Unraveling Biased Agonism, Partial Agonism and the Importance of Comprehensive Functional Screening in Drug Development

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Introduction

In the rapidly evolving landscape of pharmacology, the concept of biased agonism has gained significant attention for its potential to revolutionize drug development, particularly for G-protein-coupled receptors (GPCRs). Biased agonism, also known as functional selectivity, refers to the ability of a ligand to selectively activate specific signaling pathways over others, leading to distinct physiological responses. This nuanced understanding of receptor signaling has opened new avenues for the development of more targeted and effective therapeutics, especially in areas like pain management and cardiovascular health.

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However, as with any emerging field, challenges and misconceptions have arisen. One of the key issues is the misclassification of certain drugs as biased agonists when, in fact, they are partial agonists. This misclassification can lead to unintended clinical outcomes, as partial agonists do not provide the same level of pathway selectivity as biased agonists, potentially resulting in suboptimal therapeutic effects or increased side effects. Two notable examples of this are TRV130 (oliceridine/Olynvyk™) and PZM21, both of which were initially classified as biased agonists but later, upon closer examination of their signaling properties, actually found to be partial agonists.

In this blog, we will explore the fundamental concepts of biased agonism and partial agonism, delve into the intricacies of GPCR signaling pathways, and highlight the importance of comprehensive functional screening in drug development. We will also discuss how Gifford Bioscience offers an array of methods for measuring G-protein activation, β-arrestin recruitment, and downstream signaling events such as calcium release and cyclic AMP (cAMP) modulation, ensuring accurate drug classification and optimized therapeutic outcomes.

GPCR Signaling Pathways: A Primer

GPCRs are one of the largest and most versatile families of cell surface receptors, playing a crucial role in mediating a wide range of physiological processes. These receptors are activated by a diverse array of ligands, including hormones, neurotransmitters and drugs, which bind to the receptor and induce a conformational change. This change activates intracellular signaling pathways, primarily through the coupling of the receptor to G-proteins or the recruitment of β-arrestins.

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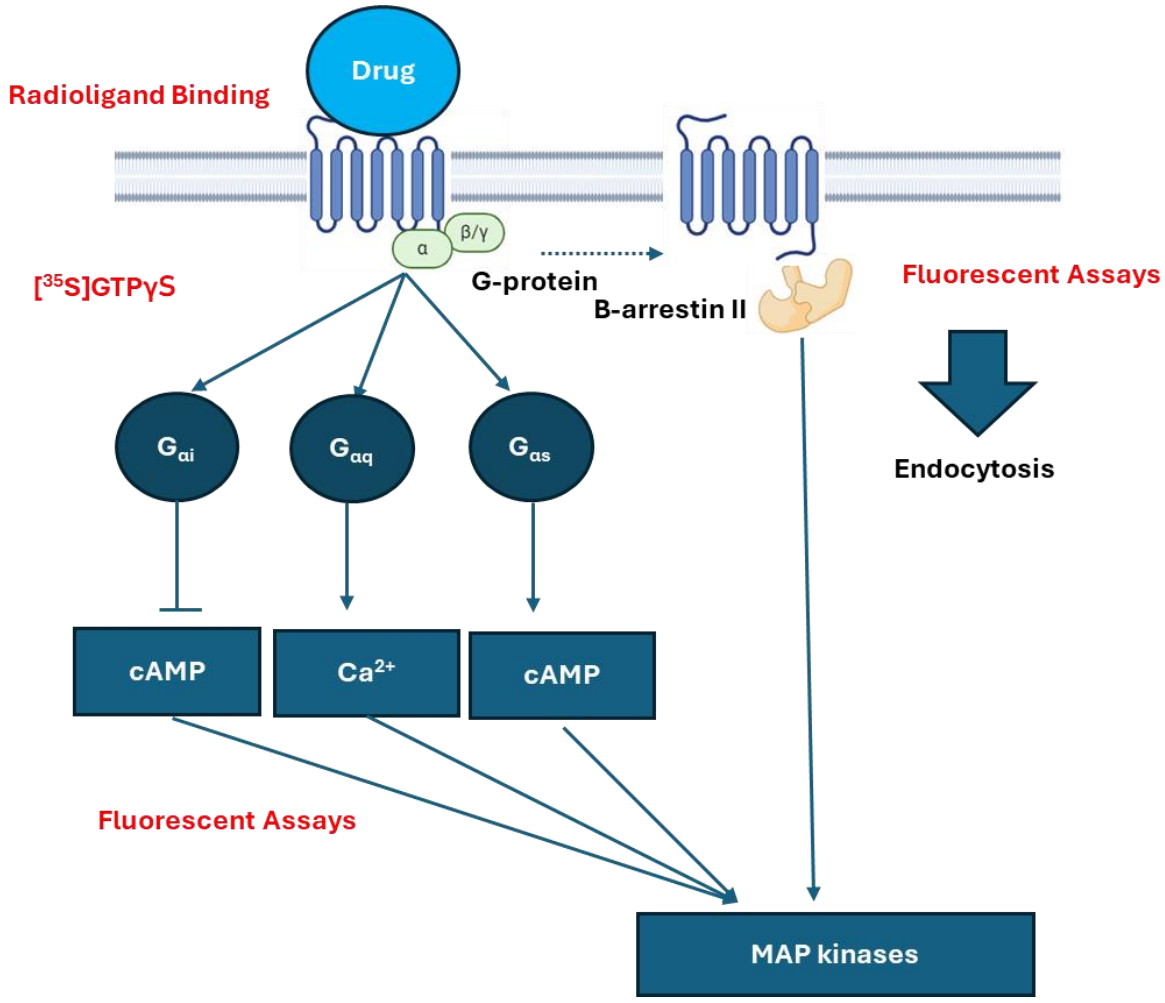
Traditionally, GPCR signaling was thought to follow a linear pathway, where ligand binding led to G-protein activation, followed by downstream signaling events such as the production of second messengers like cAMP or the activation of protein kinases. However, it is now understood that GPCRs can activate multiple signaling pathways, including both G-protein-

