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Strategies for the discovery of biased GPCR ligands

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G-protein-coupled receptors (GPCRs) represent important drug targets with complex pharmacological characteristics. Biased signaling represents one important dimension, describing ligand-dependent shifts of naturally imprinted signaling profiles. Because biased GPCR modulators provide potential therapeutic benefits including higher efficiencies and reduced adverse effects, the identification of such ligands as drug candidates is highly desirable. This review aims to provide an overview of the challenges and strategies in the discovery of biased ligands. We show different approaches for biased ligand discovery in the example of G-protein-biased opioid analgesics and discuss possibilities to design biased ligands by targeting extracellular receptor regions.

Introduction

G-protein-coupled receptors (GPCRs) represent complex and highly dynamic signaling systems, which transfer chemically encoded information from the extracellular space to intracellular compartments [1,2]. Owing to their omnipresence in human tissues and their relevance in regulating the majority of (patho-)physiological processes, it is not surprising that 35% of all currently marketed drugs directly bind to GPCRs [3]. Many other drugs act indirectly on GPCR functionality by interfering with production (e.g., angiotensin-converting enzyme inhibitors), transport (e.g., serotonin reuptake inhibitors) or metabolism (e.g., acetylcholinesterase inhibitors) of endogenous ligands. GPCR-modulating drugs cover a broad range of therapeutic fields including cardiovascular and metabolic diseases, central nervous system (CNS) disorders, pain and pulmonary diseases, among others [3,4].

Most of the currently marketed drugs were developed under the assumption that GPCRs are simple on–off switches, which is also reflected by the naming of many drug classes (e.g., beta-blockers or dopamine agonists) [5]. In the past decade, X-ray crystallography and novel approaches for pharmacological and biophysical characterization of receptor functions unveiled the functional complexity of GPCRs [6–8]. This includes partial receptor activation, allosteric modulation, receptor oligomerization and biased signaling — also referred to as functional selectivity. The latter phenomenon results from the fact that GPCRs can bind to and signal through various intracellular binding partners such as several G proteins and β-arrestins (Fig. 1) [1]. Every receptor has a naturally imprinted signaling profile, referred to as receptor bias and includes the preference of a receptor for a certain pathway (e.g., the M_2 receptor preferentially couples to G_i and the M_3 receptor to G_q proteins). Biased signaling can further be controlled by the relative expression of intracellular transducers resulting in system bias. The term 'ligand bias' describes how chemically defined ligands shift a receptor's signaling repertoire toward one or a set of distinct pathway(s) [9]. Based on the model of the ternary complex formed by the receptor, a ligand and an intracellular binding partner [10,11], GPCRs can be seen as allosteric systems connecting extra- and intra-cellular regions. On the one hand, agonist binding to the receptor increases the affinity for intracellular transducers but, on the other hand, the fully active receptor conformation requires stabilization by coupling to intracellular binding partners [12,13]. This allosteric coupling mechanism renders GPCRs flexible proteins with dynamic properties that can be controlled and shifted by chemically defined entities. Because different active receptor states exist in a conformational

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https://doi.org/10.1016/j.drudis.2019.02.010

