Biased G Protein-Coupled Receptor Signaling: New Player in Modulating Physiology and Pathology

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Abstract

G protein-coupled receptors (GPCRs) are a family of cell-surface proteins that play critical roles in regulating a variety of pathophysiological processes and thus are targeted by almost a third of currently available therapeutics. It was originally thought that GPCRs convert extracellular stimuli into intracellular signals through activating G proteins, whereas β-arrestins have important roles in internalization and desensitization of the receptor. Over the past decade, several novel functional aspects of β-arrestins in regulating GPCR signaling have been discovered. These previously unanticipated roles of β-arrestins to act as signal transducers and mediators of G protein-independent signaling have led to the concept of biased agonism. Biased GPCR ligands are able to engage with their target receptors in a manner that preferentially activates only G protein- or β-arrestin-mediated downstream signaling. This offers the potential for next generation drugs with high selectivity to therapeutically relevant GPCR signaling pathways. In this review, we provide a summary of the recent studies highlighting G protein- or β-arrestin-biased GPCR signaling and the effects of biased ligands on disease pathogenesis and regulation.

Key Words: β-arrestin, biased signaling, G protein-coupled receptor, G protein

INTRODUCTION

G protein-coupled receptors (GPCRs) represent the largest family of cell surface molecules involved in signal transduction. More than 1000 receptors for sensory (e.g., odor and light) and chemical stimuli (e.g., catecholamines, amino acids, peptides, and ions) have been identified based on their common structural and biochemical properties (Muller, 2000). Because GPCRs represent 1-5% of the total cell surface proteins in mammals, it is not surprising that nearly 30% of United States Food and Drug Administration (FDA)-approved drugs target GPCRs (Overington et al., 2006).

Historically, GPCRs were assumed to exist in equilibrium between active and inactive states, and thus activation of GPCRs would equally affect all downstream signaling pathways. However, accumulating evidences indicate that GPCRs exist in multiple conformational states where each conformation confers different downstream effects. In this context, some ligands are able to induce a differential receptor conformation which activates a different subset of signaling events, causing bias receptor signaling (Liu et al., 2012). Biased GPCR signaling has been mainly studied on adrenergic and angiotensin receptors, but accumulating evidences indicate that this phenomenon may be extended to a wide variety of GPCRs currently targeted by pharmacological agents. This notion further complicates drug discovery efforts, but also holds the promise to design specific biased ligands that antagonize detrimental signaling pathways while stimulating beneficial downstream processes. Here, we seek to summarize the current knowledge of biased signaling on GPCRs and to discuss how identified biased ligands of selected receptors modulate disease outcomes.

ACTIVATION OF GPCRs

GPCRs are often referred to as seven-transmembrane receptors (7TMRs) because their structures are characterized by the presence of seven α-helices crossing the plasma membrane. GPCRs are consisted of intracellular and extracellular loops. The NH₂ terminus is exposed to the extracellular environment and the COOH terminus is located in the
intracellular part. The intracellular domains and loops mediate
the interaction between the receptor and intracellular signaling
partners such as G proteins (Gether, 2000; Hermans, 2003).
The binding of exogenous ligands alters the conformation of
critical domains of the seven-transmembrane helix pocket,
which in turn causes the conformation changes of intracellular
domains of the receptor. These changes promote the associa-
tion of the receptor with a variety of heterotrimeric G pro-
teins. They are composed of an α-subunit interacting with a
βγ complex. Activation of the receptor promotes the exchange
of a molecule of GDP by a molecule of GTP within the active
site of the α-subunit. The binding of GTP to α-subunit causes
the dissociation of the heterotrimeric complex, and both the
GTP-bound α-subunit and the released βγ complex are then
able to interact with intracellular or membrane effectors (e.g.,
enzymes or ion channels). The intrinsic GTPase activity of the
α-subunit hydrolyzes GTP into GDP, restoring its initial inac-
tive conformation and its affinity for the βγ complex [for detailed
reviews, see (Wess, 1997; Bockaert and Pin, 1999; Gether,
2000; Hermans, 2003)]. Up to now, at least 23 α-subunits
derived from 17 different genes have been identified and are
classified into four families (Gaα₁, Gaα₂, Gaα₃, Gaα₄). At least
6 different β-subunits and 12 γ-subunits have been also
discovered (Gautam et al., 1998; Vanderbeld and Kelly, 2000).
Upon dissociation from the heterotrimeric complex, the vari-
ous Gaα subunits interact with the well-studied and classical
effector enzymes in a highly specific manner. For instance,
Gaα₁ activates (and Gaα₂ inhibits) adenylyl cyclase (AC), Gaα₃
activates photoreceptor cGMP phosphodiesterase (PDE), and
Gaα₃ activates phospholipase C (PLC) (Skiba et al., 1996;
Hamm, 1998). On the other hand, various Gβγ subunits can
activate or deactivate AC, activate PLCs or phosphatidylinosi-
tol 3-kinase (PI3K). Gγ subunits can activate various kinases
including protein kinase D (PKD) (Morris and Malbon, 1999;
Vanderbeld and Kelly, 2000) (Fig. 1).

In addition to signaling through G proteins, GPCRs can also
activate G protein-independent signaling pathways mainly
through multi-functional adaptor proteins called arrestins. The
arrestins are a small family of proteins originally discovered
in the visual system. Arrestins, which include arrestin-1 and
-4 (expressed in retinal rods and cones) and ubiquitously ex-
pressed arrestin-2 (β-arrestin1) and arrestin-3 (β-arrestin2),
were initially characterized for their roles in GPCR desensiti-
zation (uncoupling of the G protein from the cognate receptor)
(Shukla et al., 2011; Lefkowitz, 2013). Homologous desensiti-
zation is initiated by stimulation of the receptor with high con-
centrations of its agonist, resulting in a change in the receptor
conformation to its active state. G protein-coupled receptor
kinases (GRKs) can then phosphorylate the specific serine/
threonine residues at C-terminus or intracellular loops of the
activated receptor, which increases the affinity of β-arrestin
for the receptor, thus resulting in the uncoupling of the Gaα
subunit from the receptor. By interacting with components of
the endocytic machinery such as clathrin and the adaptor pro-