mediated and β-arrestin-mediated pathways. These pathways often lead to different, and sometimes opposing, physiological outcomes.

1. **G-Protein-Mediated Signaling**: When a ligand binds to a GPCR, it can activate heterotrimeric G-proteins, which consist of three subunits: Gα, Gβ, and Gγ. Upon activation, the Gα subunit exchanges GDP for GTP, dissociates from the Gβγ dimer, and both the Gα and Gβγ subunits go on to activate various downstream effectors. For example, $G\alpha_s$ stimulates adenylate cyclase to increase cAMP levels, while $G\alpha_i$ inhibits this enzyme to reduce cAMP production. These second messengers can then activate protein kinases, such as protein kinase A (PKA), which phosphorylates target proteins to elicit cellular responses.

2. **β-Arrestin-Mediated Signaling**: In addition to G-protein signaling, GPCRs can also signal through β-arrestins, which were originally thought to function solely in receptor desensitization and internalization. β-arrestins are recruited to the receptor after it has been phosphorylated by G-protein-coupled receptor kinases (GRKs). Once bound to the receptor, β-arrestins not only prevent further G-protein signaling but also act as scaffolds for signaling complexes that can activate downstream pathways, such as the mitogen-activated protein kinase (MAPK) cascade. These β-arrestin-mediated pathways often lead to longer-term changes in cellular function, such as alterations in gene expression or cell migration.



Biased Agonism: A New Paradigm in Drug Discovery

Biased agonism refers to the ability of a ligand to selectively activate one signaling pathway over another. In the context of GPCRs, this typically means favoring either G-protein-mediated signaling or β-arrestin-mediated signaling. Biased agonists are attractive from a drug development perspective because they offer the potential to enhance therapeutic efficacy while minimizing side effects. For example, in the case of opioid receptors, a biased agonist that preferentially activates G-protein signaling while avoiding β-arrestin recruitment may provide strong analgesia without the respiratory depression and constipation associated with traditional opioids.

Examples of Biased Agonism in Drug Development:

1. **TRV130 (Oliceridine)**: TRV130 was initially heralded as a biased agonist of the mu-opioid receptor, thought to preferentially activate G-protein pathways while avoiding β-arrestin recruitment. This was expected to result in effective pain relief with reduced opioid-related side effects, such as respiratory depression. Early clinical studies supported this hypothesis, and TRV130 received FDA approval as oliceridine for the treatment of moderate to severe pain. However, subsequent research revealed that TRV130 is not a true biased agonist but rather a partial agonist, meaning it activates both G-protein and β-arrestin pathways, though to a lesser extent than full agonists like morphine. This partial agonism may explain the lingering side effects observed with TRV130, challenging the initial assumption that it would be a safer alternative to traditional opioids.
2. **PZM21**: Similarly, PZM21 was initially classified as a biased agonist of the mu-opioid receptor, with the hope that it would provide analgesia without the side effects of conventional opioids. However, like TRV130, further research revealed that PZM21 is a partial agonist, not a true biased agonist. While it does preferentially activate G-protein pathways, it still recruits β-arrestins to some extent, resulting in a mixed signaling profile that may limit its therapeutic potential.

