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Biased ligands at opioid receptors: Current status and future directions

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Abstract

The opioid crisis represents a major worldwide public health crisis that has accelerated the search for safer and more effective opioids. Over the past few years, the identification of biased opioid ligands capable of eliciting selective functional responses has provided an alternative avenue to develop novel therapeutics without the side effects of current opioid medications. However, whether biased agonism or other pharmacological properties, such as partial agonism (or low efficacy), account for the therapeutic benefits remains questionable. Here, we provide a summary of the current status of biased opioid ligands that target the μ- and κ-opioid receptors and highlight advances in preclinical and clinical trials of some of these ligands. We also discuss an example of structure based biased ligand discovery at the μ-opioid receptor, an approach that could revolutionize drug discovery at opioid and other receptors. Last, we briefly discuss caveats and future directions for this important area of research.

Introduction

Opioids, such as morphine, have traditionally been and continue to be among the most potent painkillers in clinical settings (1, 2). However, the notorious worldwide “opioid epidemic” (3) has ignited the search for safer opioids, bringing this area to the forefront of novel drug discovery. A major problem with current opioids is that, at high doses, they suppress respiration and, with overdose, can be lethal. In the past few decades, the use of

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prescribed opioids (morphine, oxycodone, and hydrocodone) or nonpharmaceutical drugs (heroin and carfentanil) has risen at an alarming rate (2) for pain relief and various recreational uses. Common outcomes due to repeated or long-term licit and illicit use of these opioids are tolerance, dependence, and death. A potential framework to overcome opioid side effects has emerged in the last decade based on the design and discovery of functionally selective ligands at opioid receptors. In this review, we discuss the current status, major challenges, and future perspectives of functionally selective or biased opioid ligands as potential therapeutics to overcome the problems associated with conventional opioid-based drugs.

Opioid Receptors: Signaling And Regulation

Although opioid receptors were postulated to mediate the actions of drugs such as morphine and nalorphine more than 60 years ago (4), it was not until the 1970s that scientists were able to biochemically demonstrate the existence of opioid receptors (5). Several groups used radioligand-binding techniques to devise simple biochemical assays to distinguish agonists from antagonists based on their differential sensitivity to physiological amounts of sodium (6). Subsequently, multiple opioid receptors, which were postulated earlier based on elegant in vivo studies (7), were directly validated by radioligand-binding studies (8–10) and other approaches (11). Eventually, opioid receptors were isolated through molecular cloning (12–15), resulting in the identification of a small subfamily of receptors that are widely expressed on the surface of central and peripheral neuronal cells.

Opioid receptors belong to the class A subgroup of seven-transmembrane domain, G protein–coupled receptors (GPCRs) (16). They include three main subtypes referred to as the μ-, δ-, and κ-opioid receptors (μ-OR, δ-OR, and κ-OR, respectively), together with the nonclassical nociceptin opioid receptor (NOP). Once the opioid receptor is activated by opioids, conformational changes from the extracellular ligand-binding site to the intracellular end of the receptor occur (17–19) and rapidly lead to the coupling and activation of intracellular heterotrimeric $G_{i/o}$ family proteins (Fig. 1). The general paradigm of signaling downstream of opioid receptors mediated by heterotrimeric G proteins includes the inhibition of cyclic adenosine monophosphate (cAMP) production, which attenuates the activity of effector protein kinases, for example, cAMP-dependent protein kinase, and decreases neuronal firing (20). In addition, the Gβγ subunits modulate calcium channels to suppress Ca^{2+} influx and therefore attenuate the excitability of neurons and inhibit the release of pronociceptive neuropeptides (21–23). Moreover, Gβγ subunits can also activate G protein–coupled inwardly rectifying potassium channels (GIRKs), leading to the hyperpolarization of the cell membranes and, thereby, repression of neuronal excitation (24). Furthermore, Gβγ subunits directly interact with the soluble *N*-ethylmaleimide–sensitive factor-attachment protein receptor (SNARE) complexes, inhibiting the presynaptic release of neurotransmitters for several $Gi/_o$-coupled receptors, such as the α_2-adrenergic and 5-hydroxytryptamine 1B ($5\text{-}HT_{1B}$) serotonin receptors (25–27). However, whether the Gβγ-SNARE interaction plays a similar role downstream of opioid receptor activation requires further study.