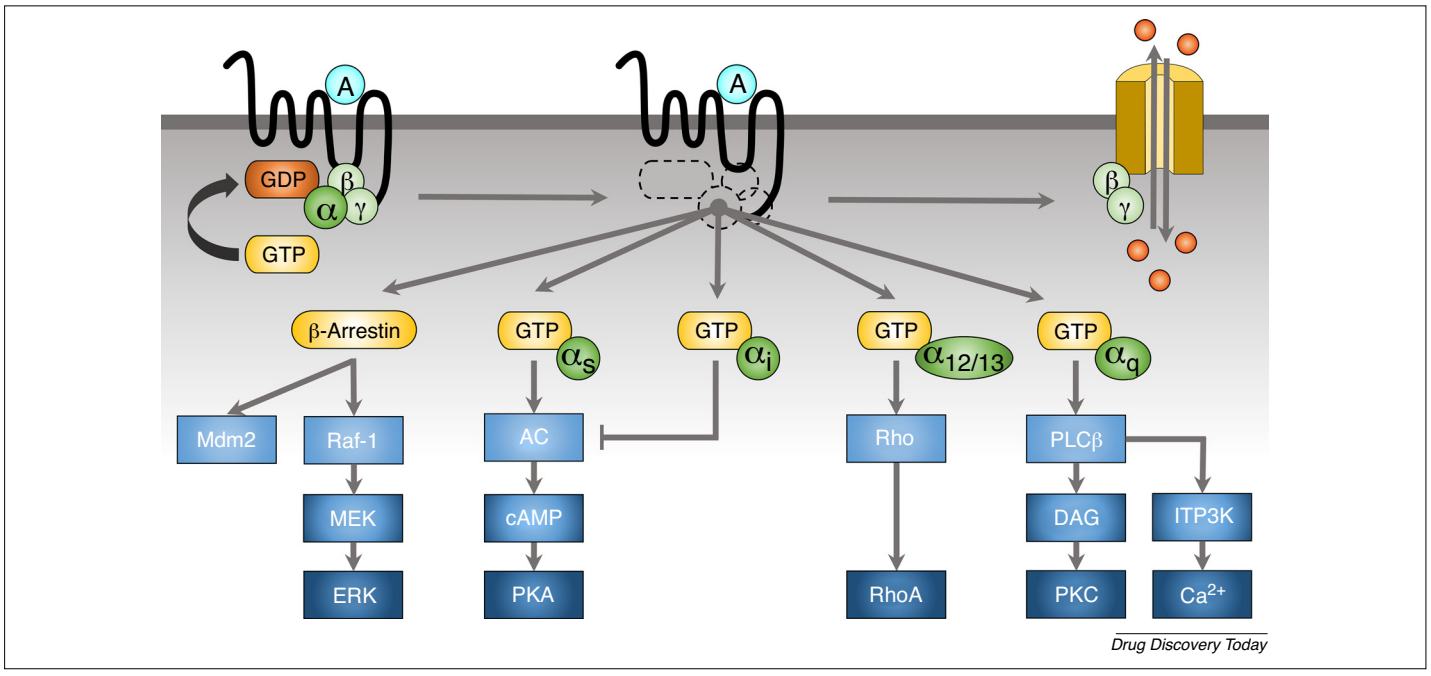

FIGURE 1

Activation, intracellular binding partners and downstream signaling of G-protein-coupled receptors (GPCRs). Abbreviations: A, agonist; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; ITP3K, inositol-trisphosphate 3-kinase; MEK, mitogen-activated protein kinase kinase (also known as MAP2K); Mdm2, mouse double minute 2 homolog; PLCβ, phospholipase Cβ; PKA, protein kinase A; PKC, protein kinase C; Raf-1, RAF proto-oncogene serine/threonine-protein kinase; RhoA, Ras homolog family member A.

equilibrium [14–16], agonists do not necessarily activate GPCRs through the stabilization of only one active state but stabilize a unique ensemble of active states in a ligand-dependent manner resulting in a signal that can be biased toward a specific subset of pathways [17]. Many types of ligand bias exist. In most cases the term 'biased signaling' is used to distinguish between G-protein activation and β-arrestin recruitment (G protein vs β-arrestin); however, shifts in the preference of the G-protein subtype (e.g., G_i over G_s) or with regard to GPCR kinases can also represent cases of biased signaling [1]. Because some of the abovementioned pathways mediate therapeutically desired effects whereas others are linked to adverse drug reactions, biased signaling offers an enormous potential to render pharmacological therapies more effective and reduce adverse effects [18]. However, it is still elusive whether biased signaling effects discovered *in vitro* can be transferred to clinical use [19–21]. In any case, more-specific ligands with tailored signaling bias are needed as pharmacological tools to evaluate the potential therapeutic benefits.

In this review, we focus on drug design strategies for the discovery of biased GPCR ligands. After giving an overview of current challenges in identifying bias, we use G-protein-biased opioid analgesics as a prominent example to describe different approaches for the development of such ligands. Furthermore, we discuss a novel concept for the rational design of biased ligands by extending the molecular ligand structure toward extracellular receptor regions. Finally, we reflect on current and future directions in biased ligand design.

Challenges in studying biased signaling

Owing to the complexity of GPCR functionality, the identification and characterization of biased signaling represents a challenging and multidimensional effort [22]. One of the key steps in early drug discovery is reliable target validation [19]. It was shown *in vivo* that G-protein-biased μ-opioid receptor (MOR) ligands could pave the way toward analgesics with reduced adverse effects [23,24]. By knocking out β-arrestin in mice, Raehal *et al.* reported enhanced and prolonged morphine-mediated analgesia with attenuation of respiratory depression and acute constipation [25]. In another study, Yang *et al.* demonstrated that morphine showed enhanced analgesia in rats that were pretreated with siRNA against β-arrestin without showing naloxone-induced withdrawal symptoms [26]. These data resulted in several different attempts to design G-protein-biased opioid analgesics as effective and safer drugs as outlined in the chapter below. However, for most therapeutic GPCR targets, reliable target validation is still incomplete, which is mainly because of the lack of well-characterized biased ligands as pharmacological tools.

Among the major challenges for generating such pharmacological tools is the limited knowledge about the underlying mechanisms for biased signaling. Recent advances in structural determination of GPCRs bound to different signaling proteins (e.g., β_2-adrenoceptor with G_s, MOR with G_i or rhodopsin with arrestin-1) or biased ligands (e.g., 5-HT_{2B} receptor with co-crystallized LSD) could help to get insights in the structural basis of ligand bias (Fig. 2) [6,27–30]. Complementarily, for many receptors molecular triggers were identified that contribute to biased signaling [31–37]; but a general mechanistic explanation of this effect that can be implemented in drug design is still missing.

Because several signaling pathways exist, many more types of bias can theoretically occur. A major limitation in detecting biased signaling is the challenge to measure and compare as

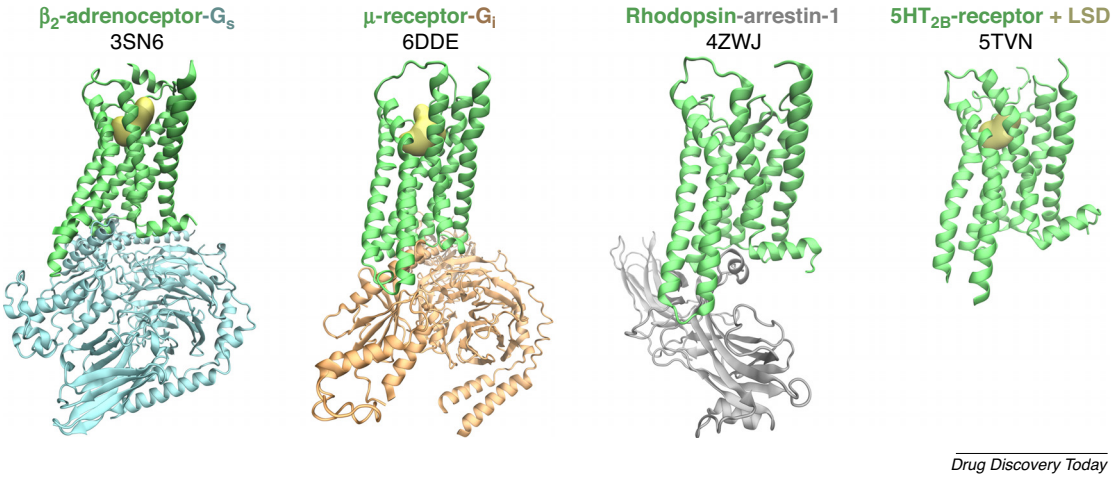

FIGURE 2

Structural insights from G-protein-coupled receptor (GPCR) complexes with different intracellular binding partners or biased ligands provide a valuable source for studying the phenomenon of biased signaling [27–30].