Fig. 1. Examples of G protein- and β-arrestin-mediated downstream signaling pathways on GPCRs. Upon agonist binding to GPCRs, both
G proteins (Gaα₁, Gaα₂, Gaα₃, Gaα₄, Gβγ, Gβγ and Gγ subunits) and β-arrestin are activated to mediate a variety of distinct downstream signaling
pathways. Stimulation of Gβγ subunit can activate PI3Kγ and Gγ subunit can activate PKD. Gaα₂ can activate Rho kinase signaling pathways
and Gaα₃ can induce the mobilization of calcium from intracellular stores through activation of PLC/IP3. Gaγ signaling activates MEK/ERK
pathway to mediate cell cycle progression. Gaα proteins promote AC-induced PKA activation. Phosphorylation of GPCRs by GRK results
in the recruitment of β-arrestin, which in turn desensitizes G protein signaling, mediates receptor trafficking to endosomes, and activates
β-arrestin-dependent signaling.
tein 2 (AP2) complex, β-arrestins also target the GPCRs for clathrin-mediated endocytosis and internalization (Lefkowitz, 1998; Ferguson, 2001). In addition to receptor endocytosis, β-arrestins were found to play prominent roles in signaling, trafficking, and ubiquitination of receptors (Shenoy et al., 2001). It has been shown that internalized receptor-β-arrestin complexes can form a scaffold for mitogen-activated protein kinases (MAPKs) including ERK1/2, p38 and c-Jun N terminal kinase-3 (JNK3) to generate signalosomes, which may mediate long-lasting cell signaling in the cytosol (Luttrell et al., 1999; McDonald et al., 2000; Gong et al., 2008; Song et al., 2009). β-Arestins have been also reported to scaffold AKT, PI3K and PDE4 in the context of various specific receptors both in vitro and in vivo [reviewed in (DeWire et al., 2007)]. Lastly, β-arrestin1 has been recently proposed to mediate nuclear signaling such as microRNA (miR) processing after activation of β,-adrenergic receptors (β,-ARs) by biased ligands ([Kim et al., 2014], see chapter 3.1). The examples of G protein- and β-arrestin-mediated downstream signaling pathways on GPCRs are summarized in Fig. 1 and 2.

MOLECULAR BASIS OF BIASED GPCR SIGNALING

As our understanding on in vivo effects of GPCR-targeting drugs becomes more profound, it has become clear that therapeutic strategies often require the modulation of a single specific downstream signaling pathway (i.e. G protein- vs. β-arrestin-mediated signaling) in a given cell type or tissue. Most drugs targeting GPCRs often lack the specificity in this regard, and as such produce undesirable side effects. In recent years, there have been significant efforts to develop ligands that preferentially activate one beneficial GPCR signaling pathway, not another detrimental pathway. These functionally selective biased ligands have demonstrated the potential to use as novel therapies and have opened new possibilities in GPCR drug discovery (Rankovic et al., 2016).

Although the detailed molecular mechanism of biased signaling is not yet well understood, it has been reported that biased GPCR ligands induce a unique receptor conformation, activating a particular signaling pathway. It is understood that the GPCR conformation stabilized by a G protein-biased ligand is distinct from the conformation stabilized by a β-arrestin-biased ligand. For example, a fluorescence-based study on activation of the arginine-vasopressin type 2 receptor by biased and unbiased ligands provided an interesting experimental notion, suggesting that the transmembrane helix 6 (TM6) and third intracellular loop at the receptor are associated with selective G protein signaling, whereas the TM7 and helix 8 (H8) regions at the receptor are required for selective

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Fig. 2. Carvedilol-mediated β-arrestin biased signaling on β,-adrenergic receptors in cardiomyocytes and hearts. Carvedilol selectively stimulates GRK5/6- and β-arrestin-dependent cardioprotective signaling without activating deleterious G protein signaling. Carvedilol-mediated GRK5/6 phosphorylation of β,-adrenergic receptors leads to β-arrestin1’s translocation into nucleus where β-arrestin1 interacts with a subset of primary miRs and components of the Drosha microprocessor complex. This results in an increased level of a subset of miRs, which act as cardioprotective miRs by repressing pro-apoptotic genes in cardiomyocytes and hearts.
β-arrestin recruitment (Rahmeh et al., 2012).

Another recent study used site-specific fluorine-19 nuclear magnetic resonance (19F-NMR) labels in the β2-AR to unveil conformational changes of the receptor upon activation by biased and unbiased ligands. It was shown that unbiased ligand’s binding to the receptor primarily shifts the equilibrium toward the G protein-specific active state of helix 6, while β-arrestin-biased ligands predominantly regulate the conformational states of helix 7 (Liu et al., 2012). Also, Woo et al. (2014) showed that phosphorylation of the receptor itself does not necessarily lead to a switching of the receptor coupling to different G proteins as once proposed (Daaka et al., 1997). It was also shown that the biased ligand-mediated signaling depends on the specific interaction between the ligand and the β2-AR’s tyrosine 308 residue positioned on transmembrane helix 7 (Woo et al., 2014). Despite these advances in biased signaling field, further structural studies are needed to unravel how the interaction between GPCR and ligand translates into the receptor conformation for selective coupling to different G proteins and β-arrestins. Such progress would move us closer to structure-based design of drugs specific to a particular signaling pathway.

It is well appreciated that the G protein-independent effector on GPCRs, β-arrestin, is recruited upon phosphorylation of GPCRs by GRKs specifically on their C terminal and intracellular loops. An interesting “barcode” hypothesis suggests that different GRKs phosphorylate distinct sites on the C terminus and internal loops of the receptor, thereby establishing a “barcode” that would instruct or determine the conformation for different β-arrestin functions. This would in turn determine the differential roles of GRKs and β-arrestins (Butcher et al., 2011). A detailed mapping of the β2AR phosphorylation by GRKs supported this hypothesis by demonstrating that the β-arrestin-biased ligand carvedilol recruited different GRKs and induced a different phosphorylation pattern from that of a full agonist isoproterenol (Nobles et al., 2011). The experimental evidence also indicates that the biased ligands can stabilize both the receptor and β-arrestin in conformations that are distinct from those associated with unbiased ligands. For example, Shukla et al. (2008) used an intramolecular bioluminescence resonance energy transfer (BRET)-based biosensor of β-arrestin2 and a combination of biased ligands and/or biased mutants of three different GPCRs to show that β-arrestin can adopt multiple “active” conformations. These findings suggest the possibility that multiple distinct β-arrestin conformations can form various complexes with different binding partners, and thereby engage in different downstream signaling pathways.