Similar to most studied GPCRs, activated opioid receptors are phosphorylated on their cytoplasmic loops and C terminus by GPCR kinases (GRKs) (28–32), which is followed by the recruitment of multifunctional proteins called β-arrestins. Initially, β-arrestins were considered as "negative" regulators that attenuate G protein signaling through steric inhibition of G protein binding (desensitization) and promotion of receptor endocytosis through a clathrin-coated mechanism (internalization and degradation) (33). Since 1999, however, it has been evident that β-arrestins can also serve as scaffolding proteins to mediate downstream signaling independently of G protein signaling (34), and studies have provided detailed insights into the structural and molecular details responsible for the activation of β-arrestins and subsequent signaling (35–39). Briefly, phosphorylation of the cytoplasmic tail of an activated GPCR can first confer high-affinity binding to β-arrestins through engagement of both the cytoplasmic tail and intracellular core region (36). In addition, GPCRs can form low-affinity complexes with β-arrestins through the receptor core alone (35). Both interaction modes could trigger arrestin-mediated signaling, including perhaps activation of extracellular signal–regulated kinases 1 and 2 (ERK1/2), members of the family of mitogen-activated protein kinases (MAPKs). Note that although ERK activation has been identified as a frequent consequence of GPCR activation, the role of G proteins and arrestins in ERK activation remains controversial (40–43). Smith *et al.* (44) found that ERK activation downstream of G_i-coupled receptors requires the engagement of both G protein and β-arrestin. Their data suggest that the G_i:β-arrestin complex directly interacts with and activates ERKs because disruption of the G_i:β-arrestin interaction impaired ERK activation. This study thus introduced an additional noncanonical signaling mechanism mediated by the G_i:β-arrestin complex, which is distinct from the classical pathways of G protein– and arrestin-mediated signaling. Although opioid receptors are also G_i-coupled receptors, whether the G_i:β-arrestin complex plays a similar role in the presence of opioids remains to be studied.

Whereas the role of G proteins in opioid-elicited analgesia has been well characterized, how arrestins are involved in both therapeutic effects and side effects remains controversial. One of the main challenges is that, so far, no unambiguous readout of signaling specific for arrestins is available. β-Arrestins nucleate many downstream signaling mediators, including MAPKs, Akt, the transcriptional regulator nuclear factor κB, and phosphoinositide 3-kinases, and thereby could regulate a diverse array of signaling pathways (Fig. 1) (45). Many of these pathways have been implicated in mediating some forms of signal transduction distinct from G protein signaling. For example, β-arrestin recruitment mediates activation of the kinase Src and subsequently regulates the activity of ERK1/2 (34). This signaling pathway is potentially important for many consequences in vivo because it is involved in regulating dopaminergic neurotransmission and behaviors (46). The arrestin-dependent Akt pathway could, on the other hand, stimulate mechanistic target of rapamycin (mTOR) signaling and subsequent protein translation (47). In this regard, the mTOR pathway was implicated through phosphoproteomic studies of κ-OR agonist–mediated aversion (48). Remarkably, mTOR inhibitors were subsequently demonstrated to repress κ-OR agonist–induced aversion (48). Again, it is important to recognize that most of these signaling pathways can also be activated by GPCR-independent receptors, such as growth factors and

integrins. Ultimately, then, the dependence of a particular opioid-dependent signaling function on arrestins remains unclear.

Biased Agonism At Opioid Receptors

Some ligands that can equally activate G protein and β-arrestin pathways are referred to as “balanced” agonists. These balanced agonists include endogenous peptides (for example, endomorphins, enkephalins, and dynorphins) and small-molecule ligands (for example, fentanyl and salvinorin A). Correspondingly, ligands that can preferentially activate G protein or β-arrestin pathways at a single receptor are called “functionally selective” or “biased” agonists. Several opioid ligands, such as TRV130 (oliceridine), PZM21, RB-64, and triazole 1.1, display G protein–biased activity in vitro. This type of functional selectivity has been proposed to be due to the distinct receptor conformations that can be stabilized by the biased agonists, thereby promoting differential coupling to signaling effectors (49). A key to understanding the molecular mechanisms for GPCR functional selectivity will be to obtain structures of receptors bound to biased agonists in multiple transition states. There are no structures of opioid receptors bound to G protein or arrestin-biased ligands currently available. With the stabilization of a llama-derived nanobody, we identified an inactive-state κ-OR (50) that displays distinct conformational features from the previously solved agonist- or antagonist-bound κ-OR structures (18, 51), which supports the existence of conformational dynamics in opioid receptors. Structural and computational studies of angiotensin II type 1, D2-dopamine, and 5-HT_{2A} and 5-HT_{2B} serotonin receptors suggest that arrestin-biased ligands could induce or stabilize specific conformational states that favor the coupling of arrestin but not G protein (52–56).

Although biased agonists have demonstrated therapeutic potential (for example, G protein–biased μ-OR agonists show analgesia with reduced side effects), how this may translate into different physiological responses in vivo in humans is still not well understood. In particular, traditional bias paradigms focusing on G proteins and β-arrestins are based on the assumption that a GPCR predominantly couples to one G protein and one β-arrestin protein (Fig. 2). However, mammalian cells encode 16 different G protein subtypes and 4 major β-arrestins. Opioid receptors, such as μ-OR, have long been known to couple to multiple G proteins (G_{i1}, G_{i2}, G_{i3}, G_oA, G_{oB}, G_z, and $G_{ustducin}$) and arrestins (β-arrestin1 and β-arrestin2) with varying efficacies and kinetics (57, 58). Therefore, it is likely that GPCRs may produce unexpected effects by coupling to other G proteins over the main subtypes through a process known as transducer bias (Fig. 2). For example, G_z is predominantly expressed in neuronal cells and couples to κ-OR and μ-OR (57). Whether G_z is involved in any opioid-mediated effects remains undefined, although reductions in the actions of morphine were observed in G_z knockout mice (59). Interestingly, G_z could selectively compete with the binding of $G\alpha_{i/o}$ to G_i-coupled GPCRs (for example, D2-dopamine or opioid family), antagonizing the function of the other G_i proteins (60). Consistent with this observation, a transducerome analysis of κ-OR agonists using cell-based assays showed that G_z is more preferred than other G protein subtypes in terms of potency and efficacy (58). On the other hand, β-arrestin1 and β-arrestin2 also mediate separate cellular responses, which suggests that their roles may be nonredundant (61). Thus, measurements of ligand bias should clarify which G protein or β-arrestin subtype is being evaluated. In vivo, another

