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Commentary

Allosteric approaches to the targeting of G-protein-coupled receptors for novel drug discovery: A critical assessment☆

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ABSTRACT

In recent years, the concept of allosteric modulation of G-protein-coupled receptors (GPCRs) has matured and now represents an increasingly viable approach to drug discovery. This is evident in the fact that allosteric modulators have been reported for every class of GPCR, and several are currently in clinical trials with one drug example approved and launched. The allosteric approach has been highlighted for the potential of identifying highly selective compounds with a minimal propensity to produce adverse effect. While much has been written regarding the promises of this approach, important challenges, caveats, and pitfalls exist that are often overlooked. Therefore, a balanced overview of the field that describes both the promises and the challenges of discovering allosteric modulators of GPCRs as novel drugs is presented.

1. The concept of orthosteric and allosteric sites

Until recently, drug discovery efforts directed at GPCRs have focused on the identification and characterization of ligands acting at orthosteric sites (from the Greek 'ortho' meaning straight, correct, or normal). The orthosteric site is that which binds the known endogenous ligand. A wide range of orthosteric ligands have been developed, including competitive agonists, antagonists, and partial agonists, all of which can be predicted and explained by traditional receptor occupancy theory [1,2]. The increase in the use of functional assays in high throughput screening (HTS) paradigms, along with directed screening designed to overcome selectivity issues at the orthosteric site, have led to the discovery of a number of allosteric modulators of GPCRs. The term allosteric, derived from the Greek 'allo' meaning other, was first used by Changeux, Monod, and co-workers in their seminal work on feedback inhibition of bacterial biosynthetic pathways [3,4]. However, the concept had its roots in earlier work on cooperative binding in a number of systems, including oxygen binding by hemoglobin[5,6], and acetylcholine gating of the nicotinic acetylcholine receptor [7], and in the induced fit theory of Koshland [8]. The same concept has been applied to GPCRs and a number of models have been proposed to quantify the effects of allosteric modulators [9,10].

A full treatment of the relevant pharmacological concepts is beyond the scope of the present commentary and the reader is directed to several recent reviews [11,12]. The basic concepts and modes of action are essential to understanding both the promise and caveats of the allosteric approach. Allosteric modulators of GPCRs may be characterized as altering either ligand affinity, efficacy, or both of these properties. Interestingly, it is also possible for a ligand to bind to an allosteric site without altering receptor function. These so-called silent modulators act as neutral antagonists at the allosteric site. While these silent modulators provide useful research tools, they may also hold therapeutic value, as GPCR allosteric

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modulators become more common in the clinic, to potentially control or reverse the effects of another allosteric modulator. This would be analogous to the use of the benzodiazepine antagonist flumazenil to reverse benzodiazepine-induced sedation and adverse effects [13].

Taken in isolation, the effect of an allosteric ligand on the affinity of the orthosteric ligand can be explained by a model which has come to be known as the allosteric ternary complex model [9]. This model incorporates a cooperativity factor (α) which acts as a multiplier modifying the dissociation constant of the ligand at the orthosteric site. The factor, α may take on a range of positive values which determine how affinity is altered. For α values less than 1, the allosteric ligand produces a decrease in affinity for the orthosteric ligand and is thus termed an allosteric inhibitor. When α is greater than 1, the allosteric ligand produces an increase in affinity for the orthosteric ligand, and thus is termed an allosteric potentiator. If $\alpha = 1$, there is no effect of the allosteric ligand on the affinity for the orthosteric ligand and the compound is termed a neutral allosteric ligand.

In addition to effects on affinity, allosteric ligands can produce changes in the intrinsic efficacy of the receptor–orthosteric ligand complex. An allosteric two state model has been proposed that provides an additional cooperativity factor governing the transition of the receptor between active and inactive states in the presence of an allosteric ligand [10]. Since the model is required to take into account the presence of active receptors that are not ligand-bound, it becomes clear that an allosteric modulator can alter the probability of the receptor signaling in the presence or absence of orthosteric ligand. Essentially, this allows for allosteric ligands that have intrinsic efficacy. Therefore, a full range of positive and negative allosteric modulators as well as allosteric agonists and inverse agonists are theoretically possible, and have been identified.