Another interesting phenomenon in biased signaling observed for multiple GPCRs in vitro is the ability of biased ligands to recruit different subtypes of β-arrestins within a single receptor. Such differential involvement of β-arrestins can determine ligands’ downstream subtypes of β-arrestins within a single receptor. Such differential involvement of β-arrestins can determine ligands’ downstream effects on receptor such as trafficking, ubiquitination, or signaling. For example, it is known that the recruitment of β-arrestin1 and 2 can mediate internalization of mu-opioid receptor (MOR). Interestingly, morphine, an agonist with the low rate of MOR internalization primarily recruits β-arrestin2, whereas another agonist DAMGO can recruit either β-arrestin1 or 2, which leads to the

### Table 1. Overview of biased ligands on selected GPCRs

| Receptor                     | Biased ligand | Pathway       | Therapeutic area   | References                                                                 |
|------------------------------|---------------|---------------|--------------------|-----------------------------------------------------------------------------|
| Angiotensin I receptor       | SII           | β-arrestin    | Cardiovascular & Renal | Ahn et al., 2009; Boerrigter et al., 2011; Violin et al., 2010              |
|                             | TRV120027     |               |                    |                                                                             |
| Apelin receptor              | MM07          | Gαs          | Cardiovascular     | Brame et al., 2015                                                          |
| Arginine-vasopressin V2      | MCF14         | Gαs          | Renal              | Jean-Alphonse et al., 2009                                                  |
|                             | MCF18          |               |                    |                                                                             |
|                             | MCF57          |               |                    |                                                                             |
| β1-adrenergic receptor      | Carvedilol    | β-arrestin    | Cardiovascular     | Kim et al., 2008, 2014                                                      |
|                             | Alprenolol     |               |                    |                                                                             |
| β2-adrenergic receptor      | Fenoterol      | Gαs          | Pulmonary           | Woo et al., 2009; Xiao et al., 2003                                        |
|                             | Carvedilol     | β-arrestin    | Cardiovascular     | Wisler et al., 2007                                                        |
| Dopamine D1 receptor        | SKF83959      | Gαs          | Neurology & Behavior | Conroy et al., 2015; Rashid et al., 2007                                   |
|                             | SKF38393      |               |                    |                                                                             |
|                             | SKF82957      |               |                    |                                                                             |
|                             | SKF75670      |               |                    |                                                                             |
| Dopamine D2 receptor        | UNC9975       | β-arrestin2   | Neurology & Behavior | Chen et al., 2012; Park et al., 2016                                       |
|                             | UNC9994       |               |                    |                                                                             |
|                             | MLS1547       | Gαq          | Neurology & Behavior | Free et al., 2014                                                           |
| Histamine H2 receptor       | Famotidine     | Gαq          | Gastrointestinal   | Alonso et al., 2015                                                        |
| Histamine H3 receptor       | JNJ7777120    | β-arrestin2   | Inflammatory       | Rosethorne and Charlton, 2011                                              |
| k-Opioid receptor (KOR)     | Isoquinoline   | Gαq          | Neurology & Behavior | Zhou et al., 2013                                                           |
|                             | 2.1            |               |                    | Rives et al., 2012                                                         |
|                             | 6-GNTI         |               |                    | White et al., 2015                                                          |
|                             | RB-64          |               |                    |                                                                             |
| µ-Opioid receptor (MOR)     | TRV130         | Gαo          | Neurology & Behavior | Chen et al., 2013; DeWire et al., 2013                                    |
|                             | NAP            | Gαo          | Neurology & Behavior | Zhang et al., 2016                                                         |
| Serotonin 5-HT2 receptor    | Ergotamin      | β-arrestin    | Neurology & Behavior | Wacker et al., 2013                                                        |
|                             |               |               |                    |                                                                             |

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high level of receptor internalization. DAMGO was also shown to induce receptor ubiquitination in a β-arrestin1-dependent manner (Groer et al., 2011). Similarly, in delta-opioid receptor (DOR), an agonist SNC80, which causes the high internalization of the receptor, was shown to preferentially recruit β-arrestin1 over β-arrestin2. In contrast, agonists ARM390 and JNU20788560 preferentially engage with β-arrestin2, resulting in the low rate of receptor internalization (Pradhan et al., 2016). Endogenous ligands for the C-C chemokine receptor 7 also showed differential internalization properties. The ligand CCL19 with high-internalizing capacity preferentially recruits β-arrestin1 over 1, whereas CCL21 with low-internalizing capacity engages with neither (Byers et al., 2008). In addition, binding of ATP induces the greater interaction between P2Y2 receptor and β-arrestin1, whereas UTP nonselectively recruits both β-arrestin1 and 2 (Hoffmann et al., 2008). These studies support a novel interesting notion that different ligands for the same receptor can form distinct receptor-arrestin complexes.

Altogether, recent advances on GPCR biased signaling field suggest that biased GPCR ligands may have an important therapeutic potential in various diseases including cardiovascular diseases, neurological diseases, and cancers. As summarized in the following chapter and Table 1, we seek to provide recent scientific progress on identifying novel biased ligands on selected highly-profiled GPCRs.

BIASED SIGNALING ON SELECTED GPCRS

β-adrenergic receptors and their biased ligands

β-adrenergic receptors (β-ARs), prototypical members of GPCR superfamily, are known for their regulation of contractile function in the heart. The stimulation of cardiac β1- and β2-AR by catecholamines such as adrenaline and noradrenaline activates the canonical Gβγ-cAMP-PKA signaling cascade, which increases calcium mobilization across different cellular compartments and sensitizes contractile proteins to cytosolic calcium. The overall physiological effect of cardiac β-AR stimulation is an increase in heart contractility (inotropic effect) and heart rate (chronotropic effect) (Rodefeld et al., 1996). The major subtype, β1-AR couples to the Gαs protein, whereas β2-AR is able to couple to both Gαs and Gαi proteins (Kilts et al., 2000; Xiao and Kobilka, 2003; Perrino and Rockman, 2007). Physiologically, the inotropic response to catecholamine stimulation is mediated mainly by β1-AR because the β2-AR-Gαs-mediated AC-cAMP-PKA response is inhibited by the co-activated β2-AR-Gαi signaling (Xiao et al., 1995). However, β2-AR can regulate the effect of β1-AR on excitation-contraction coupling by activating Gαi signaling. It is also known that activation of the β2-AR-Gαi signaling protects the cardiomyocytes from the pro-apoptotic stimuli of excessive β1-AR stimulation and activates a pro-survival PI3K-Akt signaling cascade (Chesley et al., 2000; Zhu et al., 2001). However, prolonged activation of Gαs through a synthetic receptor construct has been shown to lead to a depressed cardiac function and eventually the development of dilated cardiomyopathy in mice (McCloskey et al., 2008). Switching of β2-AR coupling from Gαi to Gαs was found to play an important role in ischemic preconditioning-induced cardioprotection in the mouse heart (Tong et al., 2005). In addition, the enhanced β2-AR-Gαi signaling contributes to the dysfunction of both β1-AR and β2-AR in the failing heart (Xiao et al., 2003; Xiao and Balke, 2004).