many pathways as possible. Naturally, only bias types can be detected and subsequently characterized, if the participating pathways were investigated before. The selection of assays used is essential for the detection of biased ligands. An excellent recent review by Smith *et al.* provides an overview about assays commonly used to investigate biased signaling [11], and a comparative analysis of bias detection methods is given by Onaran *et al.* [38]. A theoretical framework for describing GPCR agonism is provided by the operational model from Black and Leff, including a system-independent transduction coefficient τ/K_A [17,39]. By selecting two pathways of interest and a reference ligand, the ratio of transduction coefficients ($\Delta\Delta\log(\tau/K_A)$) is probably the soundest method to quantify ligand bias *in vitro* [17]. This shows that the selection of a suitable reference ligand is essential and not trivial, because it should be an 'unbiased' ligand in theory. In many cases, the endogenous ligand is used as the reference, because it is assumed to be unbiased. However, most GPCRs have multiple endogenous ligands, for example epinephrine and norepinephrine physiologically bind to adrenergic receptors. For chemokine receptors, it was demonstrated that several chemokines bind to a set of receptors (e.g., CCR2, CCR5 and CCR7) and activate different pathways, which underlines that signaling bias exists for natural ligands [40]. Recently, it was reported for several chemokine receptors that fine-tuning chemokine receptor signaling could provide beneficial therapeutic effects [41]. Furthermore, many GPCRs are recognized and regulated by metabolites in an allosteric manner [42]. Finally, allosteric modulators can also induce biased signaling, but the type of bias can change depending on the orthosteric ligand [43]. Despite these challenges, several strategies were successfully applied to discover novel GPCR modulators with biased properties.

Strategies for identifying biased ligands

Despite the multitude of challenges, the number of newly identified biased ligands constantly increases. For their identification, three main approaches were followed and represent a promising toolset for drug design. Here, we focus on dopamine receptor studies as examples to highlight the different approaches.

Characterization of existing ligands

The characterization of existing ligands in multiple assays provides a valuable source to identify 'old' drugs or molecules as biased ligands. Only a few studies focus on clinically used ligands with regard to biased signaling (e.g., ligands for the β_2-adrenoceptor by van der Westhuizen *et al.*) [44]. Because most existing GPCR ligands were identified without prior knowledge about (or without the focus on) signaling bias, many of them are likely to be characterized as biased modulators once tested in several pathways. This could be applied to small ligand sets but also in the form of a high-troughput screening (HTS). Weiwer *et al.* performed an HTS of 57 000 compounds for β-arrestin signaling that led to the identification of 609 active compounds, which were subsequently tested for G-protein activation. This resulted in the identification of BRD5814–a β-arrestin-biased D_2 receptor antagonist [45].

Structure-based drug design

Structure-based drug design (SBDD) represents a powerful technique to identify biased ligands. A strength of SBDD approaches is the possibility to discover novel scaffolds, which broaden the chemical ligand space. Structural information on GPCRs in distinct conformations is constantly increasing (Fig. 2), which will foster virtual screening campaigns aimed at identifying ligands with a desired function. Importantly, several GPCR crystal structures in complex with biased ligands have been resolved (e.g., 5-HT1BR and 5-HT2BR in complex with ergotamine), providing valuable insights into structural features causing biased signaling [47]. Increasing structural information is also helpful for the generation and validation of homology models to access structurally undetermined GPCRs [48]. In a recent study a homology model of the D_2 receptor was used to virtually screen a focused library consisting of ~13 000 compounds, all of which consisted of a 2,3-dichlorophenylpiperazine headgroup virtually connected with fragments from the ZINC database via an oxybutyl or an amidopropyl spacer. From 18 selected and synthesized compounds, 16 were active and three showed a bias toward β-arrestin signaling [49].

Synthesis-driven SAR

Synthesis-driven structure-activity relationship (SAR) of biased ligands can explore the chemical space of biased ligands to gain some mechanistic insights that can help to rationally design new molecules based on previously used SAR. Following this approach, Chen *et al.* discovered a partial D_2 receptor agonist with G-protein bias by systematically modifying the well-characterized aripiprazole scaffold [46]. One advantage of this approach is that it provides potential starting points for subsequent lead optimization.

Safe analgesics by biased agonism at opioid receptors

The severe side effects of MOR ligands, such as respiratory depression, constipation and addiction, are a major concern during the development process of new analgesics. Currently marketed drugs show reliable analgesic efficacies, but still express the risk of potentially lethal adverse effects. The correlation of adverse effects with the activation of MOR β-arrestin downstream signaling could be shown in several mouse models [25,26]. Results unveiled enhanced analgesic effects of morphine with reduced constipation, respiratory depression and tolerance in mice. These findings suggested a new path in the development of safer analgesics based on pathway-specific ligands. In the following, we highlight three main approaches for the discovery of biased ligands taking G-protein-biased opioid analgesics as examples (Fig. 3).