Of course, in most cases the actual effect of an allosteric ligand is complex and involves changes in both efficacy and affinity. More complex models can be derived, but the core properties outlined above suggest a potentially rich pharmacology that provides an attractive lure for drug discovery.

2. The promise of the allosteric approach

The allosteric approach has been frequently cited as a novel path for drug discovery that will help overcome traditional hurdles of efficacy and side effect liability. Theoretically, a drug discovery campaign directed at producing an allosteric modulator should provide a more tractable approach to selectivity and to obtaining ligands for peptide receptors, antagonist activity that is less dependent upon levels of endogenous ligand, a selectivity for receptors at the relevant site of action that cannot be obtained with orthosteric agonists, and an overall decrease in adverse effects resulting in an enhanced safety margin.

2.1. *Enhanced selectivity*

Most GPCRs are members of large families of closely related receptors that share an endogenous ligand. The muscarinic M_1–M_5 receptors, metabotropic $mGlu_1$–$mGlu_8$ receptors, and the approximately 13 members of the serotonin 5-HT receptor family exemplify this property. While selectivity for a given ligand family is usually achievable (e.g. a compound that interacts with mGlu receptors and not with 5-HT receptors), it is often difficult to obtain selectivity among members of a family. Theoretically, the orthosteric site is under selective pressure and it would be evolutionarily unfavorable for a broad divergence to develop in the amino acid sequence that composes the binding domain. Thus selective orthosteric ligands, which presumably interact with the same amino acid sequences, would tend to be non-selective within a family. Assuming that the allosteric sites on GPCRs are not bound by an endogenous ligand (a concept that is by no means universally accepted) the amino acid sequence composing these sites would be less restricted by selective pressure and therefore, free to vary. As described below, several examples exist where selectivity at the allosteric site allowed for the pharmacological separation of receptors that previously could not be distinguished using orthosteric ligands.

A somewhat related argument has been made to suggest that GPCRs that have peptides as endogenous orthosteric ligands may be more amenable to small molecule discovery efforts directed at producing allosteric ligands. This is based on the concept that any compound acting at the orthosteric site would likely need to bridge distant binding partners engaged by the larger peptide (a problem more evident in the discovery of small molecule agonists). Therefore, an allosteric interaction with the receptor may be able to produce a significant conformational change while acting in a more spatially restricted fashion. It can also be argued that lipid receptors may be more amenable to the allosteric approach as more soluble compounds could be developed that those that would be required to interact at the hydrophobic lipid binding site.

2.2. *Impact of endogenous ligand*

The actions of negative allosteric modulators are non-competitive and therefore, to an extent are not influenced by ambient levels of endogenous orthosteric ligand. This property is particularly promising in terms of establishing more reliable clinical dosing. If a dose of a negative allosteric inhibitor produces 50% occupancy at the allosteric site *in vivo*, it is expected that this will occur independent of changes in endogenous orthosteric ligand due to individual variability, disease state, etc. However, this potential benefit must be tempered by the understanding that the allosteric ligand is producing its negative modulation by either decreasing the affinity of orthosteric ligand, or by decreasing efficacy of the active receptor within the bounds set by the cooperativity factors discussed above. Therefore, these effects are saturable and can be overcome by the orthosteric ligand.

2.3. *State dependency*

An interesting application of the allosteric approach is the development of a state-dependent drug. This concept is based on the fact that a positive allosteric modulator will not produce a global activation of the targeted receptor, but will

rather modulate receptors present at sites where the orthosteric ligand is available. Theoretically this will limit the adverse effects that might be produced by an orthosteric agonist, and will furthermore avoid issues of receptor desensitization that commonly frustrate agonist-based approaches. These two issues, adverse effect and receptor desensitization, largely account for the fact that there are few direct agonists of any receptor that are clinically useful drugs for chronic administration. In contrast, there are many examples of compounds that enhance the effects of endogenous transmitters by increasing their extrasynaptic levels. Examples include the 5HT and norepinephrine reuptake inhibitors, inhibitors of catabolic enzymes, such as monoamine oxidase and acetyl cholinesterase, and the use of selective allosteric potentiators of specific receptor subtypes. The classic example of the latter is the use of benzodiazepines as allosteric potentiators of $GABA_A$ ligand-gated ion channel that provide an effective and safe approach to treatment of anxiety disorders that would not be possible with orthosteric GABA receptor agonists [14]. It is therefore hoped that positive allosteric modulators of GPCRs may similarly allow for the development of new drugs that are both selective and safe.

