WHITE PAPER

GPCRs

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THE NEW WORLD OF GPCR ALLOSTERIC MODULATION: ANOTHER SHOT ON GOAL

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Although historically GPCRs have been a rich source of new drug molecules, the discovery of unique drug types for this target class has waned in the last 3 decades. In the same time frame, the emergence of functional screening and the appreciation of the allosteric nature of GPCRs has revitalized the field and led to an explosion of activity that has transformed GPCR discovery. Thus allosteric screening and development are seen as another shot on goal for many mined out and/or intractable GPCR targets. It is useful to consider the particular mechanistic interactions of allosteric ligands with receptors, the unique molecules that emerge from these interactions, and finally the key role of pharmacological assays in their discovery and development.

The Emergence of Receptor Allostery in Pharmacology

The emergence of allostery in pharmacology supports the notion that whether a discovery program seeks an allosteric ligand or not, it is increasingly likely that allosteric hits and leads will emerge from functional screening campaigns. Thus it is incumbent upon pharmacologists to at least be aware of how to study these unique molecules and develop their full potential in discovery programs.

The study of allosterism was pioneered with enzymes, but the pursuit of this mechanism for receptors has lagged behind. One important reason for this is the historical reliance of high-throughput screening on basically orthosteric assays (binding). In all probability, many allosteric ligands were not detected leading to a paucity of these molecules for development. The advent of functional assay screening, where allosteric effects are readily seen, has led to a precipitous increase in the publication of papers on receptor allosterism beginning in 1992 and continuing through to the present day. With this trend has come a resurgence of interest in allosteric receptor ligands (and its offshoot, biased receptor signaling) and an increase in the number of molecules available to study allosteric receptor mechanisms.

Allosteric Mechanisms and New Drug Discovery

GPCRs are nature's prototypical allosteric protein (everything the receptor does is allosteric) since these proteins simply act as energy conduits for extracellular molecules and intracellular signaling proteins. The molecular dynamics of the receptor suggests that they can form a multitude of active and inactive conformations through selective stabilization. These concepts also support the notion that efficacy and affinity are thermodynamically linked since binding is not a passive process. Therefore, the pursuit of possible signaling effects (pluridimensional efficacy[1]) for all molecules detected in a screening assay could lead to unique therapeutic profiles. In this regard, the assay assumes a key role in GPCR drug discovery.

What Makes Allosteric Molecules So Special?

There are at least three unique features of allosteric ligand-receptor interactions that lead to potentially valuable therapeutic behaviors[2].

- Allosterism can change the very nature of receptors (efficacy, functional signaling)
- The fact that allosteric molecules bind to separate sites on the receptor allows them to modulate or potentiate receptor effects (re-set physiology)
- Allosteric effects are probe dependent, allowing precise discrimination between endogenous molecules and signaling proteins (effects can be molecule-specific)

Allosteric Modulation Can Create a New Receptor

Allosteric modulators have the unique capability of allowing the natural endogenous agonist to concomitantly bind to the receptor and, therefore, they can potentiate physiological response. An active research area in the pharmacology of drug discovery is the search for Positive Allosteric Modulators (PAMs) to revitalize failing physiological systems in disease. PAMs possess unique advantages over orthosteric agonists in that the systems affected respond only when activated. For example, a current therapy for diabetes employs GLP-1 agonists that potentiate the release of insulin. However, a limitation of this approach is that the constant activation of the GLP-1 receptors with an agonist causes intractable nausea in patients. In contrast, a GLP-1 PAM potentiates GLP-1 enhancement of insulin release in diabetes only when the system is activated, such as when the patient has a meal but otherwise produces no effect. This intermittent activation will eliminate the current GLP-1 based nausea seen with agonist therapies for diabetes. The potentiation of muscarinic response by the PAM BQCA is illustrated with the Eurofins Discovery, M1 receptor β-arrestin recruitment assay shown in Figure 1. Patterns of concentration-response curves such as these can be fit to the functional allosteric model[3-5] to yield universal parameters that characterize allosteric function in a system-independent manner.