In 2007, Noma et al. showed that β1-AR-mediated β-arrestin signaling confers cardioprotection independent on G protein-mediated second messenger signaling (Noma et al., 2007), bringing up a concept of biased signaling. Here, we summarize recent advances on β-AR biased signaling and highlight some of biased ligands which are used in clinic for a therapy.

**Fenoterol:** Racemic fenoterol is unique among the β2-AR agonists because it has been identified as a biased β2-AR ligand selectively coupling β2-AR to Gαs protein. In the study by Xiao et al., (2003) dysfunction of β2-AR but not β1-AR in the model of failing spontaneous hypertensive rats was induced by enhanced Gαs signaling. Disruption of Gαs signaling by pertussin toxin restored the blunted β2-AR contractile response in the failing heart. Interestingly, β2-AR agonist fenoterol had similar beneficial effects, which were due to selective activation of β2-AR-Gαs signaling (Xiao et al., 2003). Functional selectivity of fenoterol was shown to depend on the stoichiometry at its two chirality centers (Woo et al., 2009). Based on these observations, at least two other fenoterol derivatives (S, R’)-4’-methoxy-fenoterol and (S, R’)-4’-methoxy-1-naphthyl-fenoterol have been identified to selectively activate only Gαs signaling pathways on β2-AR. These biased β2-AR ligands are therapeutically used for bronchial asthma and chronic obstructive lung disease (Reinartz et al., 2015). These studies suggest that identifying novel Gαs-biased β2-AR ligands, which display a better efficacy, may provide a new therapy with the sustained efficacy by switching off β-arrestin-dependent receptor desensitization and down-regulation.

**Carvedilol:** Carvedilol is a well-known neurohormonal antagonist with multiple activities efficiently used in patients with congestive heart failure, stable angina pectoris (Dunn et al., 1997), myocardial infarction (Doughty et al., 2001), and myocardial ischemia-reperfusion injury (Brunvand et al., 1998). It blocks both the β1- and β2-AR, resulting in the improved myocardial function and attenuation (or reversal) of adverse myocardial remodeling in heart failure. It also reduces the peripheral vascular resistance via vasodilation caused by antagonism of α1-AR. In addition to these well-known properties, carvedilol has a number of ancillary activities including antioxidant, anti-inflammatory, and antiapoptotic actions (Ohtsuka et al., 2005). In 2007, carvedilol was shown to confer a range of cardioprotective effects, which were hypothesized to stem from its antioxidant effects and/or from direct inhibition of proapoptotic pathways (Schwarz et al., 2003).

More importantly, carvedilol has been identified as a biased ligand on β1-AR and β2-AR, which selectively stimulates GRK5/6- and β-arrestin-dependent cardioprotective signaling without activating G proteins (Wisler et al., 2007; Kim et al., 2008). Our group also showed that after stimulation of β1-AR by carvedilol, β-arrestin1 promotes the processing of five miRs (miR-125a-5p, miR-125b-5p, miR-150, miR-199a-3p and miR-214) in murine hearts and human cells (Kim et al., 2014). MiRs, a class of ~22 nucleotide small noncoding RNAs governing post-transcriptional repression of target miRNAs, were found to play important roles in normal cardiac physiology including the control of myocyte growth, contractility, and maintenance of cardiac rhythm as well as the pathogenesis of various heart diseases [reviewed in (Quiat and Olson, 2013)]. The hypothesis for the mechanism by which carvedilol promotes miR processing is that carvedilol-induced GRK5/6 phosphorylation of β1-AR mediates the recruitment of β-arrestin1 to the
ligand-occupied receptor, resulting in the translocation of β-arrestin1 to the nucleus where it interacts with a subset of primary miRs and components of the Drosha microprocessor complex. Formation of a nuclear complex of β-arrestin1 with the heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) and Drosha, which are crucial nuclear RNA-binding proteins involved in miR processing (Lee et al., 2003; Gull and Caceres, 2007), leads to activation of RNA helicase-independent miR processing (Kim et al., 2014). In the mouse hearts, 7-day carvedilol infusion induced the upregulation of miR-150, miR-214, miR-125b-5p and miR-199a-3p, which were shown to be cardioprotective in the mouse models of heart failure (Salloum et al., 2010; Aurora et al., 2012; Wang et al., 2014; Tang et al., 2015). Moreover, our group also showed that 7-day infusion of carvedilol induced a unique gene signature on multiple genes related to cardiac disease. Genes upregulated by carvedilol included those encoding proteins in the tight junctions, malar-ia and viral myocarditis pathway, while downregulated genes were those encoding proteins in the glycosaminoglycan biosynthesis and arrhythmogenic right ventricular cardiomyopathy (Teoh et al., 2015). These findings make us speculate that carvedilol-responsive miRs can regulate the expression of various detrimental genes to confer cardioprotection (Fig. 2). We postulate that β-AR-mediated β-arrestin1 biased signaling provides an additional mechanism for the clinical efficacy of this β-blocker (Table 1).

**Insulin signaling:** Interestingly, a recent study suggests that insulin signaling can also mimic the effects of a biased β2AR ligand and selectively activate a Gαi-biased signaling pathway (Fu et al., 2014). Insulin and adrenergic stimulation represent two divergent regulatory systems that interact with overlapping downstream signaling pathways in adipocytes, liver, and skeletal and cardiac muscle. Stimulation of insulin receptor (IR) as well as βARs increases the glucose uptake in cardiac and skeletal muscle cells (Nevzorova et al., 2006; Ciccarelli et al., 2011). In the heart, IR and β2AR form a complex and the stimulation of both receptors shares common downstream signaling components including GRK2 (Cipolletta et al., 2009; Ciccarelli et al., 2011), Gαi (Song et al., 2001) and β-arrestin (Luan et al., 2009). Stimulation with either insulin or adrenergic receptors antagonizes the ability of the other to activate glucose transport (Morisco et al., 2006) and to modulate myocyte survival (Rane et al., 2010).