2.4. *Functional selectivity*

All of the potential benefits of the allosteric approach discussed above are consistent with basic pharmacological models and the assumption that the allosteric effect is due to a simple modulation of affinity or intrinsic efficacy. However, this ignores the interesting possibility that allosteric ligands may be able to take advantage of functional selectivity. The concept of functional selectivity (also termed ligand-directed trafficking or biased agonism) has recently been gaining acceptance as it has been demonstrated that a number of ligands for a variety of GPCRs exhibit diverse signaling properties while acting at a single receptor (for review, see ref. [15]). This suggests that the basic concept of intrinsic efficacy is, in some circumstances, an over simplification and in fact orthosteric ligand–receptor complexes may exhibit different intrinsic efficacy for different signaling pathways (see for example, ref. [16]). This conceptually opens up a whole area of potentially novel orthosteric ligand effects. For example, a compound could theoretically act as an agonist along one signaling pathway and a neutral antagonist at another. It is therefore not surprising that allosteric ligands have also been demonstrated to elicit functionally selective responses. While this is clearly a new and emerging concept in GPCR pharmacology, it has the potential to provide a remarkable precision in the targeting of new drugs, and the additional benefits of the allosteric approach applied to this effort may prove fruitful.

3. Examples of allosteric modulators of GPCRs

In recent years, allosteric modulators have been discovered that act at the family A, B, and C GPCRs [12]. While many of these compounds remain early stage discovery tools, a few have advanced to clinical studies. Below, we briefly review a few key compounds from each of the GPCR families in order to demonstrate the overall applicability of the allosteric approach to GPCR-targeted drug discovery.

3.1. *Class A GPCRs*

Muscarinic receptors, and in particular the M_1 muscarinic receptor, have been the subject of intense research focus and have been touted as holding promise in a number of therapeutic areas, including cognitive enhancement in Alzheimer's disease and schizophrenia. Several companies have attempted to develop M_1-selective agonists [17,18]. While these programs produced potent compounds that improved cognition, both in pre-clinical animal models [18] and in the clinic [19], these compounds proved to be non-selective and produced significant dose-limiting side effects, including nausea, vomiting, and diarrhea. Recent studies in knockout mice suggest that these side effects are likely due to activation of the M_2 and M_3 muscarinic receptor subtypes [20]. While this suggests that a truly selective M_1 agonist would have significant therapeutic potential, the historical difficulties experienced with developing selective orthosteric site muscarinic ligands makes the development of such drugs unlikely. However, there is a body of evidence suggesting that the M_1 muscarinic receptor is amenable to allosteric modulation. The first evidence for this was the finding that brucine, a naturally occurring alkaloid, produces a selective allosteric enhancement of acetylcholine activation of the M_1 receptor [21]. The first M_1-selective allosteric ligand to arise from a drug discovery effort was AC-42 (Fig. 1), a compound with unprecedented selectivity for the M_1 muscarinic receptor over other muscarinic subtypes [22]. While AC-42 and the structural analog AC-260584 (Fig. 1) function as agonists, these compounds interact with an allosteric site (termed the ectopic site) unrelated to the orthosteric binding site [22,23]. Recently, the mode of action of AC-42 has been further characterized using a combination of functional, equilibrium binding, and dissociation kinetic assays [24]. These studies have confirmed that AC-42 is, in fact, an allosteric agonist by providing strong evidence for interaction at a site distinct from the orthosteric site. Interestingly, the *N*-desmethyl metabolite of clozapine also acts as a selective allosteric agonist at the M_1 muscarinic receptor [25]. Clinical trials with *N*-desmethylclozapine as ACP-104 (Fig. 1) are ongoing in schizophrenia based on the ability of the compound to selectively activate M_1 muscarinic receptors and findings that *N*-desmethylclozapine levels correlated with outcome in clozapine-treated schizophrenic patients [26].