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manifestation of biased signaling is secondary or so-called “system bias,” which is due to variations in the relative abundances of receptor, G protein, and arrestin subtypes between different cellular or tissue environments, for example, plasma membrane versus nucleus membrane and striatum versus cortex (Fig. 2). System bias imposes unique bias paradigms because the stoichiometry between receptors and G proteins or β-arrestins can determine distinct signaling profiles (62, 63).

Another well-studied example of system bias relates to GRKs, which display cell type– and tissue type–specific expression patterns. It is Ligands may elicit differential coupling of heterotrimeric G proteins versus β-arrestins, as well as differential coupling of G protein subtypes and β-arrestin isoforms. In addition, some ligands may also elicit context-specific bias, for example, in different tissues expressing opioid receptors.not known at present which GRKs (for example, GRK2, GRK3, and GRK5) are coexpressed with the localized receptors at specific neurons. Given that GRK-mediated phosphorylation is subtype specific (64), differential GRK accessibility could, conceivably, directly affect the degree of arrestin coupling and therefore shift signaling in either a balanced or biased direction (65). Accordingly, the consequence of GPCRs coupling to one GRK or arrestin subtype can be distinct, similar to the aforementioned consequences of distinct G protein subtype engagement. Future evaluation of biased agonism needs to take into consideration the potential complexities of the signal transduction pathway.

Therapeutic Potential And Challenges Of Biased Agonism In Opioid Receptors

Studies focused on the traditional bias (G proteins versus β-arrestins) have suggested distinct functional outcomes of each pathway when activated by opioid receptors. In particular, opioid-related analgesia is proposed to be mediated by the G_i-dependent pathway, whereas several adverse effects, such as tolerance, addiction, and respiratory depression, are mediated by the β-arrestin pathway at μ-OR. For example, Bohn and colleagues reported that β-arrestin2 knockout mice display enhanced morphine-mediated analgesia but have reduced side effects (66, 67). Similarly, Bruchas *et al.* and Ehrich *et al.* (68, 69) reported that the analgesic actions of κ-OR agonists require different signaling pathways from those responsible for their aversive effects. Additional studies showed that β-arrestin2 knockout mice display differential responses to κ-OR agonists when compared with their wild-type littermates (70).

Inspired by these studies, the search for safer and more efficacious opioids has focused on G protein–biased agonists at opioid receptors. Several such ligands, including natural products (her-kinorin and mitragynine), synthetic small molecules (TRV130, SR-17018, and PZM21), and peptides (cyclopeptide) have been shown in vitro by some groups to display preference toward G protein signaling at the μ-OR (Fig. 2). A few of these compounds have advanced to animal or clinical studies and they have maintained their therapeutic potential in vivo (Table 1). In particular, TRV130 (oliceridine), as a G protein–biased agonist at μ-OR, was approved by the Food and Drug Administration (FDA) in 2020 for the treatment of moderate to acute pain. Similarly, a number of studies have identified, characterized, and optimized G

protein–biased κ-OR ligands over the past several years, such as 6-GNTI, RB-64, triazole 1.1, and nalfurafine (Fig. 2 and Table 2). κ-OR agonists produce analgesia without the side effects associated with μ-OR agonists, such as euphoria or respiratory depression. However, κ-OR activation in vivo is frequently associated with other side effects, such as dysphoria and psychotomimesis (71). Preclinical studies of G protein–biased κ-OR agonists suggest that they could avoid such side effects, which supports their therapeutic potential as nondysphoric (for example, triazole 1.1) or nonhallucinogenic (for example, nalfurafine) analgesics (72, 73). Furthermore, coadministration of nalfurafine and morphine could substantially enhance the analgesic activity but inhibit the addictive potential of morphine (74), which provides an additional option in the applications of G protein–biased agonists.