In both diabetes and heart failure, circulating insulin levels are chronically elevated, leading to persistent stimulation of IRs. Despite the insulin resistance of adipocytes and skeletal muscle cells, the heart retains its insulin sensitivity to activate IR signaling cascades in type 2 diabetes (Wright et al., 2009; Cook et al., 2010). Hyperactive insulin signaling was shown to significantly accelerate adverse left ventricular remodeling in pressure overload-induced hypertrophy in rodents (Shimizu et al., 2010). In the animal model of ischemia/reperfusion, insulin inhibited β-AR action in the hearts (Yu et al., 2008). It was shown that in the animal hearts, insulin could directly impair adrenergic signaling pathways for contractile function via an IR-β-AR signaling complex. Insulin stimulation promotes crosstalk with βAR pathways via insulin receptor substrate (IRS) and GRK2-mediated phosphorylation of the βAR, which selectively activates a Gαi-biased β-AR signaling cascade to inhibit cAMP/PKA activities. Consequently, this IR-β-AR crosstalk leads to impaired β-AR-induced contractile function in cardiomyocytes and perfused mouse hearts (Fu et al., 2014).

However, these findings still need to be confirmed in humans, as it is known that diabetes is one of the risk factors for heart failure (Nichols et al., 2001), and heart failure is an insulin-resistant state (Cook et al., 2010). If the hyperinsulinemia can indeed inhibit β-AR signaling via Gαi-biased β2AR signaling in humans, it is possible that hyperinsulinemic subjects with type 2 diabetes and heart failure might have increased sensitivity to the cardio-depressive effects of nonselective or β1-blockade, which is the standard care for managing patients with heart failure (Fu et al., 2014).

**Biased ligands on angiotensin receptors**

Angiotensin II (AngII) type I receptor (AT-R), a primary regulator of blood pressure, is a prototype GPCR in the study of biased agonism. Upon binding of its natural ligand AngII to the receptor, Gαs proteins are activated, resulting in intracellular inositol triphosphate (IP3) production, calcium mobilization, protein kinase C (PKC) activation, which altogether mediate the physiological effects of AT-R such as vasoconstriction and fluid retention. Moreover, the conformational rearrangement of 7 transmembrane α helices of the receptor also leads to the recruitment of β-arrestins, which mediates G protein-independent signaling, leading to overall positive inotropic and cardioprotective effects (Ikeda et al., 2015). Angiotensin receptor blockers (ARBs) are clinically used for their anti-hypertensive activity, and some ARBs also show variable efficacies toward the protection against organ damage in diabetic nephropathy, cardiac hypertrophy, arrhythmia, and renal failure (Burnier and Brunner, 2000). Multiple studies suggest that such additional tissue-protective benefits of ARBs may be mediated by β-arrestin signaling (Kim et al., 2005; Miura et al., 2013), thus the development of biased ligands has resulted in a promising therapy.

**SII:** One of the first β-arrestin-biased ligands to be described was the peptide [Sar1, Ile4, Ile8]-Ang (SII). This peptide was reported to exert anti-apoptotic cytoprotective effects in rat vascular smooth muscle cells (Ahn et al., 2009) and induce positive inotropic and lusitropic effects in rat primary cardiomyocytes by stimulating endogenous AT-R-β-arrestin signaling (Rajagopal et al., 2006). SII was also able to activate MAPK signaling in perfused rodent hearts (Aplin et al., 2007).

**TRV120027:** Utilizing SII as a pharmacological probe in vitro and ex vivo was helpful in elucidating downstream AT-R-mediated β-arrestin signaling. However, due to its low-affinity for the receptor, it has been difficult to study SII’s potential pharmacological benefit in in vivo models. To overcome this limitation, custom-synthetic peptides have been developed based on SII sequence. Among the identified ligands, TRV120027 (TRV027) exhibited an improved potency compared to AngII in stimulating β-arrestin signaling, but no detectable G protein activation. TRV027 was shown to stimulate β-arrestin recruitment with subsequent activation of several kinase pathways such as p42/44 mitogen-activated protein kinase, Src, and Akt-endothelial nitric-oxide synthase pathways. TRV027 was also shown to increase cardiomyocyte contractility in vitro and decrease mean arterial pressure in rats in vivo, similar as unbiased ARBs such as losartan. However, unlike the unbiased ARBs, which decrease cardiac performance, TRV027 increased cardiac performance and preserved cardiac stroke volume (Violin et al., 2010). Moreover, in healthy and heart failure canines, TRV027 also reduced pulmonary capillary wedge pressure, systemic and renal vascular resistance while...
preserving renal functions (Boerrigter et al., 2011). Because of this unique pharmacological profile, TRV027 has already entered phase II clinical trial as a novel therapeutic agent for acute heart failure (Ikeda et al., 2015) (Table 1).

**Biased signaling in apelin receptor**

The apelin receptor (also known as APJ, APLNR, AGTRL1) is a class A GPCR discovered in 1993 based on its sequence similarity with the AT1R (O’Dowd et al., 1993). APJ is widely expressed in brain and peripheral organs including heart, lung, kidney, or placenta (Hosoya et al., 2000; Pope et al., 2012). Since its discovery, a number of physiological and pathophysiological roles for the receptor have been identified, including regulation of cardiovascular function, fluid homeostasis, and the adipoinuscular axis [reviewed in (Pitkin et al., 2010)]. APJ does not bind AngII but its natural ligand, apelin, is a potent inotropic and vasodilatory agent. Apelin induces coupling of APJ to Goα (Habata et al., 1999) with additional evidence for the involvement of Goαq-coupled activation of PLC and PKC (Japp and Newby, 2008). Interestingly, the apelin receptor in the heart may act as a mechanosensor for stretch in an apelin-independent/G protein-independent manner through recruitment of β-arrestin (Scimia et al., 2012).