Allosteric modulators have been reported for a number of other Class A GPCRs, including those for chemokines. Chemokines are a class of small proteins that regulate the trafficking of leukocyte and other biological processes, including cell growth, angiogenesis, and hematopoiesis [27]. Disregulation of chemokine networks has been implicated in a variety of diseases, including rheumatoid arthritis, asthma, and chronic pulmonary obstructive disease [28,29]. The allosteric approach has been successfully employed to develop repertaxin (Fig. 1), an allosteric inhibitor of CCRX1/2, for the indication of ischemia-reperfusion injury after lung transplantation [30]. This compound is in phase II clinical trials. A striking example of the potential power of the allosteric approach is the discovery of GW873140 (Fig. 1), a CCR5 receptor negative allosteric

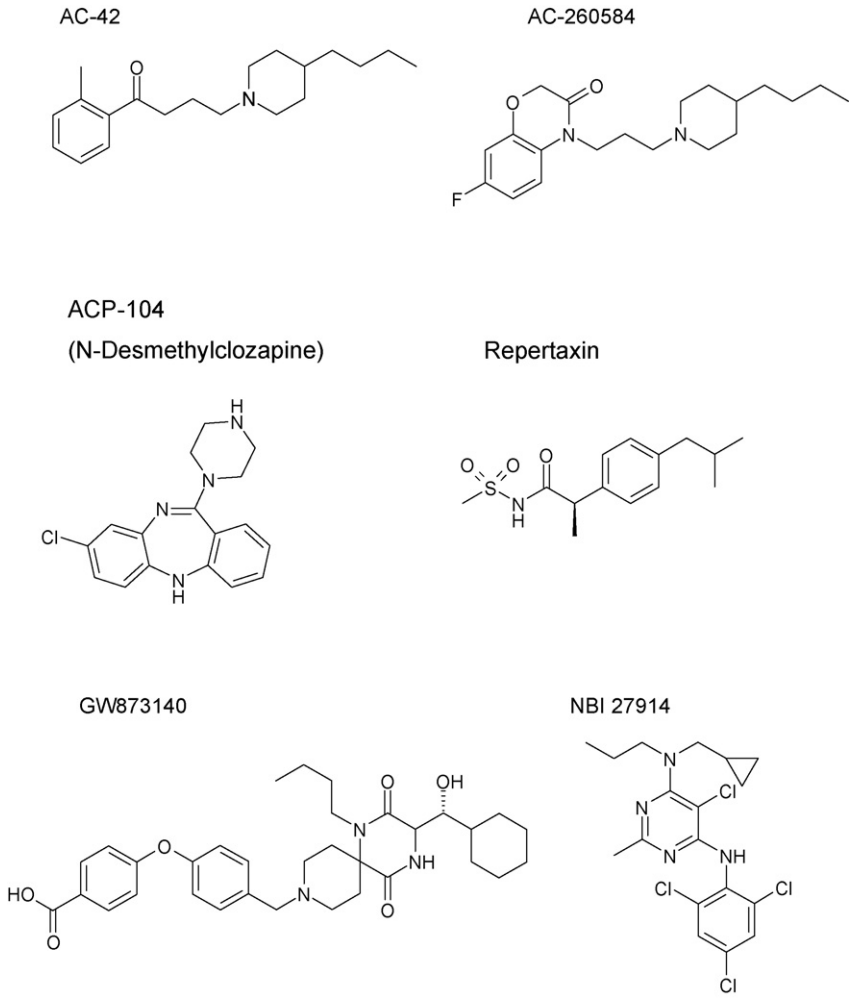

Fig. 1 – Chemical structure of allosteric modulators of Class A and Class B GPCRs described in the text.

modulator. This compound exhibits functional selectivity in that it produces a non-competitive antagonism of MIP-1α binding and only minimally antagonizes binding of RANTES [31] allowing GW873140 to block HIV entry into the cell while allowing physiological chemokine signaling [32].