Such analyses yield parameters for modification of endogenous agonist affinity (through a cooperativity term α) and efficacy (through a cooperativity term β) to guide medicinal chemistry structure-activity studies. For the data shown in Figure 1, the cooperativity factor for BQCA effect is 24 indicating that BQCA will produce a 24-fold potentiation of acetylcholine β-arrestin response in all systems.

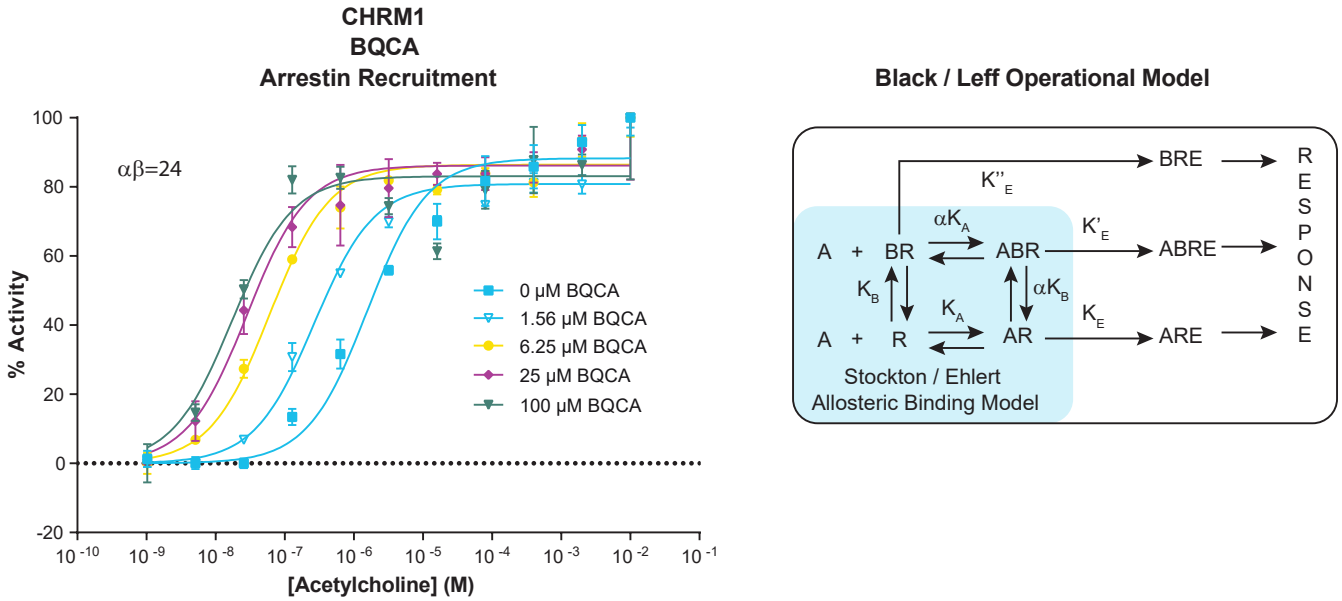


Figure 1. Concentration-response curves to acetylcholine for the receptor recruitment of β-arrestin in the absence and presence of a range of concentrations of the PAM BQCA. Concentration-dependent shifts of the CR curves to the left indicate allosteric potentiation of acetylcholine response. Fitting these curves to the functional allosteric model, schematically shown on the right, yields universal parameters quantifying the allosteric effect in terms of the co-operative effects on acetylcholine binding and efficacy. Data from Eurofins Discovery.

Allosteric Modulators Re-Set Target Physiology

Another feature of allosteric molecules is that they can produce a redefinition of target responsiveness including complete inhibition, reduced sensitivity, increased sensitivity, and full activation. Figure 2 shows the effect of the negative allosteric modulator (NAM) UCB35625 on the binding of the chemokine CCL3. It can be seen that the receptor can still bind CCL3, but with a 2-fold reduced affinity; Such fine-tuning of responsiveness can be useful therapeutically.