The first example TRV-130 (oliceridine) was discovered in 2013 by Chen and co-workers and is one of the first compounds to exhibit the desired high analgesic potency and diminished adverse effects with a high selectivity for MOR (Fig. 3, left) [50,51]. The initial lead compound with G-protein activation in the nanomolar range was discovered through HTS of the internal compound library of Trevena measuring MOR G-protein activation and β-arrestin recruitment. In a campaign studying SAR it was possible to enhance potency and lower β-arrestin recruitment. The lead optimization considered different enantiomers, potential metabolic effects, such as potential glucuronidation and avoiding hERG inhibition, and resulted in the final compound: TRV-130. Studies in mice showed, in equimolar concentration to morphine, similar analgesic efficacies with less respiratory depression and constipation [12]. Hints from a Phase II clinical study suggest a fast and potent pain relief without serious adverse effects [52]. Initial results of Phase III clinical trials indicate positive trends in the safety profile of equimolar TRV-130 compared to morphine supporting the *in vitro* and *in vivo* results (unpublished).

In the second example, Manglik *et al.* utilized an *in silico* SBDD approach for the development of the G-protein biased ligand PZM21 (Fig. 3, center) [53]. Noteworthy, this approach cannot yet reliably find lead structures with tailored signaling efficacies but it can reliably identify entirely new scaffolds and chemotypes, even if the biased properties of PZM21 were probably fortuitous, as reflected by the authors. Three million compounds from the ZINC lead-like set were docked into the inactive MOR crystal structure and were evaluated based on a ligand–receptor complementary score. The 2500 top-scoring compounds have been manually inspected for novelty and key interactions. As an optimization step, 500 analogs of the best three compounds were scored from which the 15 best were tested. Seven out of the 15 tested showed K_i

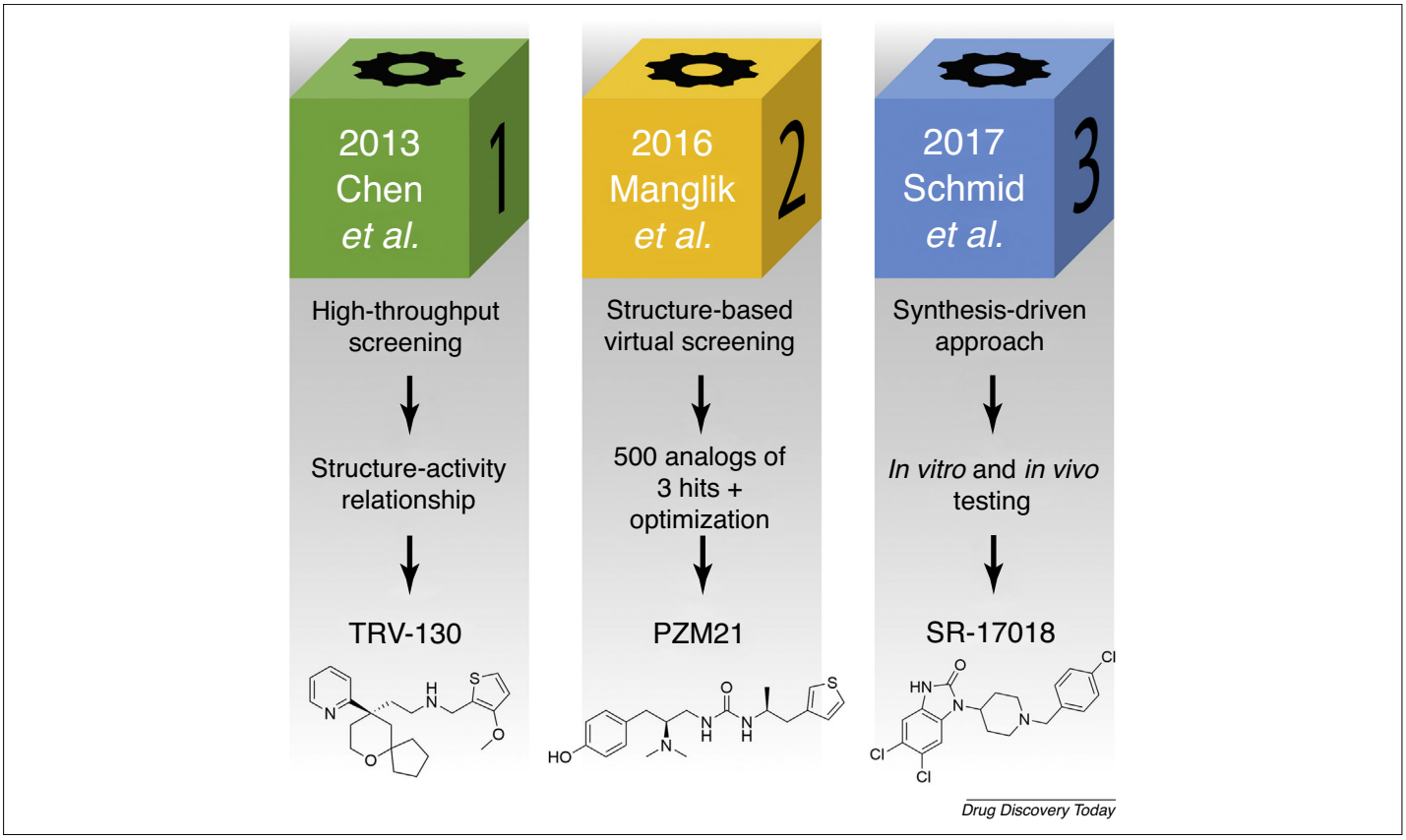

FIGURE 3

Three main approaches for the discovery of biased ligands illustrated by three key examples for designing G-protein biased μOR analgesics [50,53,56].