3.2. *Class B GPCRs*

Class B GPCRs include a number of peptide receptors, such as corticotropin releasing factor (CRF), vasoactive intestinal polypeptide, and pituitary adenylyl cyclase-activating peptide (PACAP) receptors. This class has been particularly recalcitrant to small molecule drug discovery directed at developing orthosteric ligands. However, Class B GPCRs are amenable to allosteric approaches which have been successful in developing small molecule allosteric antagonists of the CRF_1 receptor. CRF-1 receptor allosteric antagonists are under study for the potential treatment of anxiety, depression, and irritable bowel syndrome. A series of tetraazaacenaphthylenes have shown promising results in pre-clinical models [33]. Evidence for the allosteric nature of these effects was found in studies employing the CRF_1 antagonist NBI 27914 (Fig. 1). Site-directed mutagenesis studies showed that NBI 27914 bound to key residues in the transmembrane regions of the CRF_1 receptor [34], these residues are non-overlapping with the orthosteric binding site [35,36].

While there have been relatively few studies on the Class B GPCRs, these findings suggest that the allosteric approach presents a viable path forward for discovering small molecules directed against these receptors.

3.3. *Class C GPCRS*

Class C GPCRs have not only proved amenable to allosteric potentiation, but hold the distinction of being the first class of GPCRs for which an allosteric modulator has been approved and marketed as a novel therapeutic. Cinacalcet (NPS-1493; Fig. 2) is a positive allosteric modulator of the calcium-sensing receptor (CaR) [37]. Representing a first in class oral calcimimetic for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease (CKD) on dialysis, and for hypercalcemia and primary hyperparathyroidism in patients with parathyroid carcinoma or end stage renal disease [38]. Cinacalcet acts as a calcimimetic by increasing CaR sensitivity to activation by extracellular calcium, resulting in a leftward shift in the concentration–response curves for extracellular calcium leading to a decrease in parathyroid hormone production and a subsequent decrease in serum calcium.

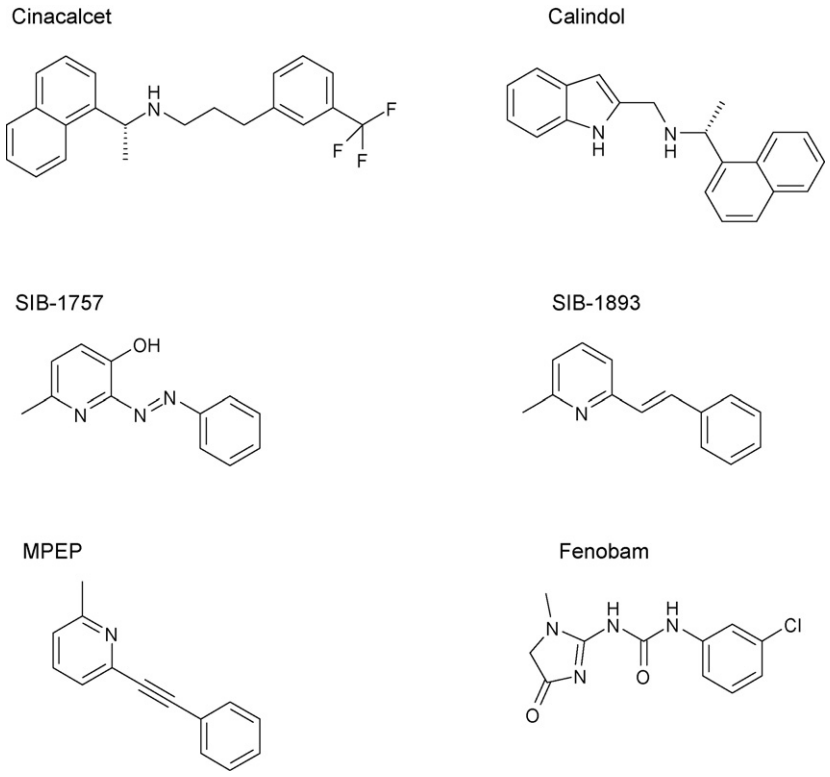

Fig. 2 – Chemical structure of allosteric modulators of Class C GPCRs described in the text.

Cinacalcet interacts with the extracellular loops on the CaR, and not with the large N-terminal extracellular orthosteric binding domain that is unique to the Class C GPCRs [39]. Removal of the extracellular domain from the CaR converts the positive allosteric modulator calindol (Fig. 2) into a direct acting agonist that exhibits synergy with additional calcium binding sites that are apparently within the remaining seven transmembrane domains [40]. This highlights not only interactions evident between the allosteric and orthosteric sites on these receptors, even over large distances on the receptor, but also the inherent complexity in these systems that must be considered in drug discovery efforts.