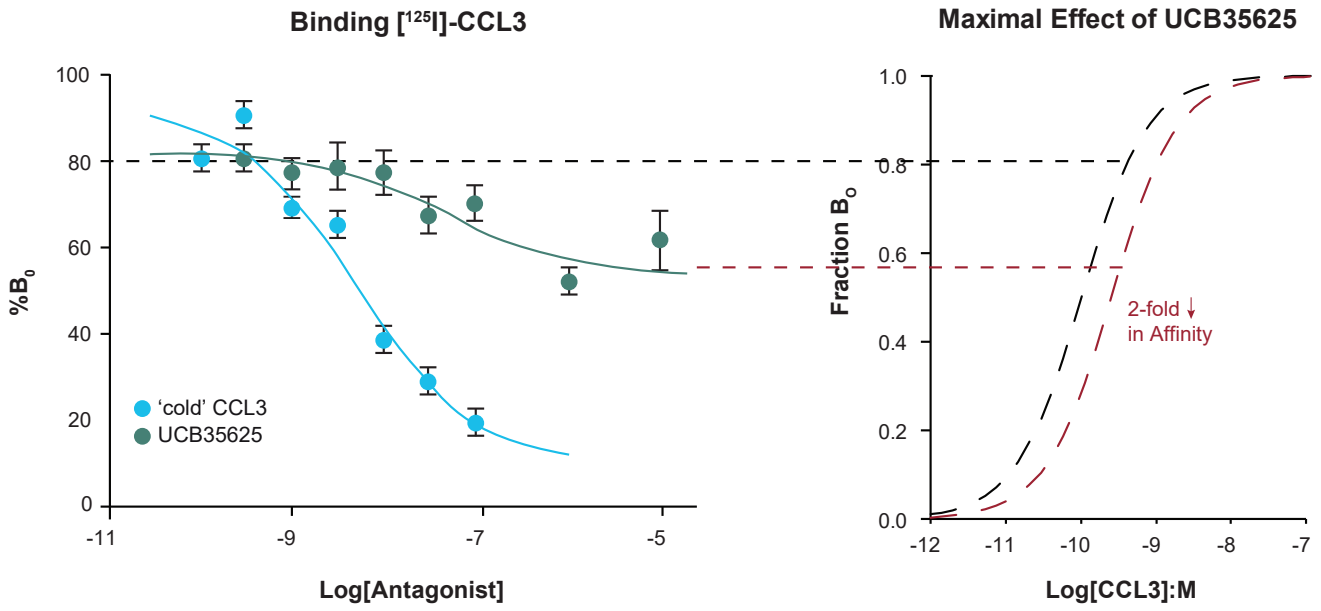
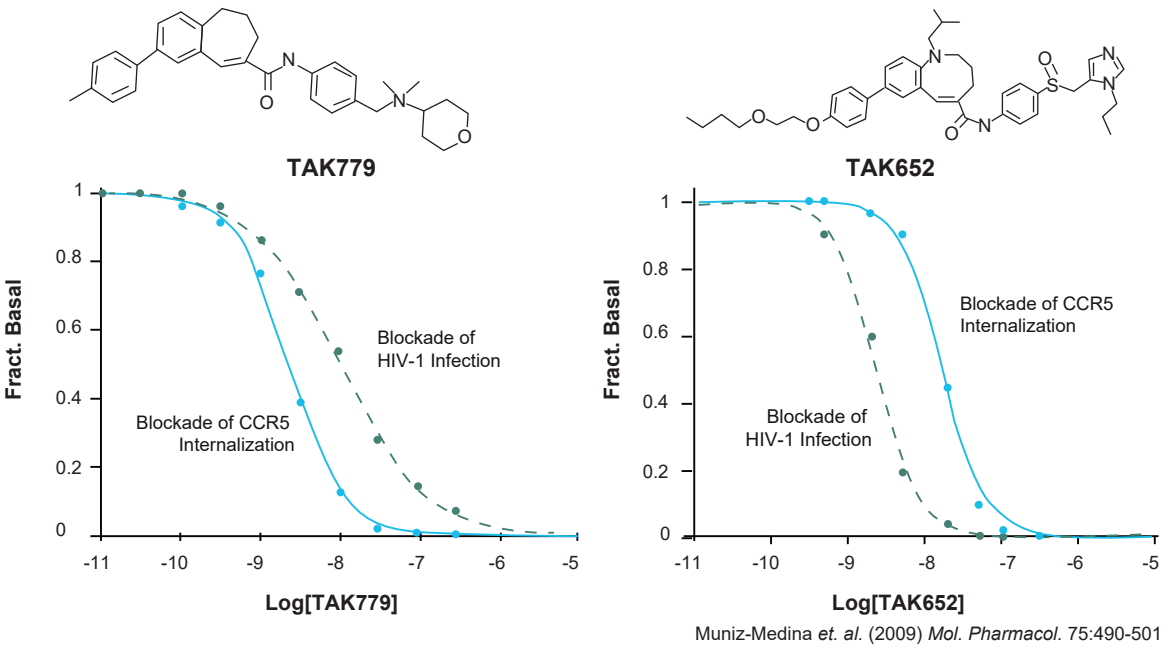