values from 42 nM to 4.7 μM. The best compound served as the lead structure for further optimization resulting in the final compound: PZM21. Remarkably, PZM21 not only shows low receptor internalization and no detectable β-arrestin recruitment but also leads to KOR antagonism and no relevant activity during a counter-screen against 316 other GPCRs. High analgesic efficacy, which surprisingly is only CNS and not spinally mediated, low constipation and low respiratory depression in mice support these findings. Controversially, Hill *et al.* measured β-arrestin recruitment of PZM21 *in vitro* with no significant difference compared to morphine [54]. Moreover, respiratory depression was found for PZM21 using the same strain of mice and the utilization of naloxone during application of PZM21 could reverse the respiratory depression completely [54]. Similarly, TRV130 has been found to retain undesirable constipating and abuse-related effects during repeated treatment of mice despite its G-protein bias [55]. Owing to the direct contrast of these studies, a final evaluation remains elusive and indicates challenges of biased ligands used in *in vivo* systems.

Third, a study released in 2017 by Schmid *et al.* focuses on an extensive characterization of newly synthesized analogs of 10 1-(1-(1-phenylethyl)piperidin-4-yl)-1,3-dihydro-2H-benzo[d]imidazol-2-one, morphine, fentanyl and sufentanyl (Fig. 3, right) [56]. They addressed the context-based problems of bias factors through a comprehensive comparison of different assay types (cAMP inhibition versus GTPγS assay) and cell–receptor systems (human MOR versus mouse MOR). They observed that high bias factors are less influenced by the assay types or cell systems, which was not the case for low factors. Fentanyl depicts exactly this phenomenon being β-arrestin-biased when calculating the factor based on GTPγS assay over β-arrestin recruitment (bias factor 0.18) but being G-protein biased using cAMP inhibition over β-arrestin recruitment (bias factor 2.8). In general, the GTPγS assay showed a better correlation with *in vivo* data and the calculated therapeutic window. Out of the ten novel and selective MOR analogs, SR-17018 shows the strongest bias toward G-protein signaling (human MOR: GTPγS/β-arrestin bias factor 85; cAMP/β-arrestin bias factor 40) and high analgesic efficacies combined with low respiratory depression.

Although *in vitro* and *in vivo* studies for new biased MOR agonists have revealed promising results supporting the mechanism of biased signaling, it has yet to be shown to what extent these results translate to therapeutic benefits. However, the potential of biased signaling for safe opioid analgesics is not exploited and certainly has considerable therapeutic potential that has been underestimated for decades.

Targeting extracellular receptor regions

GPCRs exist in a conformational equilibrium, including multiple active receptor conformations of which some are specific for a distinct signaling pathway. The ligand-dependent stabilization of pathway-specific receptor conformations therefore represents a promising approach to design biased GPCR modulators. Recently, several studies highlighted the role of extracellular receptor regions for signaling bias [31,32,34,35,37,49,57].

The first mechanistic insights came from studies with the M_2 receptor in which ligand-induced conformational constraints in the extracellular receptor region were found to be the cause for Gi bias of dualsteric (bitopic) ligands such as iper-6-phth and iper-6-naph (Fig. 4) [31,32,57]. The conformational restriction of the extracellular receptor region by allosteric moieties is mainly mediated by ligand contacts with the middle of extracellular loop 2 (EL2) and the beginning of TM7 [31]. A study by McCorvy *et al.* reported similar ligand–receptor contacts of an aripiprazole derivative (Fig. 4) with the EL2, which is linked to β-arrestin bias in the D_2 receptor [34]. This ligand–receptor contact was also found to be involved in ligand bias for serotonin receptors [35].

The influence of extracellular loop conformations on intracellular signaling recently received attention. In a structural analysis

