxamides is also reported (Figure 2).

Figure 3 depicts the 1,2,4-triazadiazole derivative SCH-202676 which modulates the activity of many GPCRs including dopaminergic, adrenergic, enkephalins, and muscarinic receptors. It appears as a promiscuous agent and it supposes that certain allosteric sites are common across distinct receptors. As a result, the identification of such molecules during the early stages of the allosteric drug discovery process, using functional selectivity screening, is crucial. Allosteric modulation of adenosine receptors has been a
Recent developments

Numerous examples of allosteric modulators have been reported in the cited reviews; in this article, we would like to stress some recent discoveries and new avenues in the field.

We have focused this article on small molecules; however, lipidated fragments of intracellular GPCR loops are developed as a novel pharmacological approach of peptide-based therapeutics with long half life. These lipopeptides so called pepducins modulate GPCR activity using an allosteric mechanism; a recent pepducin agonist of the chemokine receptor CXCR4 demonstrates in vivo activity in chemotaxis model.

- The GPCR family A has been an area of intense activity with regard to discovery and characterization of novel allosteric modulators; the recent success of X-ray crystallography of active-state GPCR structure with β2 and adenosine A2a receptors has allowed descriptions of family A GPCR binding sites in the antagonist and agonist conformational states, including putative allosteric pockets. These results may boost structure-based drug discovery approaches of classical GPCR ligands; however, it should be noted that allosteric modulators have added complexity since a molecule could show affinity for its target protein with an efficacy ranging from null (SAM) or partial to full. The rhodopsin-like class A includes fairly large receptors families like the adenosine receptor and the metabotropic cholinergic receptor families. Many structurally distinct adenosine receptor PAM have been reported, however, there are still efforts to be made to identify more potent and subtype-selective molecules.

Recent progress in the discovery of selective allosteric modulators of muscarinic receptors M1 and M4 suggest that allosteric activation of these receptors has procognitive and antipsychotic potential. CYM2503, a selective Galanin R2 receptor PAM having anticonvulsant effect in animal models has been disclosed. This illustrates the potential of PAM as a non-peptidergic approach to address medicinal needs.

- The secretin-like receptor family or class B GPCR family is a family of peptide-binding receptors including corticotropin releasing factor (CRF), glucagon, glucagon-like peptide-1 (GLP-1), and calcitonin receptors, all of which are involved in major physiological functions. Besides CRF1 non-competitive antagonists, few small molecule modulators of this receptor class have been discovered. A sulfonyl quinoxaline derivative allosteric agonist of GLP-1 receptor has been reported by Novo Nordisk. This molecule which modulate GLP-1R response in a peptide–agonist dependent manner has shown in vitro glucose dependent potentiation of insulin secretion in pancreatic islet cells. Such studies highlight the potential of small-molecules modulators of this receptor class and the complexity of the pharmacology.

- Family C GPCRs have been proven to be the most amenable to allosteric modulation, in particular PAM. Glutamate is the major excitatory amino acid neuromediator in the brain; allosteric modulators of mGluR have a very attractive therapeutic potential; many small drug-like molecules have been developed against mGluR1.
mGluR2, mGluR4 and mGluR5. Table 1 illustrates the recent progress leading to clinical candidates in mGluR5 NAM and mGluR2 PAM series.

### The Most Advanced GPCR Allosteric Modulators

Table 1 also shows two allosteric modulators of GPCR, which are currently on the market, cinacalcet and maraviroc, and several clinical leads. The search for follow-up candidates of these two drugs is very competitive. Besides these drugs, several allosteric modulators have reached clinical proof of concept. NBI30775, a CRF1 NAM discovered at Janssen Pharmaceuticals and Neurocrine Biosciences showed efficacy in a major-depression clinical trial. The mGluR5 NAM ADX10059 was the first compound of this class reported to improve the clinical symptoms in GERD patients. This molecule has also shown a significant benefit for the acute treatment of migraine. AFQ056 has shown a positive outcome in

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<th>Target</th>
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patients with Parkinson’s disease levodopa-induced dyskinesia (PD-LID) in two phase IIa clinical trial and one Phase IIb trial when given in combination with levodopa. In addition to AFQ056, the Michael J. Fox Foundation has recognized ADX48621 specifically and mGluR5 NAM generally as a promising approach for the treatment of PD-LID. Addex initiated Phase IIa testing of ADX48621 in PD-LID patients earlier this year. mGluR5 NAM appears also as an interesting therapeutic approach towards Fragile X syndrome which is an inherited cause of mental retardation and autism; a Phase II clinical study with fenobam suggests beneficial effect. Addex also announced earlier this year the entry into a Phase IIa clinical schizophrenia trial for ADX71149, which is a mGluR2 PAM, developed in collaboration with Ortho-McNeil-Janssen Pharmaceuticals, which discovered and developed risperidone (Risperdal), a leading anti-psychotic.

In conclusion, allosteric modulation of GPCRs is benefiting from major advances in the understanding of the functioning, detection and optimization of allosteric modulators. The first marketed GPCR allosteric modulators and the growing number of clinical proof of concept studies using allosteric molecules suggest that PAM and NAM are perceived as differentiated and that they are likely to become more common therapeutic agents.

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Reference list
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