Partial Agonism: Understanding the Misclassification

Partial agonism refers to a drug's ability to activate a receptor but with less efficacy than a full agonist. In the context of GPCRs, partial agonists can engage both G-protein and β-arrestin pathways, but they do so at submaximal levels compared to full agonists. This is an important distinction from biased agonism, where the ligand selectively activates one pathway over the other.

The misclassification of TRV130 and PZM21 as biased agonists instead of partial agonists highlights the complexity of receptor signaling and underscores the need for comprehensive functional screening during drug development. While partial agonists may still provide therapeutic benefits, they do not offer the same level of pathway selectivity as biased agonists, which can lead to unintended side effects or reduced efficacy.

The Clinical Implications of Partial Agonism

In clinical settings, the distinction between partial agonists and biased agonists is crucial. Partial agonists may offer some advantages over full agonists, such as a reduced risk of overdose or tolerance development, but they may also be less effective at producing the desired therapeutic response. Moreover, because partial agonists can activate both G-protein and β-arrestin pathways, they may still trigger side effects associated with β-arrestin recruitment, such as desensitization or receptor internalization.

A drug that truly avoids β-arrestin recruitment (i.e. a biased agonist) would be expected to offer a better safety profile compared to a partial agonist

For example, in the case of opioids, β-arrestin recruitment is linked to many of the negative side effects of opioid therapy, including respiratory depression, constipation, and tolerance. Therefore, a drug that truly avoids β-arrestin recruitment (i.e. a biased agonist) would be expected to offer a better safety profile compared to a partial agonist, which may still engage this pathway to some extent.

The Importance of Comprehensive Functional Screening

The misclassification of TRV130 and PZM21 underscores the need for comprehensive functional screening during the drug discovery process. Traditional methods of evaluating drug efficacy often focus on downstream signaling events, such as second messenger production, without fully characterizing the upstream signaling pathways involved. This can lead to an incomplete understanding of a drug's mechanism of action and, in some cases, result in misclassification as a biased agonist.

The author, Dr Mark Bird, is a Senior Scientist at Gifford Bioscience Limited. Mark has significant experience in GPCR pharmacology, developing an increasing interest for the effects of receptor heterodimerisation on downstream signalling pathways.

At Gifford Bioscience, we offer a range of advanced screening techniques to accurately assess a drug's signaling profile. Our methods include:

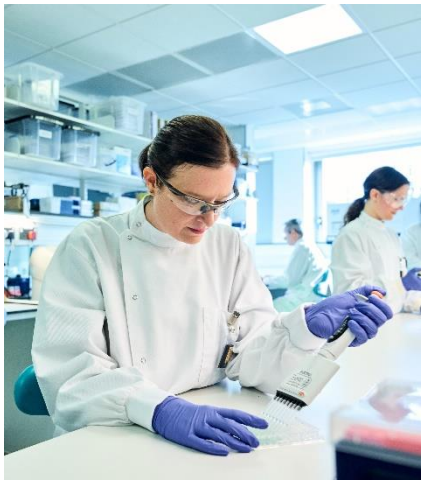
1. **G-Protein Activation Assays**: These assays measure the activation of G-proteins in response to ligand binding, providing a direct assessment of G-protein-mediated signaling. By comparing the levels of G-protein activation to other signaling events, such as β-arrestin recruitment, we can determine whether a drug is truly a biased agonist or a partial agonist.
2. **β-Arrestin Recruitment Assays**: β-arrestin recruitment is a key indicator of GPCR desensitization and internalization, as well as an important signaling pathway in its own right. Our assays allow for the quantification of β-arrestin recruitment, helping to distinguish

between drugs that selectively activate G-protein pathways and those that engage both pathways.

3. **Downstream Signaling Assays**: In addition to measuring G-protein and β-arrestin activation, we offer assays to assess downstream signaling events, such as calcium release, cAMP production and kinase phosphorylation. These second messengers play critical roles in mediating cellular responses to GPCR activation and provide valuable insights into a drug's overall signaling profile.

By combining these assays, we provide a comprehensive analysis of a drug's functional activity, ensuring accurate classification and helping to optimize its therapeutic potential.

Why Choose Gifford Bioscience?



The discovery of biased agonism has transformed our understanding of GPCR signaling and opened new possibilities for drug development. However, as the examples of TRV130 and PZM21 demonstrate, accurately classifying a drug as a biased agonist requires a thorough understanding of its signaling properties. Misclassification can lead to unintended clinical outcomes, highlighting the importance of comprehensive functional screening.

At Gifford Bioscience, we are committed to providing cutting-edge screening services that go beyond traditional methods. By measuring G-protein activation, β-arrestin recruitment, and downstream signaling events, we offer a complete picture of a drug's signaling profile, helping you make informed decisions during the drug development process. Whether you're developing new therapeutics or optimizing existing ones, our expertise in functional screening can help you achieve your goals.