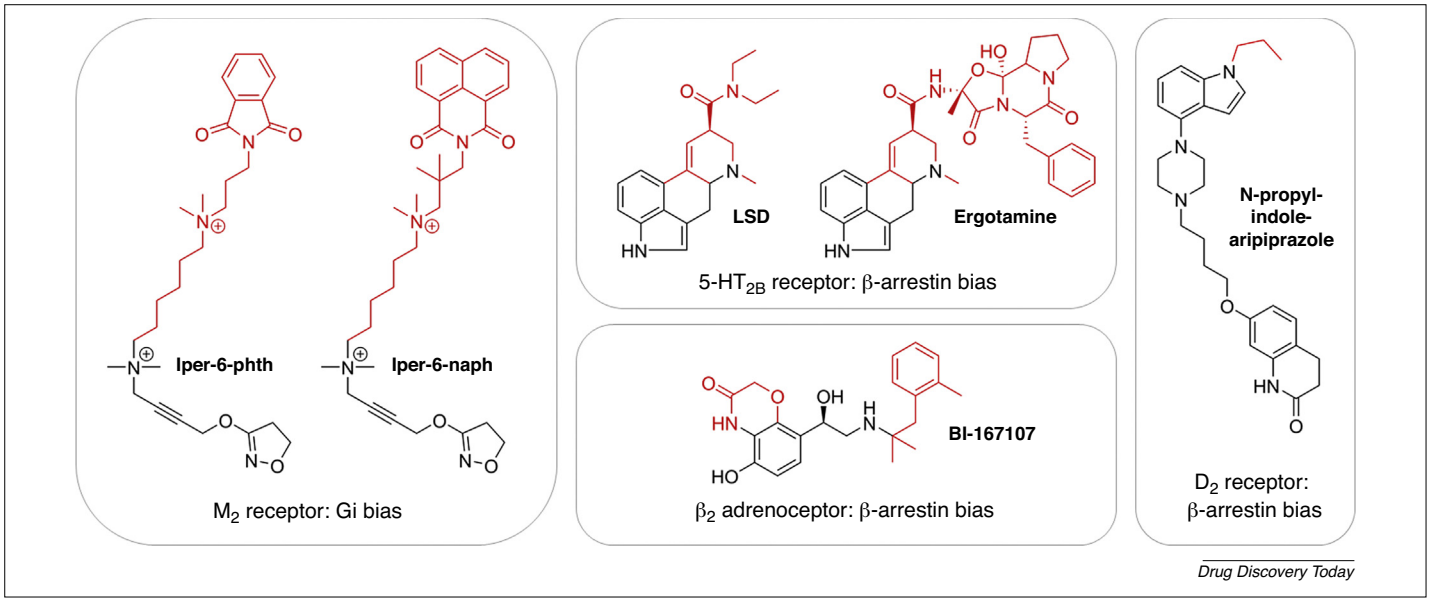

FIGURE 4

Examples of biased ligands with extended binding modes. The core scaffolds of the unbiased mother compounds are represented in black (M2 receptor: iperoxo; 5-HT2B receptor: serotonin; β2 adrenoceptor: adrenaline; D_2 receptor: indole-aripiprazole) and the ligand extensions, which interact with the extracellular receptor domain region, are shown in red.

of crystal structures with biased and unbiased ligands bound to their receptors together with modeling data, Bermudez and Bock surmised a more general concept for the rational design of biased ligands, based on the concept of extending the ligand structure toward extracellular receptor regions [58]. Given that every receptor has a physiological repertoire of signaling pathways that might be activated, it is proposed that signaling bias in many cases results from diminishing a single pathway in a more pronounced way than others. Therefore, ligand-dependent signaling bias could be achieved upon binding of agonists that restrict the conformational receptor space in the orthosteric binding site or the extracellular region [58]. Among the analyzed examples are iperoxo-based dualsteric M_2 agonists showing G_i bias, the β-arrestin-biased compound BI-167107 for β_2-adrenoceptors and LSD and ergotamine at 5-HT_{2B} receptors [30,31,35]. The latter example is particularly interesting, because the ligands were co-crystalized with the 5-HT_{2B} receptor but differ in their strength of β-arrestin bias. Noteworthy, the conformational restriction of the extracellular region is more pronounced for ergotamine, which is in correlation with its stronger bias and its more extended ligand structure compared with LSD (Fig. 4) [30]. This suggests that ligand bias by structural extension might be a titratable phenomenon [58].

These examples indicate that the concept of ligand-dependent conformational restriction of the extracellular receptor region holds the potential to specifically disable one signaling pathway while keeping others active. Recent observations for the biased partial agonist salmeterol at the β_2-adrenoceptor are in accordance with this concept and underline the importance of extracellular receptor regions for signaling bias [33]. The major challenge for tailored design of biased ligands will be the identification of pathway-specific epitopes for the target receptors, because different structure elongations could lead to different bias types. However, for the future, we assume that an increasing number of identified biased ligands resulting from ligand structure extensions toward epitopes of the extracellular loop region will be developed.

Future directions in biased ligand design

Although the number of reported biased ligands constantly increases, most of them were identified by serendipity, because mechanistic understanding of ligand bias remains incomplete. The growing amount of available structural data (X-ray crystallography, Cryo-EM and NMR) of GPCRs in complex with biased ligands or with intracellular binding partners provide a valuable source for structural and dynamic insights in biased signaling and the underlying mechanisms on a molecular level (Fig. 2) [6–8]. Increasing structural information will also foster the application of computational approaches aimed at understanding dynamic properties of GPCRs as a prerequisite for signaling bias [59,60]. We expect that, once these mechanisms are better understood, it will be possible to rationally design drug candidates with tailored signaling properties. A complementary approach could be the ligand-based analysis of biased GPCR modulators in comparison to unbiased ligands. This requires consistent data in terms of the assays used for bias detection, the choice of reference compounds and the cell systems used.

The example of G-protein-biased opioid analgesics demonstrates the importance of target validation and the consequent translation of *in vitro* data to disease-relevant *in vivo* models. A reliable target validation and *in vivo* models represent essential requirements for potential clinical development [19,20]. Another emerging issue in the field of biased signaling is the temporal dimension of GPCR signaling and bias, because intracellular pathways often operate on different timescales [61]. This is particularly important for biased signaling, which is caused by allosteric GPCR modulators [43]. With regard to time-dependent signaling outcomes driven by ligand binding and coupling kinetics, the time at which a measurement takes place could strongly influence the detection and characterization of ligand bias and has therefore to be considered in experiment design [22]. It is still elusive how time-encoded signaling bias might contribute to pharmacological effects *in vitro* and *in vivo*. Furthermore, biased signaling properties might be influenced by many other factors such as tissue-specific characteristics [11,20,22], natural GPCR variants [4] or receptor oligomerization [62].

In addition, active compounds in drug development campaigns are typically subjected to multiparameter optimization including physicochemical properties, target selectivity and ADME properties, among others. In terms of GPCR modulators it must be taken into account that subtle changes in molecular structure can influence ligand bias [63]. By contrast, this could also mean that unbiased lead compounds become biased upon structural modifications during lead optimization, which would change their pharmacological characteristics. Therefore, detection and characterization of ligand bias is not only necessary when aiming at biased ligands but also required for the development of unbiased ligands. Finally, it is very likely that many marketed drugs show an unidentified bias, which would be worth investigation in a systematic manner.