Figure 2. Displacement binding of [^{125}I]-CCL3 by non-radioactive CCL3 (blue circles) and the allosteric NAM UCB35625 (green circles). It can be seen that the maximal concentrations of UCB35625 do not displace bound [^{125}I]-CCL3 but rather re-set the receptor affinity for the radioligand by a factor of 2. Data are redrawn from [6].

Allosteric Modulators Practice Probe-Dependence

Finally, allosterism is probe dependent, that is, an allosteric molecule can produce an effect for one probe of the receptor (i.e. agonist, radioligand), but have no effect on another (a different agonist, radioligand, etc.). This can be extremely valuable therapeutically as shown in Figure 3 for the allosteric HIV-1 entry inhibitors TAK779 and TAK652 in the prevention of HIV-1 infection and AIDS. Specifically, these molecules demonstrate probe dependence in the form of a 10-fold differential activity for blocking HIV-1 vs. the blockade of a beneficial effect for AIDS patients (CCL3-L1-induced CCR5 receptor internalization found to be correlated with increased survival[7]). It can be seen that while TAK779 is 10-fold more potent at blocking CCR5 internalization over HIV-1 infection, TAK652 reverses this profile to yield a beneficial 10-fold margin for blockade of HIV-1 infection while sparing the beneficial CCL3L1-induced internalization effect[8]. In general, the judicious application of different target probes in assays can uncover such therapeutically relevant probe dependence.

In general, allosteric modulators can re-format receptor sensitivity (including the revitalization of failing systems) and alter responses to agonists. They can also make targets 'smart' in that they will discern different activators and respond to some but not others. These effects re-define the drug discovery playing field.



Muniz-Medina *et. al.* (2009) *Mol. Pharmacol.* 75:490-501

Figure 3. Inhibition of HIV-1 infection of HOS cells (green circles, dotted line curves) and blockade of CCL3L1-induced CCR5 internalization (blue circles, solid line curves). Data are shown for two allosteric inhibitors of HIV-1 entry, TAK779 and TAK652- redrawn from [8].

New Players as Drug Target Molecules in Pharmacological Therapy

Allosteric mechanisms have spawned new types of molecules for therapeutic application. These new players can generally be classified as PAMs that increase an endogenous agonist response and thus can be used to augment failing physiology in disease. NAMs (Negative Allosteric Modulators) decrease endogenous agonism (and are basically antagonists, but with some very special qualities that differentiate them from standard competitive blockers. In addition, a special type of PAM that augments receptor-signaling protein interaction (and thus is an agonist) practices probe dependence for cytosolic signaling proteins; these are a new and important class of ligand referred to as the biased agonist. Considering two probes of the receptor as being two points of interaction with different cytosolic signaling proteins (i.e. G protein, and β-arrestin), biased agonists differentially activate one probe at the expense of another to produce a biased cell signal. These types of effects can be of enormous value in that beneficial signals can be emphasized (i.e. β-arrestin PTH response for osteoporosis[9]), deleterious signals can be de-emphasized (respiratory depression and addiction for opioids[10]) and de-emphasized with blockade of natural activation of the same pathway (G_q protein-mediated vasoconstriction by angiotensin in heart failure[11]). In addition, the editing of pleiotropic signaling may allow pursuit of previously forbidden drug targets for therapeutic advantage (i.e. κ-opioid receptors[12]).