Apelin receptor system represents an attractive target in pathologies such as pulmonary hypertension and heart failure (Chong et al., 2006; Chandra et al., 2011), which encourages the development of its synthetic ligands. However, the limitation in translation to clinic is that chronic administration of an agonist would likely cause receptor desensitization with subsequent β-arrestin-mediated downregulation and loss of therapeutic efficacy. Thus, the development of agonists biased toward G protein signaling is essential. MM07, a cyclic apelin peptide, was shown to preferentially activate G protein responses with the low potency toward β-arrestin and receptor internalization. In rats, systemic infusions of this peptide caused a dose-dependent increase in cardiac output greater than apelin. Moreover, MM07 was an effective vasodilator in human forearm without loss of effects on repeat dosing, providing proof-of-concept of a clinical potential for biased ligands (Brame et al., 2015). Another APJ ligand, K17P was also reported to be G protein-biased and displayed the strong impairment of β-arrestin-dependent signaling. This molecule lacks the vasodilator capacity of its mother molecule, which is due to the deletion of single C-terminal phenylalanine (Ceraudo et al., 2014). This reflects the impact of the specific structure of the ligand in determining the signaling pathway activated in the receptor (Table 1).

**Biased signaling in histamine receptors**

The human histamine H3 receptor (H3R) belongs to the GPCRs and is considered as an important receptor in immune and inflammatory processes (Leurs et al., 2009). H3R was originally thought to signal only through Go proteins and recently shown to also recruit and signal via β-arrestin2. This discovery made by its antagonist JNJ7777120, which was identified as a biased ligand in a β-arrestin2 recruitment assay (Rosethorne and Charlton, 2011). The therapeutic potential of JNJ7777120 has been successfully studied in the mouse model of chronic dermatitis, where it inhibited pruritus and skin inflammation when used in combination with H1R antagonist (Ohsawa and Hirasawa, 2012). Based on its indolocarboxamide structure, various JNJ7777120 analogues have been recently developed with the similar biased affinity toward β-arrestin2 (Nijmeijer et al., 2013). The potentially improved therapeutic efficacy of these substances is yet to be evaluated.

Currently, one of the most clinically relevant therapies for histamine receptors is achieved through the regulation of H2R, which is pathologically implicated in gastric acid-related diseases but widely expressed in most tissues. The H2R stimulation increases adenylyl cyclase activity and induces cAMP accumulation. The most commonly used H2R blocker, famotidine was shown to act as an “inverse agonist” by diminishing G protein-mediated increase of cAMP. Interestingly, famotidine also mimicked the effect of histamine, and induced receptor desensitization and internalization along with increased ERK phosphorylation in gastric epithelial cells (Alonso et al., 2015) (Table 1).

**Biased signaling in dopamine receptors**

Dopamine receptors are another well-studied family of GPCRs largely because dopamine neurotransmission is important in multiple neuropsychiatric disorders. Among the dopamine receptors, dopamine receptor D2 (D2R) is one of the most validated drug targets in neurology and psychiatry. However, most drugs targeting the D2R are problematic, either being less efficacious than desired or possessing adverse side effects due to the activation or blockade of a subset of downstream signaling pathways.

D2R couples Gq/o to negatively regulate cAMP-PKA pathways and modulate intracellular Ca2+ levels by acting on ion channels or by triggering the release of Ca2+ from intracellular stores. In addition, more recent discoveries showed that dopamine receptors exert their in vivo effects through β-arrestin2-mediated protein kinase B (Akt)- glycogen synthase kinase 3 (GSK3) signaling cascades (Beaulieu et al., 2005). In humans, the Akt1/GSK3β signaling pathways are implicated in schizophrenia as evidenced by the low levels of Akt1 protein and reduced phosphorylation of GSK3β in the brain and lymphocytes of schizophrenic patients (Emamian et al., 2004). As Akt and GSK3 responses are mediated through ligands that are biased for β-arrestin signaling (Beaulieu et al., 2005), this may represent a novel approach to develop drugs with fewer side effects, greater therapeutic selectivity, and enhanced efficacy for treating schizophrenia. Indeed, two newly synthesized β-arrestin-biased ligands for D2R, UNC9975 and UNC9994 have already shown some promise by displaying robust antipsychotic drug-like activities in wild-type mice, which were abolished in β-arrestin2 knockout mice (Chen et al., 2012). These two biased ligands also reduced schizophrenia-like behaviors in phencyclidine-treated or NR1-knockdown hypoglutamatergic mice, where they increased performance in various neurobehavioral tests, and elicited a lower level of catalepsy than standard antipsychotic drug and D2R antagonist haloperidol (Park et al., 2016).

Interestingly, biased ligands with the opposing pharmacology for the D2R, that is, the stimulation of G protein signaling pathways without activation of β-arrestin recruitment have been recently identified. The first example is MLS1547, which was shown to robustly activate G proteins while antagonizing β-arrestin recruitment to the D2R (Free et al., 2014). Identification of such functionally selective ligands should help to dissect the roles of both signaling arms of the D2R in physiology and pathology. Moreover, functionally selective G protein-
biased ligands may also result in improved therapies for certain neuropsychiatric disorders such as Parkinson’s disease, in which D2R stimulation is desired (Table 1). Several recent advances on D1R-biased signaling are also summarized in Table 1.

Biased signaling in opioid receptors

Opioid receptors are GPCRs, which are widely studied due to their crucial roles in pain management, drug abuse/addiction, and mood disorders. There are three major subtypes of opioid receptors: δ-receptor (DOR), κ-receptor (KOR), and µ-receptor (MOR). Majority of opioids exert their analgesic activities primarily via activating MOR. Upon activation, MOR predominately couples to Gαi, which orchestrates downstream signaling cascades including those contributing to antinociception. On the other hand, activation of β-arrestins, especially β-arrestin2 induces receptor internalization and desensitization, diminishing G protein-mediated signaling. Recent studies have shown that some MOR agonists such as fentanyl and [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO) have the high efficacy to recruit β-arrestin, whereas other opioids such as morphine are biased toward G protein signaling (McPherson et al., 2010; Molinari et al., 2010). MOR-mediated β-arrestin activation has been associated with the adverse effects of opioids such as dependence, and gastrointestinal and respiratory dysfunction. Indeed, in the absence of β-arrestin2, morphine produced less tolerance, dependence, constipation, and respiratory suppressive side effects. Moreover, a significantly enhanced and prolonged analgesic effect of morphine was observed in loss-of-function of β-arrestin2 (Thompson et al., 2015). Therefore, the development of biased G protein ligands for MOR holds a greater therapeutic potential compared to an unbiased one. In fact, a G protein-biased MOR ligand TRV130 (made by Trevena Inc., King of Prussia, PA, USA) with little β-arrestin recruitment activity has recently been reported to show potent analgesic effects with reduced respiratory depression and constipation compared to morphine (DeWire et al., 2013). TRV130 was delivered intravenously and has successfully completed phase I trials for safety in healthy volunteers (Soergel et al., 2014). The first encouraging results from phase II trials were reported in a randomized and double-blinded study in patients experiencing moderate-to-severe post-operative pain after bunionectomy. The results showed that TRV130 rapidly produces profound analgesia with no serious adverse effects, suggesting that G protein-biased MOR activation is a promising target for novel analgesics (Viscusi et al., 2016).