Several independent studies focused on μ-OR agonists have questioned the role of arrestin signaling in mediating μ-OR–related side effects. Because the recruitment of β-arrestin to μ-OR is dependent on receptor phosphorylation, Kliewer *et al.* (75) generated phosphorylation-deficient μ-OR mutants that fail to recruit β-arrestin and subsequently generated knock-in mouse lines expressing these mutant receptors. Compared to wild-type mice, these phosphorylation-deficient knock-in mice exhibit a substantially greater analgesic response to fentanyl and morphine. This enhanced response presumably arises from reduced receptor desensitization because of the lack of β-arrestin recruitment. Surprisingly, however, both fentanyl and morphine induce profound respiratory depression, constipation, and hyperlocomotion in these knock-in mice, which were the opposite responses to what one would predict based on the biased signaling paradigm discussed earlier. Interestingly, fentanyl- and morphine-induced tolerance was markedly blunted in these knock-in models, albeit at different levels. In another study, Kliewer *et al.* (76), together with two other groups, in experiments with β-arrestin2 knockout mice found that morphine- or fentanyl-induced respiratory depression is maintained. Regarding G protein–biased μ-OR agonists, Gillis *et al.* (77) showed that TRV130, PZM21, and SR-17018 had low intrinsic efficacies compared with drugs such as fentanyl and that their reduced side effect profile was correlated with partial agonism rather than biased signaling per se. Accordingly, efforts aimed at only reducing β-arrestin recruitment to μ-OR might not improve the safety profile of opioids (78). In considering these studies, however, one must acknowledge that many of the aforementioned experiments relied on mice in which these genetic manipulations were maintained over the entire life span of the animal and that subsequent compensatory changes could complicate the interpretation of the results.

Whereas the studies described earlier originate from a number of independent laboratories and use rigorous experimental frameworks to establish the therapeutic potential of biased agonism, additional studies will be required to better understand and corroborate the intriguing findings described here. In particular, we advocate for an even more careful and extensive analysis of biased signaling going forward (Fig. 2) because opioid receptors can interact with multiple G proteins and arrestins (58). The consequences of signaling by individual G proteins or arrestins remain understudied. In addition, the available G protein–biased agonists are not particularly biased with respect to G protein signaling, and extremely biased tool compounds will be needed to test the hypothesis definitively. Note also that almost all of the currently marketed opioid drugs exert their analgesic effects through the μ-OR (79). Thus, it is likely that ligands targeting other pain-related opioid receptors (for

example, κ-OR, δ-OR, and NOP) together with nonopioid receptors (for example, cannabinoid receptors) could be potential alternatives to current opioids. Although not within the scope of this review, G protein–biased δ-OR agonists also demonstrate therapeutic potential without proconvulsive activity or analgesic tolerance as typical δ-OR agonists do (80).

Approaches To Identify New Scaffolds For Biased Ligands At Opioid Receptors

There have been two major approaches for discovering biased ligands at opioid receptors and other pain-related therapeutic targets. The first involves physically screening available libraries of compounds and ultimately testing derivatives on cells and animals to identify those with the desired characteristics (81–84). Although this approach is time-consuming, almost all of the clinically approved drugs were found in this way, including most of the biased opioid ligands described earlier. The advantage of this approach is that the tested ligands can be directly evaluated in assays to examine pathway selectivity. Several assays are now available to investigate the signaling pathways of interest, such as G protein activation, for example, with cAMP inhibition or bioluminescence resonance energy transfer (BRET) assays, and β-arrestin recruitment, for example with parallel receptorome expression and screening via transcriptional output, with transcriptional activation following arrestin translocation (PRESTO-Tango) or BRET assays (85–87). The advantages and disadvantages of these assays have been previously discussed (88). Again, these assays can robustly monitor G protein association or arrestin recruitment. When using these assays, similar levels of amplification should be used to test the same ligand, and a known unbiased ligand should be included in parallel as a reference (for example, DADLE for δ-OR, U50,488 for κ-OR, and DAMGO for μ-OR) to minimize systematic bias introduced by the different assays. The potencies and efficacies of the tested ligands in each signaling pathway are then normalized to the reference ligand to minimize the influence of system bias. This normalized curve (G protein or arrestin pathway) is then integrated into pharmacological models to quantify bias propensity toward one signaling pathway over the other (also referred to as a biased factor; for example, the biased factor of a reference ligand is 1) (89–91). Thus, this bias factor is a parameter that enables one not only to evaluate preference for a pathway (G protein or β-arrestin) but also to compare the bias potential between multiple ligands at a single receptor.

The second approach for the rapid discovery of biased ligands involves structure-guided ligand screening and optimization (Fig. 3). In this approach, hundreds of thousands (92) to more than 100 million compounds (93) can be docked into the binding pocket of a GPCR structure (92, 94) or a homology model (95, 96), and the top-ranking compounds can be physically tested in vitro (96) and in vivo (95). Because this is a computation-based approach, some potential ligands might be missed during the triaging of potentially active compounds. Nonetheless, the explosion of high-resolution GPCR structures provides the potential that this method represents a fruitful approach to discover and optimize novel ligands using a structure-guided approach. Thus, from such a large-scale docking campaign followed by rational design, Manglik *et al.* (97) identified a new chemotype, PZM21 (Fig.