The allosteric approach has also met with success when applied to the Class C GPCRs, the metabotropic glutamate (mGlu) receptors. The mGlu receptors are a family of eight GPCRs that fall into three distinct groups; group I ($mGlu_1$ and $mGlu_5$) group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$ and $mGlu_{6–8}$) [41]. While selective orthosteric ligands for each group have been identified, the allosteric approach has had significant impact on the field by allowing the development of highly selective pharmacological tools for individual mGlu receptors within the groups [42,43]. Significant efforts have been directed at developing selective positive allosteric modulators of the $mGlu_2$ receptor [44–46] for potential indications of schizophrenia and anxiety. However, the most advanced efforts have focused on the development of selective allosteric inhibitors of the $mGlu_5$ receptor.

The first negative allosteric modulators of the $mGlu_5$ receptor were SIB-1757 and SIB-1893 (Fig. 2) that exhibited both selectivity for the $mGlu_5$ receptor and nanomolar potency [42]. Subsequent structural analogs include MPEP (Fig. 2) [47] and MTEP [48] that have improved potency, selectivity, and brain penetrance. MPEP is anxiolytic in a number of pre-clinical models (for review, see ref. [49]). These pre-clinical findings may be clinically relevant with the finding that the atypical anxiolytic, fenobam (Fig. 2) is a selective and potent allosteric antagonist of the $mGlu_5$ receptor [50] showing clinical efficacy as an anxiolytic in a double blind placebo controlled trial [51] at a time when the molecular target was unknown. While there has been some concern raised by pre-clinical studies suggesting that inhibition of the $mGlu_5$ receptor may produce cognitive impairment [52] the evidence that allosteric modulators of this receptor can exhibit functional selectivity suggest that it may be possible to generate compounds selective for a particular signaling pathway [53].

In summary, all of the three main classes of GPCRs have proven amenable to allosteric modulation, and these efforts are beginning to have a visible impact on clinical treatment with several compounds in clinical trials and one approved and marketed. Based on this apparent success, and the potential benefits outlined above, the allosteric approach has rapidly become a Zeitgeist presenting a tempting path for those engaged in drug discovery research.

4. Challenges, caveats, and potential pitfalls

4.1. *Practical aspects of identification of new chemical entities*

Many of the properties of allosteric ligands that provide potential therapeutic advantages also create added challenges

in pre-clinical drug discovery. Since the modulators that have been discovered demonstrate a wide array of effects on the affinity and efficacy of endogenous ligands, the greatest challenges lie in determining the desired properties of this class of ligands and developing assays to appropriately quantify a variety of possible modulatory effects.

Although the establishment of functional assays as the preferred approach to HTS increases the likelihood of identifying allosteric modulators, added care must be taken in verifying and characterizing these ligands. Effects seen in functional, especially whole cell, assays may reflect artifactual changes in the assay, membrane integrity or in cell viability. Traditionally, equilibrium radioligand binding studies are used to complement functional assays and to verify ligand interaction directly with the GPCR of interest. Labeled modulators can be used to verify and quantitate interaction with known modulatory sites [54,55], however, such tools are not yet available for most receptors. Thus, mutagenesis experiments must be performed to establish sites of interaction with the receptor in the absence of modulators with known binding sites. Alternatively, radiolabeling of lead compounds becomes crucial to establish direct interaction with receptor complexes in these cases. However, establishing a radioligand binding assay prior to understanding structure–activity relationships presents a risk as multiple allosteric binding sites may exist.

In many cases, allosteric modulators can be characterized by measuring changes in the kinetics of binding of radiolabeled orthosteric ligands, most commonly by changes in dissociation rate. However, allosteric modulators do not necessarily alter binding of orthosteric ligands [56]. Furthermore, the effects on the binding of the orthosteric ligand may be ligand-dependent. The example discussed above of functional selectivity by the CCR5 receptor negative allosteric modulator GW873140 demonstrates the need for careful design of assays to characterize allosteric modulators. In other cases, the effects of allosteric modulators on binding of orthosteric ligands can be misleading. In the case of allosteric modulators of the CB1 receptor, compounds have been identified that increase equilibrium binding and slow dissociation of the agonist [^{3}H]CP 55,940, suggesting that these compounds would act as positive allosteric modulators, but these compounds conversely function as non-competitive antagonists in multiple functional assays in cells and tissues [57]. As described above, the balance between the modulation of efficacy and affinity determines the overall effect of the allosteric ligand. Therefore, multiple parameters must be tracked by characterizing compounds across a panel of assays and guiding medicinal chemistry efforts using measures of affinity, efficacy, and cooperativity factors.