Concluding remarks

Research on biased GPCR modulators represents a highly active and vibrant field in drug discovery. Biased GPCR ligands provide a directed targeting of pathomechanisms in a pathway-specific manner and are therefore thought to have a higher therapeutic efficiency with reduced adverse effects. The constantly increasing number of biased ligands and structural information will help to better understand the underlying mechanisms of biased signaling, which will allow the rational and tailored design of such ligands. Besides HTS, SBDD and synthesis-driven approaches, the extension of ligands toward the extracellular receptor region represents a promising strategy to identify novel biased ligands. However, for a successful development of biased GPCR modulators as drugs, *in vitro* results must be translated to *in vivo* models, and this represents one of the key challenges for future development.

References

1 Rajagopal, S. *et al.* (2010) Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat. Rev. Drug Discov.* 9, 373–386
2 Vass, M. *et al.* (2018) Chemical diversity in the G protein-coupled receptor superfamily. *Trends Pharmacol. Sci.* 39, 494–512
3 Sriram, K. and Insel, P.A. (2018) G protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? *Mol. Pharmacol.* 93, 251–258
4 Hauser, A.S. *et al.* (2017) Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discov.* 16, 829–842

5 Seifert, R. (2018) Rethinking pharmacological nomenclature. *Trends Pharmacol. Sci.* 39, 785–797
6 Glukhova, A. *et al.* (2018) Rules of engagement: GPCRs and G proteins. *ACS Pharmacol. Transl. Sci.* 1, 73–83
7 Hilger, D. *et al.* (2018) Structure and dynamics of GPCR signaling complexes. *Nat. Struct. Mol. Biol.* 25, 4–12
8 Piscitelli, C.L. *et al.* (2015) A molecular pharmacologist's guide to G protein-coupled receptor crystallography. *Mol. Pharmacol.* 88, 536–551
9 Wootten, D. *et al.* (2018) Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nat. Rev. Mol. Cell Biol.* 19, 638–653
10 Kenakin, T. (2017) Theoretical aspects of GPCR–ligand complex pharmacology. *Chem. Rev.* 117, 4–20
11 Smith, J.S. *et al.* (2018) Biased signalling: from simple switches to allosteric microprocessors. *Nat. Rev. Drug Discov.* 17, 243–260
12 DeVree, B.T. *et al.* (2016) Allosteric coupling from G protein to the agonist-binding pocket in GPCRs. *Nature* 535, 182–186
13 Weis, W.I. and Kobilka, B.K. (2018) The molecular basis of G protein-coupled receptor activation. *Ann. Rev. Biochem.* 87, 897–919
14 Bock, A. *et al.* (2016) Ligand binding ensembles determine graded agonist efficacies at a G protein-coupled receptor. *J. Biol. Chem.* 291, 16375–16389
15 Kofuku, Y. *et al.* (2012) Efficacy of the beta(2)-adrenergic receptor is determined by conformational equilibrium in the transmembrane region. *Nat. Commun.* 3, 1045. http://dx.doi.org/10.1038/ncomms2046
16 Nygaard, R. *et al.* (2013) The dynamic process of beta(2)-adrenergic receptor activation. *Cell* 152, 532–542
17 Kenakin, T. and Christopoulos, A. (2013) OPINION signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nat. Rev. Drug Discov.* 12, 205–216
18 Rankovic, Z. *et al.* (2016) Biased agonism: an emerging paradigm in GPCR drug discovery. *Bioorg. Med. Chem. Lett.* 26, 241–250
19 Gundry, J. *et al.* (2017) A practical guide to approaching biased agonism at G protein coupled receptors. *Front. Neurosci.* 11 . http://dx.doi.org/10.3389/fnins.2017.00017
20 Kenakin, T. (2017) Signaling bias in drug discovery. *Expert Opin. Drug Discov.* 12, 321–333
21 Michel, M.C. and Charlton, S.J. (2018) Biased agonism in drug discovery -is it too soon to choose a path? *Mol. Pharmacol.* 93, 259–265
22 Costa-Neto, C.M. *et al.* (2016) A pluridimensional view of biased agonism. *Mol. Pharmacol.* 90, 587–595
23 Chan, H.C.S. *et al.* (2017) Designing safer analgesics via mu-opioid receptor pathways. *Trends Pharmacol. Sci.* 38, 1016–1037
24 Madariaga-Mazon, A. *et al.* (2017) Mu-Opioid receptor biased ligands: a safer and painless discovery of analgesics? *Drug Discov. Today* 22, 1719–1729
25 Raehal, K.M. *et al.* (2005) Morphine side effects in beta-arrestin 2 knockout mice. *J. Pharmacol. Exp. Ther.* 314, 1195–1201
26 Yang, C.H. *et al.* (2011) Antinociceptive potentiation and attenuation of tolerance by intrathecal beta-arrestin 2 small interfering RNA in rats. *Br. J. Anaesthesia* 107, 774–781
27 Kang, Y.Y. *et al.* (2015) Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser. *Nature* 523, 561–567
28 Koehl, A. *et al.* (2018) Structure of the mu-opioid receptor-G(i) protein complex. *Nature* 558, 547–552
29 Rasmussen, S.G.F. *et al.* (2011) Crystal structure of the beta(2) adrenergic receptor-Gs protein complex. *Nature* 477, 549–U311
30 Wacker, D. *et al.* (2017) Crystal structure of an LSD-bound human serotonin receptor. *Cell* 168, 377–389
31 Bermudez, M. *et al.* (2017) Ligand-specific restriction of extracellular conformational dynamics constrains signaling of the M-2 muscarinic receptor. *ACS Chem. Biol.* 12, 1743–1748