2), which is a G protein–biased agonist at μ-OR that is very similar to TRV130. Briefly, PZM21 reduces affective pain responses in mice and is devoid of both respiratory depression and constipation. It also does not produce conditioned place preference or stimulate locomotion activity (97). With partial efficacy in the hot plate assay, however, PZM21 does not show effectiveness in the tail-flick test or spinal reflexive response. Together, these findings suggest that the actions of PZM21 are complicated, which may involve other pathways or targets. For example, PZM21 also displays antagonist activity at the κ-OR. Another study (98) reported that PZM21 acts as a balanced agonist at μ-OR and causes respiratory depression similarly to morphine. Thus, the in vivo actions of PZM21 and its pharmacodynamics require further study (99). Furthermore, Ehrlich *et al.* (100) reported that PZM21 retains G protein–biased activity in native neurons. Note that both PZM21 and TRV130 have modest degrees of assay-dependent G protein bias (97) and that compounds with more extreme bias would be better compounds to ultimately test the hypothesis.

Another study used structure-based virtual screening approaches with the crystal structure of κ-OR and identified 11 previously uncharacterized submicromolar κ-OR ligands (101). Of these, the best ligand exhibited a binding affinity of approximately 100 nM for the κ-OR, and another ligand identified in this screen, referred to as “compound 81,” was identified as a potent G protein–biased agonist with minimal β-arrestin2 recruitment [median effective concentration (EC_{50}) = 0.53 μM for G protein activation and an EC_{50} = 8.1 μM for β-arrestin2 recruitment]. Together, these two examples underscore the inherent potential of the structure-based discovery of biased opioid receptor ligands and the likelihood that it will emerge as an even more powerful approach when more structures of opioid receptors in complex with biased ligands become available. Note that both the physical and virtual screening approaches usually do not directly lead to the identification of biased ligands and that the compounds identified typically require further extensive medicinal chemistry optimization.

Concluding Remarks

G protein–biased opioid receptor ligands provide a potentially valuable framework to develop novel therapeutics with minimized side effects aimed at overcoming the increasing burden of the opioid crisis. The approval of TRV130 by the FDA, although with safety concerns, together with other promising preclinical studies at κ-OR or δ-OR will continue to make biased agonists as candidates for potential opioid alternatives. Going forward, a more detailed understanding of opioid receptor signaling and regulation, particularly using more profoundly biased ligands for in vivo studies, will be of crucial importance to ensure that the hypothesis is adequately tested in clinical trials. Assays that unambiguously reflect G protein or β-arrestin–dependent signaling are also urgently needed for screening purposes before these probes can be advanced to animal studies or clinical trials. The rapid emergence of structures of GPCRs in complex with ligands of distinct efficacy profiles and specific signal transducers is likely in the next few years. Information from the atomic-level images will improve our current understanding of the structural determinants that drive biased signaling and therefore are likely to be a major guiding platform for the efficient discovery of biased ligands. For example, the identification of specific ligand-receptor interactions that control the equilibrium between receptor functional states can critically facilitate the design of

biased ligands. Strong evidence for this approach is already emerging (54, 55). Because GPCRs share many features for activation-related conformational changes, it is likely that the knowledge gained from nonopioid receptor systems will also be applied and translated to opioid receptors for the design of highly biased ligands.

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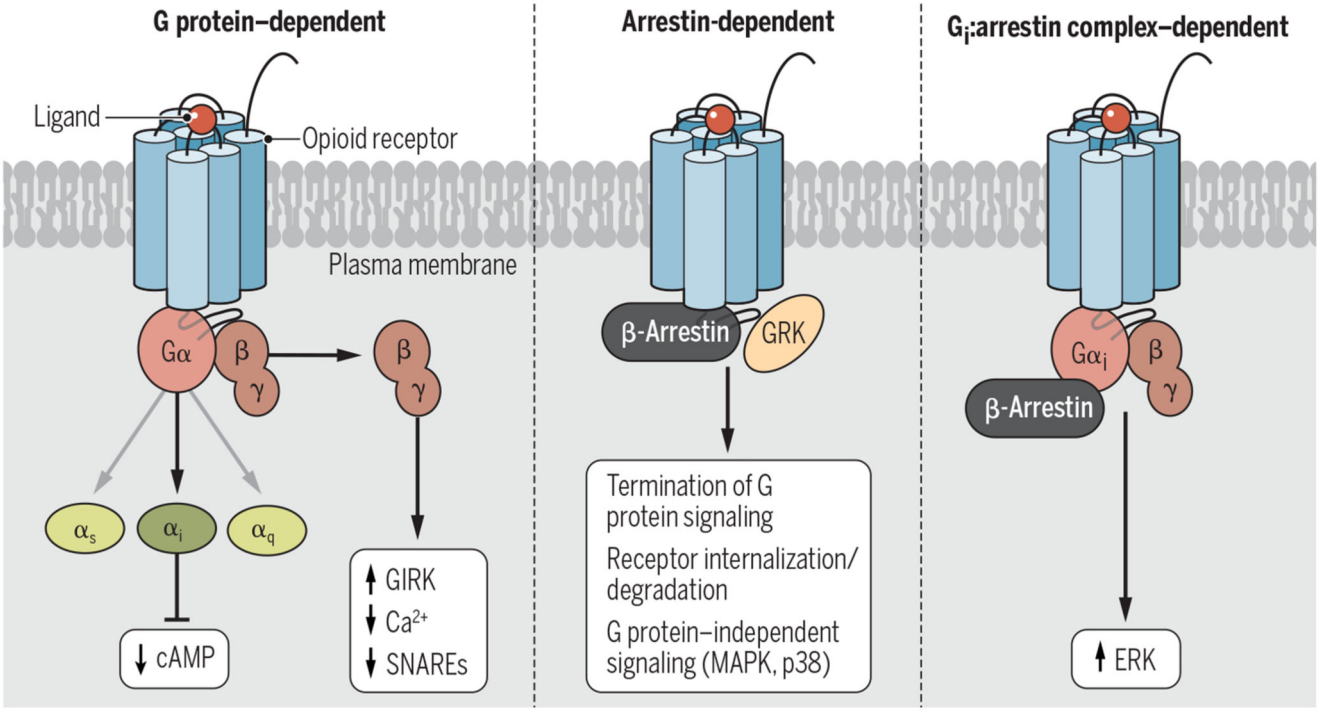