The increased likelihood of identifying receptor subtype-selective ligands due to the lack of evolutionary selection of allosteric sites provides an excellent opportunity for drug discovery. However, this potential advantage also results in a greater likelihood that the allosteric site will be further divergent in different species than will the orthosteric site. Since drug discovery relies on testing compounds in species other than man (usually rodent) this could be problematic. For example, a non-peptide, allosteric antagonist of the glucagon-like peptide 1 (GLP-1) receptor, T-0632 [58] binds with 100-fold lower affinity at the recombinant rat relative to human GLP-1 receptor. While the species homologs are 91% identical in amino acid sequence, this difference may reflect interaction with a single tryptophan residue in the allosteric binding site of the human receptor that is absent in the rat receptor. This would suggest difficulty in optimizing binding affinity to both receptors. On the other hand, a number of endogenous allosteric ligands have been identified [12] that show activity in multiple species suggesting there may be more conservation of these binding sites across species than originally predicted. While this may decrease the concern over identifying compounds that are species-selective, it may actually suggest that the allosteric sites are also evolutionarily conserved and may therefore not provide as much leverage in producing highly selective compounds as expected. Clearly the potential issue of species-selectivity should be recognized as an increased risk and addressed by early testing of compounds at receptors from both human and the pre-clinical species of interest.

Characterization of compounds in drug discovery paradigms relies heavily on recombinantly expressed receptor systems. Use of these systems to characterize orthosteric ligands brings with it the risk that factors known to produce allosteric interactions with the receptors in their own right, such as G-protein coupling and the complement of accessory proteins, are almost certainly different in the recombinant cell line than those found in the *in vivo* context. Therefore, the activities of an allosteric ligand, which may be even more sensitive to changes in the receptor conformational states than those of orthosteric ligands, may differ dramatically between *in vitro* and *in vivo* or native *in vitro* assays, or may even be altered by subtle differences between orthosteric ligands. For example, the activity of a competitive antagonist should not depend on the nature of the orthosteric agonist. However, the effects of allosteric modulators have been shown to be exquisitely sensitive to different agonists (see for example, ref. [59]). This makes use of the endogenous agonist critical for characterizing allosteric ligands even in cases where the chemical properties of the endogenous agonist (for example, stickiness, instability, or insolubility) can make them difficult to work with in screening situations. Potential issues also exist with varying receptor/G-protein stoichiometry as is the case for modulators of the M_2 muscarinic receptor [60]. In this study, the activity of alcuronium, a positive allosteric modulator of muscarinic receptors, was sensitive to receptor/G-protein ratios in artificial lipid membranes incorporating purified proteins demonstrating that the effects often seen with differing receptor expression levels in cell lines could be recreated in this more simple system and do not require other changes to occur in the cell. Allosteric ligands must therefore be tested under conditions closely mirroring those of the targeted *in vivo* system necessitating the use of native systems to confirm results from recombinant assays.

In short, the issues and risks of identifying and verifying allosteric ligands are similar to those familiar to investigators working with orthosteric ligands. However, it is important to understand the areas where these risks are amplified by the nature of allosteric interactions in order to develop the most appropriate assay systems. While the potential advantages to allosteric ligands are becoming clearer over time, this novel

class of new chemical entities requires additional efforts in the early drug discovery stages. Understanding the mechanisms of allosterism will allow investigators to design appropriate assay systems and identify the most useful agents for therapeutic advantage.

4.2. *Unanswered questions concerning efficacy*

Several of the potential benefits of the allosteric approach described above suggest that allosteric modulators will provide enhanced efficacy while limiting the occurrence of adverse effects. While this would certainly be beneficial, it must be recognized that there are still many unknowns regarding the underlying pathophysiology of disease states that cast uncertainties on the therapeutic potential of allosteric modulators.