Interestingly, biased ligands that are selective agonists at one desired pathway could also act as biased competitive antagonists for the undesired pathway. In general, MOR ligands with low efficacy for G protein activation also have low efficacy for β-arrestin2 recruitment, and in fact partial agonists such as buprenorphine do not significantly recruit β-arrestin2 in cell models (McPherson et al., 2010). In clinic, partial MOR agonists with bias toward antagonism of the β-arrestin2 would be beneficial for the treatment of opioid-induced constipation, which often complicates an analgesic therapy in patients. The pursuit of highly selective and potent non-peptide MOR ligands has yielded several more promising compounds including NAP, which acts as a peripherally selective MOR partial agonist. Being a P-glycoprotein substrate, NAP has limited access to the central nervous system. Moreover, it has no apparent analgesic effect due to its low efficacy in activating G protein-mediated signaling with no apparent effect on β-arrestin2 recruitment. However, its therapeutic potential lies in its ability to antagonize MOR full agonist-induced intracellular calcium flux and β-arrestin2 recruitment (Zhang et al., 2016). NAP dose-dependently restored the morphine-impaired intestinal motility without precipitating significant withdrawal of symptoms and thus held great promise in the treatment of opioid-induced constipation (Yuan et al., 2012) (Table 1). In addition to multiple GPCRs as aforementioned, we summarize recent advances on serotonin 5-HT2A receptor- and arginine-vasopressin V2 receptor-mediated biased signaling and highlight some biased ligands in Table 1.

Biased GPCR signaling in cancer

In contrast to the successful implementation of GPCR biased signaling concept for clinical benefit in the cardiovascular, neurological, behavior fields, there have been no reports demonstrating the utility of GPCR biased signaling for the treatment of cancer. However, a few recent studies reported the scientific progress in the potential use of biased signaling on endothelin receptors in cancer treatment. Endothelin-1 (ET-1) is a peptide belonging to a family of the most potent vasoconstrictors. In addition to this function in the circulation, it has been implicated in various physiological and pathological conditions such as development, cell proliferation, differentiation, cardiac function and cancer (Schorlemmer et al., 2008; Rosano et al., 2013b). ET-1 is a well-recognized growth factor that is present in plasma and is produced by stromal and tumor cells. The ET-1 receptors, endothelium type A receptor (ETₐᵣ) and endothelin type B receptor (ET₂ᵣ) are members of the GPCR family. ETᵣ is coupled to Gᵣ and Gᵦ, and expressed mainly in endothelial cells, while ET₂ᵣ, which is coupled to Gᵦᵣ, Gᵦᵣ, and Gᵦ₁₃, is expressed in vascular smooth muscle cells and cardiomyocytes as well as solid tumors (Sakurai et al., 1990; Williams et al., 1991; Bagnato et al., 1999). In ovarian cancer, ET₁ᵣ/ET₁ᵣ axis has been shown to promote tumorigenesis by promoting anti-apoptosis, invasion, and neoangiogenesis (Spinella et al., 2004; Rosano et al., 2005). Indeed, ET₁ᵣ overexpression is associated with poor survival in patients with ovarian carcinoma (Teoh et al., 2014). However, a recent clinical study demonstrated that specific ET₁ᵣ antagonists are ineffective as auxiliary anti-cancer treatment (Cognetti et al., 2013). This might be explained by signaling bias of ET₁ᵣ, which mediates both oncogenic and tumor suppressive properties. The known oncogenic downstream effects of ET₁ᵣ are mediated by Gᵦᵣ-coupled or β-arrestin-dependent signaling pathways (Spinella et al., 2004; Rosano et al., 2013a). It was shown that GRK5/6-mediated phosphorylation of the receptor leads to the recruitment and nuclear translocation of β-arrestin, which in turn functions as an epigenetic regulator of several angiogenic/metastatic genes including β-catenin, thus promoting cell invasion (Rosano et al., 2013a; Teoh et al., 2014). On the other hand, ET₂ᵣ-mediated Gᵦᵣ activation induces AĈ/CAMP/PKA signaling, which can confer tumor suppressive effects as reported in several carcinoma-derived cell lines (Takahashi et al., 2009; Follin-Arbelet et al., 2013; Teoh et al., 2014). Given that stimulation of the ET₁/ET₁ᵣ axis can activate both tumor suppressive and oncogenic properties in cancer cells, ligands biased toward Gᵦᵣ/CAMP/PKA signaling might represent a novel potential therapy of various malignancies. Unfortunately,
such therapeutic agents are yet to be developed.

Other GPCRs, which are involved in the progression of cancer and have been suggested as potential targets for yet-to-be-identified biased ligands, are CXC chemokine receptor 4 (CXCR4) and protease activated receptor 2 (PAR2). The enhanced expression of CXCR4 and aberrant downstream signaling are implicated in several cancers, where it is involved in tumor growth, vascularization, and metastasis (Guleng et al., 2005; Rubin, 2009). Also, PAR2, a GPCR with distinct biased signaling, has emerged as one of the promising therapeutic targets to inhibit rapidly metastasizing breast cancer cells (Morris et al., 2006). Therefore, the development of novel biased ligands for CXCR4 and PAR2 may open new opportunities for cancer treatment.