Fig. 1. Signaling and regulatory paradigms of opioid receptors.

(**Left**) Agonist stimulation leads to the coupling of opioid receptors to heterotrimeric G proteins, resulting in a reduction in cAMP abundance, a decreased Ca^{2+} response, and the activation of GIRK channels. (**Middle**) Subsequently, the receptor is phosphorylated by GRKs, which results in β-arrestin recruitment, receptor desensitization, and internalization. β-Arrestins also mediate the activation of various signaling pathways, including those of the MAPKs, ERK1/2, and p38. (**Right**) $G\alpha_i$ protein and β-arrestins can also interact with each other and form a complex to mediate downstream signaling, such as ERK activation.

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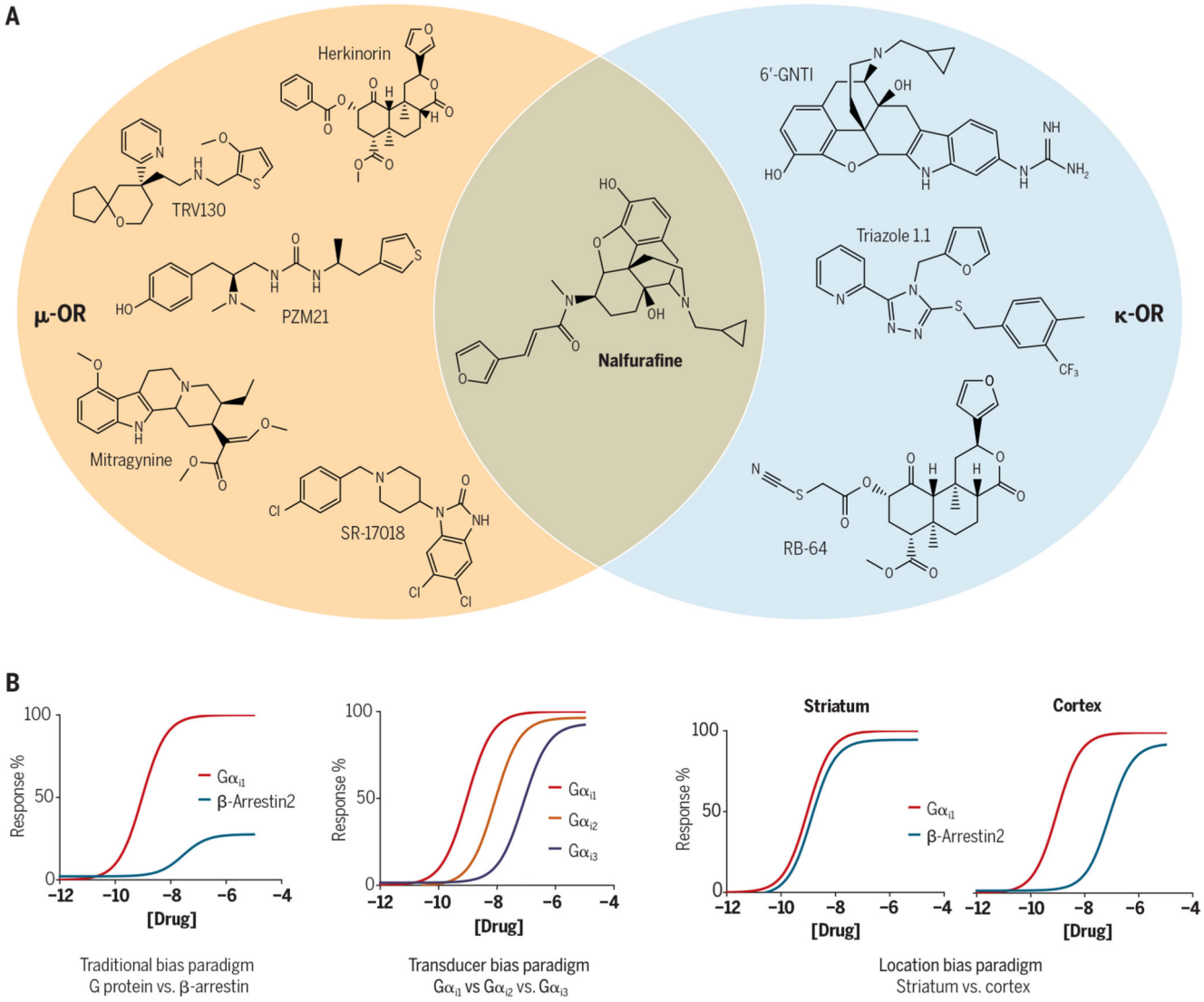

Fig. 2. Chemical space shared by the currently reported G protein–biased ligands at μ-OR and κ-OR.