Finally, the application of functional and binding assays may be used to identify a unique new class of antagonist, namely the PAM-Antagonist. These are a special subset of NAMs that actually become more potent upon activation of the functional system by the agonist allowing them to seek and destroy signaling agonist-bound receptors[13]. PAM-Antagonists can reverse persistent pathological signaling (e.g. endothelin-based pre-eclampsia) and have extraordinarily high target coverage properties *in vivo* (and long $t_{1/2}$ for clearance) due to the cooperative binding with endogenous hormones and neurotransmitters as seen for the 5-HT_3 receptor antiemetic palonosetron[14]). Figure 4 shows Eurofins Discovery data used to characterize Org27569, a PAM Antagonist for the cannabinoid CB1 receptor[5]. The key to detecting these unique profiles is the orthogonal application of functional and binding assays.

Specifically, this behavior emanates from a positive α cooperativity to increase affinity and a fractional β activity to decrease efficacy. Thus, the presence of the agonist increases the affinity of the receptor for Org27659 that then becomes incapable of signaling once Org27659 is bound.

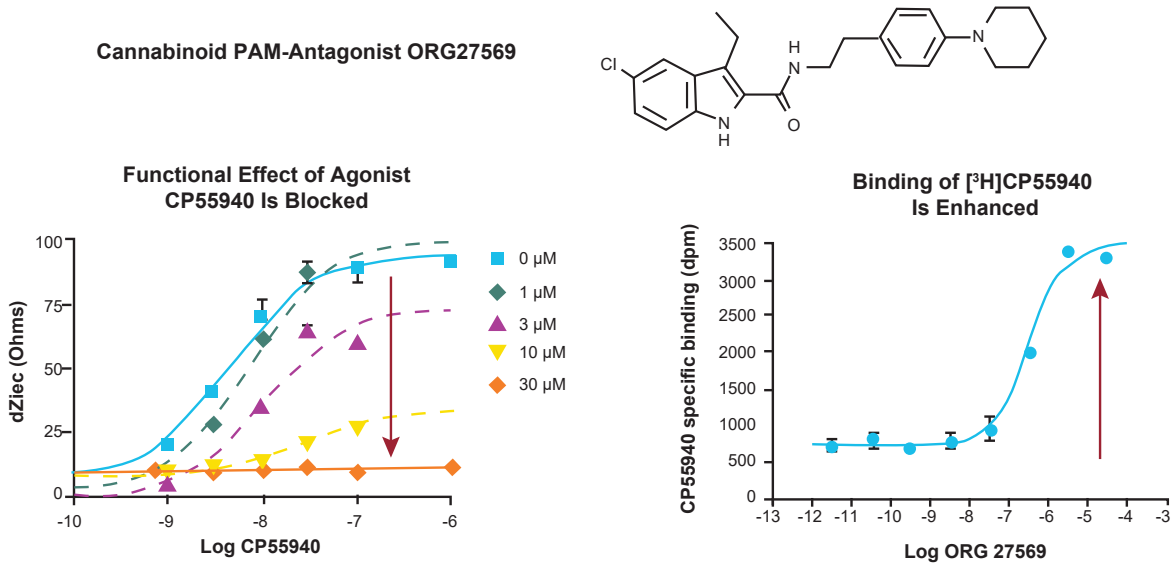


Figure 4. Opposing effects of the cannabinoid CB1 receptor PAM-Antagonist Org27659 on function (non-competitive blockade) and binding (increased agonist binding). Data from Eurofins Discovery.