In the simplest case, an allosteric modulator will alter affinity or efficacy. It is difficult to predict *a priori* which of these parameters is most relevant for a given disease. Furthermore, the cooperativity factors impose a limit or ceiling on the allosteric effects. While this may be an advantage in terms of limiting side effects, it is difficult to predict where that ceiling should be set. In cases where there is a clearly defined disease process, these caveats may be less of a concern. However, in complex disorders with pre-clinical models of questionable construct validity an allosteric modulator may be moved forward only to fail in late stage clinical trials because the ceiling was set too low.

The state dependence of an allosteric modulator has also been touted for its potential to limit adverse effects. While this may be the case, there are scenarios where this may actually be a limitation. For example, the development of an allosteric potentiator presumes the presence of the orthosteric ligand at significant concentrations. An obvious example of when this would become problematic is the case of neurodegenerative disease where the loss of neurons leads to a decrease in the availability of the endogenous agonist. In this case, the positive allosteric modulator may be able to provide efficacy early on in the disease which may be lost as the degeneration progresses. In the case of negative allosteric modulators, it is assumed that a non-competitive interaction will be superior to a competitive antagonist. However, one of the benefits of a competitive ligand may be that its effects can be overcome by increasing concentrations of the endogenous orthosteric ligand. Therefore, a competitive antagonist may shift the dynamic range over which the receptor is activated. Presumably, the effect of an allosteric inhibitor in this case would be very different. Once again, a lack of understanding of the disease pathology could lead to a mistake that would only become evident in a costly clinical trial.

Finally, the promise of functionally selective allosteric modulators providing highly selective drugs that act on a subset of signaling pathways is currently just a promise. There is a great deal that needs to be understood about the relevant roles of the various signaling systems in both the normal and disease states before a rational approach can take advantage of this property. However, the potential for selectivity also remains a significant caveat that must be kept in mind. Since it is possible to develop allosteric ligands that favor one signaling pathway over another, or one orthosteric ligand over another, there is a constant risk that a compound may be developed that unknowingly has these properties. Without knowledge of which pathways are relevant to the disease pathophysiology, it is impossible to predict which are the most critical to track in pre-clinical studies.

5. Conclusions

When critically assessed, the allosteric approach to drug discovery presents clear advantages that have stimulated efforts over the past decade. Based on the success of this

Table 1 – Table summarizes the potential benefits as well at the challenges, caveats, and pitfalls associated with the allosteric approach to drug discovery for GPCR targets

	Potential benefit	Challenges, caveats, and pitfalls
Selectivity	Lack of evolutionary conservation of binding site allows for greater degree of selectivity Allosteric sites more spatially restricted than peptide orthosteric sites	Requires functional assay for high throughput screen and binding assay for compound characterization Need to track multiple parameters (affinity, efficacy, cooperativity factors) Potential for high degree of species selectivity
Lack of effect of endogenous ligand on negative allosteric modulator	Allosteric approach may produce more consistent and predictable dosing May limit adverse effect	Not clear where to set ceiling on effects May be unknown advantages to competitive antagonism
State dependency of positive allosteric modulator	Effects limited to active circuits and proportional to endogenous ligand concentration Less propensity for desensitization Lack of global activation of receptor limits adverse effect	Makes assumption regarding levels of endogenous ligand and correlation of those levels with disease state
Functional selectivity	More diverse and subtle modulation of receptor conformation may lead to a greater degree of functional selectivity	May be difficult to generalize recombinant results to *in vivo* context May be an unknown property of a compound with unrecognized clinical significance

approach across all families of GPCRs, it is expected that it will continue to impact positively on the discovery of new chemical entities and the development of novel therapeutics. However, there are significant challenges, critical caveats, and potential pitfalls that must be kept in mind in pursuing allosteric modulators (Table 1). The technological advancements that have allowed for high throughput functional screening have essentially opened up a new area of GPCR pharmacology. However, the drug discovery process cannot be undertaken as a technological exercise. While the general importance of driving drug discovery from the standpoint of therapeutic area biology with an emphasis on native systems and *in vivo* models can never be over emphasized, the caveats outlined above make it clear that the allosteric approach requires particular attention to careful experimental design, high quality science, and an emphasis on understanding the underlying biology of the potential indication.

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