(**A**) Chemical structures of currently described G protein–biased ligands at the μ-OR and κ-OR. Of these, nalfurafine shows moderate selectivity at κ-OR compared with μ-OR and δ-OR, whereas the others exhibit substantial selectivity for the different receptors. (**B**) The different types of ligand bias that can be potentially manifested by opioid receptors.

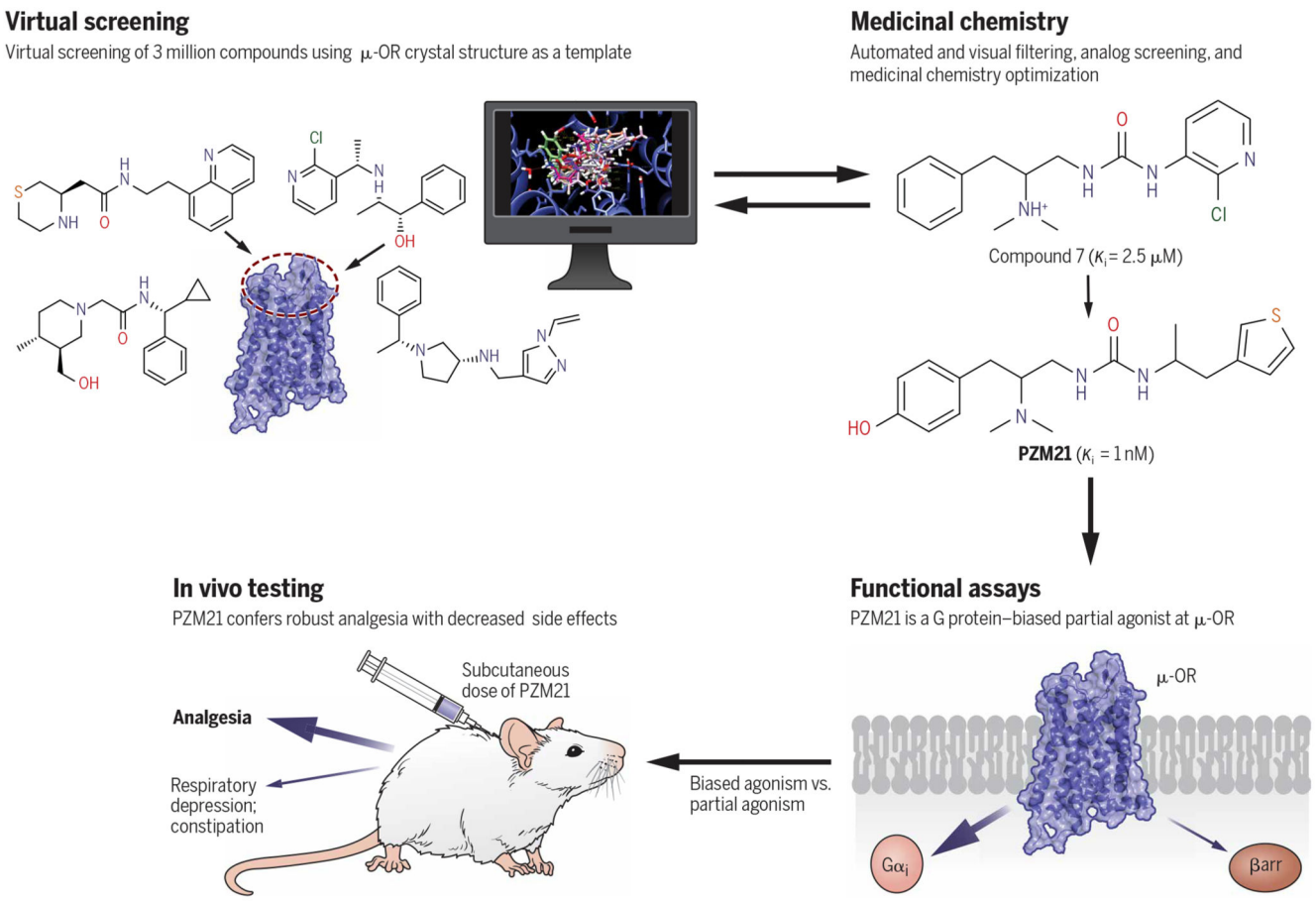

Fig. 3. Structure-based discovery and optimization of a G protein–biased μ-OR ligand, PZM21.