Pharmacological Assays as the Window into Pluridimensional Efficacy

The key to the discovery of ligand diversity is to have multiple views into pharmacological molecular activity. Classification of molecules into simple bins of agonist and antagonist has ceased to be relevant as molecules can be agonists for some signaling pathways and antagonists in others[15]. A more sophisticated classification system to characterize efficacy must be employed. For example, the multiple testing of ligand functional response yields textured patterns of ligand activity such as the display of μ-opioid signaling activity determined by clustering of data for sixteen opioid agonists in six functional assays shown in Figure 5[16]. The hope is that such detailed profiling of new molecules will lead to more informed choices for compound progression.

In general, the key to unlocking complex and potentially useful efficacy and affinity fingerprints in new molecules is the pharmacological assay. Data from multiple assays allows comparison to quantitative pharmacological models to yield universal and system-independent scales of activity for use in medicinal chemistry efforts to optimize activity.

50% of new molecules in clinical testing fail due to lack of efficacy17. While some of this is due to lack of knowledge of what needs to be corrected in some diseases, some of this may also be due to the inadequate characterization of the efficacy of the candidate molecules put forward in the clinic. With more informed characterization of ligand efficacy fingerprints, perhaps better targeting for progression will result in a reduction in compound late-stage attrition.

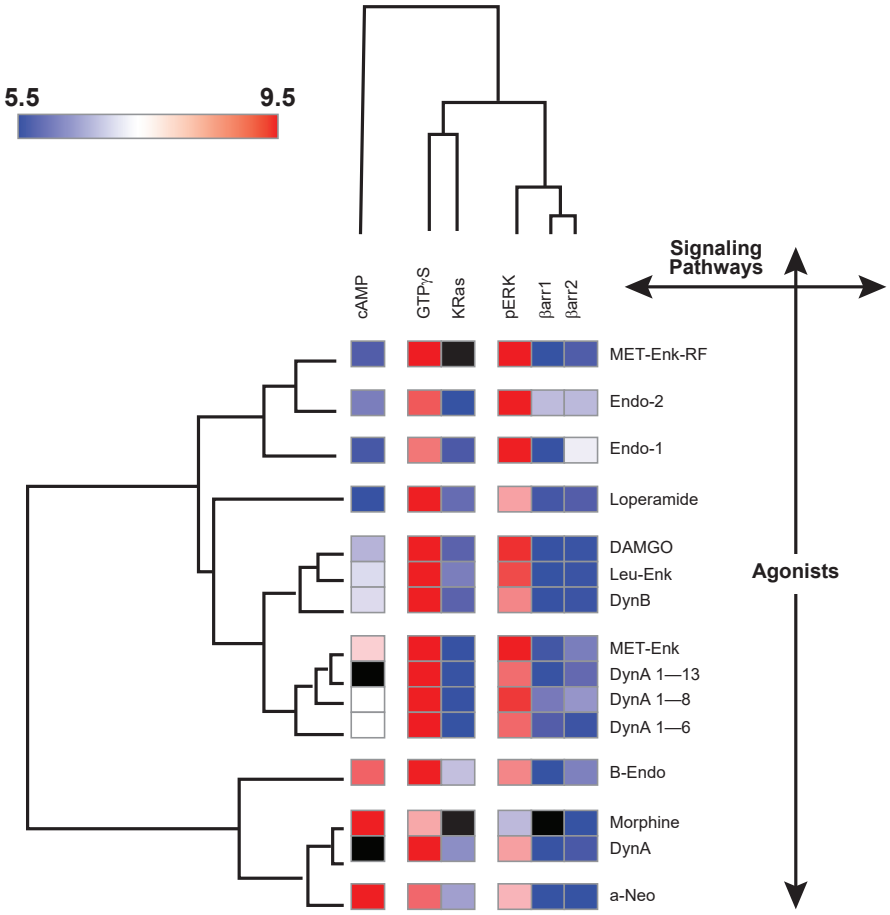


Figure 5. Cluster analysis of sixteen m-opioid agonists in six different functional assays. The gene cluster program GENE-E groups the agonists according to their Log(τ/KA) values in each assay thereby grouping agonists according to their signaling profiles- redrawn from [16].

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