DISCOVERING BIASED SIGNALING ON GPCR

Over the past decade, there has been a surge in publications to describe the identification of biased ligands at a wide variety of GPCRs. Accordingly, quantifying ligand bias has been an active area of research. One way to quantify ligand bias is to plot β-arrestin activity against G protein activity. For biased ligands, there would be different levels of β-arrestin- and G protein-mediated efficacies. Such data can also be represented as a matrix that incorporates data from multiple assays, or ligand bias factors that compare β-arrestin activity against G protein activity in different assays (Rajagopal et al., 2010). The quantification of the relative levels of bias is important in the identification of lead compounds and in the optimization of drug screening for biased ligands. Despite this effort, there are still considerable gaps in our understanding of bias. The conventional high throughput screening methodology used in the pharmaceutical industry is inadequate for the needs of novel biased ligand discovery. The drug discovery and experimental studies of biased signaling mechanisms require the use of highly sensitive assays and a standardized methodology to quantify responses related to the signaling pathways. For example, assays and analyses must be configured to remove any apparent bias resulting from the biological assay system and thus correctly identify true ligand bias. In addition, at least one of the endpoints requires the accurate quantification of a poor response. Lastly, the analysis must be compatible for use with a large number of compounds, and the data must be presented to enable medicinal chemistry to derive the relationship between structure and activity (Winpenny et al., 2016).

In the past years, most groups have relied on comparing the maximal effects (E_{max}) and potencies (EC_{50}) of ligands for different signaling pathways. However, they are prone to errors in the interpretation in the setting of receptor reserve. For example, these parameters failed to account for the differences in the receptor reserve and amplification of different assays (Rajagopal et al., 2010). In assays with significant amplification such as second-messenger assays (e.g., cAMP formation), both full and partial agonists can reach the same maximal response, whereas in assays with little amplification such as assays that monitor the recruitment of β-arrestin to a receptor by enzyme complementation (Egleng et al., 2007), partial agonists have significantly lower maximal responses than full agonists (Rajagopal et al., 2010). Therefore, a partial agonist that reaches the maximal effect in one assay and half-maximal effect in another assay would be incorrectly identified as being biased compared with a full agonist, which reaches the maximal response in both assays. Also, the difference in potencies between the full agonist and partial agonist may be smaller in assays with less receptor reserve (Rajagopal et al., 2010, 2011). The current best practice in the identification and quantification of biased agonism is thus complex and an ongoing topic of debate.

The key requirements for measuring bias signaling are a common reference compound to overcome observational and systemic bias as well as a scale, which accounts for both potency and maximal response of ligands. They allow the relative activity of ligands to be compared across assays. The current ‘gold standard’ method is the operational model of agonism that allows for the systematically independent quantification of agonist activity via the relative transduction ratio coefficient Δlog (τ/K_{D}). The term τ incorporates agonist efficacy, receptor density and coupling within the system. The dissociation constant (K_{D}) is the reciprocal of the conditional affinity of the agonist in the functional system [for detailed reviews, see (Kenakin and Christopoulos, 2013)]. The alternative to this method is to use the bias factor [B], which is calculated by the ratios of the efficacy of agonists for a given signaling pathway in a cell. This method was first used to quantify ligand bias and to identify weak biased compounds in β-AR and AT-R.

This method differs from the previous one by assuming that a single estimate of KA for the receptor (obtained from biochemical binding studies) should be used to fit the data with the operational model [for detailed reviews, see (Rajagopal et al., 2011)]. However, the model to use the bias factor has limitations such as a possible error in the calculation of bias if the affinity of the agonist is changed when different signaling proteins are coupled to the receptor (Kenakin and Christopoulos, 2013). Another method for quantifying agonist bias offers the comparison of ratio of the maximal response (E_{max}) to the EC_{50} value for an agonist (Δlog (E_{max}/EC_{50})). The equation has been employed to identify new biased MOR ligands. In this method, the relative signaling bias was quantified for each compound by calculating the difference in activity between β-arrestin recruitment assays (e.g., fluorescent labeling of β-arrestins and GPCRs) and G protein activation assays (e.g., cAMP assay).

In such quantification, 0 represented no bias and +1 or -1 represented 10-fold bias for one signaling pathway [for detailed reviews, see (Winpenny et al., 2016)]. These methods for the identification and quantification of bias across large compound numbers have been essential for biased drug discovery.

One of increasingly recognized techniques for identification of GPCR signaling bias is BRET, which is a sensitive and non-destructive method commonly used in live cells to investigate protein-protein interactions or changes. BRET is a naturally occurring phenomenon resulting from the nonradioactive transfer of energy between luminescent donor and fluororescent acceptor proteins. In the sea pansy Renilla reniformis, the luminescence resulting from the catalytic degradation of coelenterazine by luciferase (Rluc, donor) is transferred to the green fluorescent protein (GFP, acceptor), which in turn emits fluorescence upon dimORIZATION of the two proteins. The BRET response depends on the distance and the relative orientation of the donor and acceptor. In many studies, donor and acceptor are tagged individually on two proteins that potentially assemble in signaling complexes. In other cases, subtle changes in the protein conformation upon complex assembly
or disassembly can be studied using modified proteins that are labeled with both BRET donor and acceptor moieties engineered into the same protein in an intramolecular setting. Because of its fast reaction kinetics, this method allows for the real time detection of complexes or conformational changes that may be transient (Angers et al., 2000; Milligan, 2004). BRET has been successfully applied to study GPCR dimerization (Angers et al., 2000), and more recently to study proximal interactions of GPCRs with different signaling effectors including G proteins and β-arrestins (Shukla et al., 2008; Molinari et al., 2010; Ceraudo et al., 2014). Recent new advances in BRET technology include the discovery of biosensors, which do not involve the labeling of receptor as either a BRET donor or acceptor, and allow the identification of biased signaling from unknown compounds for any GPCR of interest (Namkung et al., 2016).

In addition to BRET, fluorescence resonance energy transfer (FRET) proximity and conformation assays as well as signaling assays such as MAPK activation have been widely used for the discovery of biased ligands (Rajagopal et al., 2010).

CONCLUSIONS

The drug discovery strategy targeting only the primary ligand binding sites of GPCRs is becoming more and more difficult. As our understanding on in vivo effects of GPCR-targeting drugs becomes more profound, therapeutic strategies have often required the modulation of only a part of downstream effector pathways of a GPCR in a specific cell or tissue. Biased ligands with functional selectivity in specific GPCRs thus represent a new generation of drugs with increased specificity and fewer adverse effects. Much has been speculated regarding the potential advantages of the pharmacological feature of biased signaling and there are several biased ligands entering clinical studies. However, many gaps still exist. For example, few large animal and human studies have been performed. To completely translate existing basic research outcomes into clinical therapeutic options, further collaborative research should be pursued. Moreover, it is important for physicians and pharmacists to be continuously informed about which currently used drugs belong to the category of biased GPCR ligands and in which cases their use is beneficial or should be avoided due to the known adverse/unwanted effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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