Schematic representation of the discovery and optimization pipeline using structure-guided virtual screening. Using the crystal structure of the μ-OR, a large set of chemical compounds was virtually screened, which was followed by the identification of a handful of lead compounds for further testing. Subsequent optimization and structure-function relationship studies yielded PZM21, which is a G protein–biased μ-OR partial agonist, and produced desirable analgesic activity in vivo without the typical side effects observed with other μ-OR agonists, such as morphine.

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Table 1

G protein-biased ligands at the μ-OR.

ND, no data available.

Ligand	In vitro		In vivo		Reference
	G protein activation	β-Arrestin recruitment	Administration and dose	Outcome	
Oliceridine (TRV130)	EC_{50} = 7.94 nM Efficacy = 84%	EC_{50} = 5.01 nM Efficacy = 15%	ND		(81)
	EC_{50} = 8 nM Efficacy = 71%	Efficacy = 14%	C57BL/6J mice Subcutaneous, 1 mg/kg	•Peak analgesia in 5 min •Reduced central nervous system depression and gastrointestinal dysfunction	(82)
	ND	ND	Phase I trial 18 healthy volunteers Intravenous, dose range 0.15 to 7 mg	•Well tolerated •Nausea and vomiting at 7 mg limited further dose escalation	(102)
	ND	ND	Phase II trial Pilot phase: 144 patients After pilot phase: 195 patients Intravenous, 0.5, 1, 2, or 3 mg every 3 hours	•2 and 3 mg mitigated severe acute pain over 48 hours	(103)
	ND	ND	Phase III trial 375 patients Intravenous: 1.5 mg loading dose followed by 0.1-mg, 0.35-mg, or 0.5-mg doses Morphine (4-mg loading dose; 1-mg demand dose)	•Superior analgesia •Reduced respiratory side effects and increased gastrointestinal tolerability	(104)
Mitragynine pseudoindoxyl	EC_{50} = 1.7 nM Efficacy = 84%	No recruitment at 10 μM	CD-1 mice Subcutaneous, 0.76 mg/kg	•Analgesia •Limited respiratory depression and constipation	(105)
SHR9352	EC_{50} = 0.77 nM Efficacy = 96%	EC_{50} = 2.5 nM Efficacy = 18%	C57BL/6J mice and Wistar rats Subcutaneous, 0.1 mg or Intravenous, 0.3 mg	•Analgesia •No constipation	(106)
SR-17018	EC_{50} = 97 nM Efficacy = 75%	No recruitment at 10 μM	C57BL/6J mice Intraperitoneal, 6 mg/kg	•Analgesia •No respiratory suppression	(107)
Herkinorin	EC_{50} = 0.5 μM	No recruitment at 10 μM	No blood-brain barrier penetration		(108)
PZM21	EC_{50} = 4.6 nM Efficacy = 76%	No recruitment at 10 μM	C57BL/6J mice Subcutaneous, 40, 20, and 10 mg/kg	•Dose dependent response •Long-lasting analgesia	(97)

Ligand	In vitro		In vivo		Reference
	G protein activation	β-Arrestin recruitment	Administration and dose	Outcome	
				•Decreased respiratory depression and constipation	
Cyclopeptide	EC_{50} = 5.2 nM Efficacy = 80%	No recruitment at 10 μM	ND		(109)

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Table 2

G protein–biased ligands at the κ-OR.

Ligand	In vivo		In vitro		Reference
	G protein activation	β-Arrestin recruitment	Administration and dose	Outcome	
6'-GNTI	EC_{50} = 1.6 nM Efficacy = 64%	No recruitment activity	C57BL/6J mice Spinal cord injection, 10 to 30 nmol	•Analgesia •No aversion •Tolerance	(110)
RB-64 (22-thiocyanatosalvinorin A)	EC_{50} = 5.22 nM Efficacy = 99%	EC_{50} = 1130 nM Efficacy = 126%	C57BL/6J mice Subcutaneous, 3 mg/kg •Aversive	•Long lasting analgesic •No sedative effect	(70)
Triazole 1.1	EC_{50} = 77 nM Efficacy = 101%	EC_{50} = 4955 nM Efficacy = 98%	C57BL/6J mice Subcutaneous dose Analgesia: 5, 15, and 30 mg/kg Antipruritic: 1 and 3 mg/kg	•Analgesia •Antipruritic •No sedation or dysphoria observed	(72)
HS666	EC_{50} = 35.7 nM Efficacy = 50%	EC_{50} = 449 nM Efficacy = 24%	CD-1 mice Intracerebroventricular, 6.02 nmol •Respiratory suppression	•Time and dose dependent •Antinociceptive response	(111, 112)
Nalfurafine	EC_{50} = 1.4 nM (pERK1/2)	EC_{50} = 110 nM (p38)	Rats and primates Subcutaneous, 1 mg/kg	•Analgesic •Antipruritic •No dysphoria or aversion	(113)
	EC_{50} = 0.11 nM Efficacy = 111%	EC_{50} = 1.4 nM Efficacy = 129%	CD-1 mice Subcutaneous, 10 μg/kg	•Analgesic •Antipruritic •No aversion	(73)