32 Bock, A. *et al.* (2012) The allosteric vestibule of a seven transmembrane helical receptor controls G-protein coupling. *Nat. Commun.* 3, 1044
33 Masureel, M. *et al.* (2018) Structural insights into binding specificity, efficacy and bias of a beta(2)AR partial agonist. *Nat. Chem. Biol.* 14, 1059–1066
34 McCorvy, J.D. *et al.* (2018) Structure-inspired design of beta-arrestin-biased ligands for aminergic GPCRs. *Nat. Chem. Biol.* 14, 126–134
35 McCorvy, J.D. *et al.* (2018) Structural determinants of 5-HT2 B receptor activation and biased agonism. *Nat. Struct. Mol. Biol.* 25, 787–796
36 Weichert, D. *et al.* (2015) Molecular determinants of biased agonism at the dopamine D-2 receptor. *J. Med. Chem.* 58, 2703–2717
37 Wootten, D. *et al.* (2016) The extracellular surface of the GLP-1 receptor is a molecular trigger for biased agonism. *Cell* 165, 1632–1643
38 Onaran, H.O. *et al.* (2017) Systematic errors in detecting biased agonism: analysis of current methods and development of a new model-free approach. *Sci. Rep.* 7, 44247
39 Black, J.W. and Leff, P. (1983) Operational models of pharmacological agonism. *Proc. Royal Soc. Series B Biol. Sci.* 220, 141–162
40 Corbisier, J. *et al.* (2015) Biased signaling at chemokine receptors. *J. Biol. Chem.* 290, 9542–9554
41 Foley, J.F. (2018) New connections: taking advantage of bias. *Sci. Signal.* 11, eaav9344
42 Husted, A.S. *et al.* (2017) GPCR-mediated signaling of metabolites. *Cell Metab.* 25, 777–796
43 Lane, J.R. *et al.* (2017) A kinetic view of GPCR allostery and biased agonism. *Nat. Chem. Biol.* 13, 929–937
44 van der Westhuizen, E.T. *et al.* (2014) Quantification of ligand bias for clinically relevant beta(2)-adrenergic receptor ligands: implications for drug taxonomy. *Mol. Pharmacol.* 85, 492–509
45 Weiwer, M. *et al.* (2018) Functionally biased D2R antagonists: targeting the beta-arrestin pathway to improve antipsychotic treatment. *ACS Chem. Biol.* 13, 1038–1047
46 Chen, X. *et al.* (2016) Discovery of G protein-biased D2 dopamine receptor partial agonists. *J. Med. Chem.* 59, 10601–10618
47 Wacker, D. *et al.* (2013) Structural features for functional selectivity at serotonin receptors. *Science* 340, 615–619
48 Bermudez, M. *et al.* (2016) More than a look into a crystal ball: protein structure elucidation guided by molecular dynamics simulations. *Drug Discov. Today* 21, 1799–1805
49 Mannel, B. *et al.* (2017) Structure-guided screening for functionally selective D-2 dopamine receptor ligands from a virtual chemical library. *ACS Chem. Biol.* 12, 2652–2661
50 Chen, X.T. *et al.* (2013) Structure activity relationships and discovery of a G protein biased mu opioid receptor ligand (3-methoxythiophen-2-yl)methyl a2 (9R)-9-(pyridin-2-y1)-6-oxaspiro- 4.5 clecan-9-yl ethylpamine (TRV130), for the treatment of acute severe pain. *J. Med. Chem.* 56, 8019–8031
51 DeWire, S.M. *et al.* (2013) A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphines. *J. Pharmacol. Exp. Ther.* 344, 708–717
52 Viscusi, E.R. *et al.* (2016) A randomized, Phase 2 study investigating TRV130, a biased ligand of the mu-opioid receptor, for the intravenous treatment of acute pain. *Pain* 157, 264–272
53 Manglik, A. *et al.* (2016) Structure-based discovery of opioid analgesics with reduced side effects. *Nature* 537, 185–190
54 Hill, R. *et al.* (2018) The novel mu-opioid receptor agonist PZM21 depresses respiration and induces tolerance to antinociception. *Br. J. Pharmacol.* 175, 2653–2661
55 Altarifi, A.A. *et al.* (2017) Effects of acute and repeated treatment with the biased mu opioid receptor agonist TRV130 (oliceridine) on measures of antinociception, gastrointestinal function, and abuse liability in rodents. *J Psychopharmacol* 31, 730–739
56 Schmid, C.L. *et al.* (2017) Bias factor and therapeutic window correlate to predict safer opioid analgesics. *Cell* 171, 1165–1175
57 Bermudez, M. *et al.* (2015) Structural characteristics of the allosteric binding site represent a key to subtype selective modulators of muscarinic acetylcholine receptors. *Mol. Inform.* 34, 526–530
58 Bermudez, M. and Bock, A. (2019) Does divergent binding pocket closure drive ligand bias for Class A GPCRs? *Trends Pharmacol. Sci.* . http://dx.doi.org/10.1016/j.tips.2019.02.005
59 Bermudez, M. and Wolber, G. (2015) Structure versus function –the impact of computational methods on the discovery of specific GPCR-ligands. *Bioorg. Med. Chem.* 23, 3907–3912
60 Lee, Y. *et al.* (2018) Recent advances in structure-based drug design targeting class A G protein-coupled receptors utilizing crystal structures and computational simulations. *J. Med. Chem.* 61, 1–46
61 Grundmann, M. and Kostenis, E. (2017) Temporal bias: time-encoded dynamic GPCR signaling. *Trends Pharmacol. Sci.* 38, 1110–1124
62 Zhou, B. and Giraldo, J. (2018) An operational model for GPCR homodimers and its application in the analysis of biased signaling. *Drug Discov. Today* 23, 1591–1595
63 Tan, L. *et al.* (2018) Biased ligands of G protein-coupled receptors (GPCRs): structure–functional selectivity relationships (SFSRs) and therapeutic potential. *J. Med. Chem.* 61, 9841–9878