Review

Opioid Analgesics: Rise and Fall of Ligand Biased Signaling and Future Perspectives in the Quest for the Holy Grail

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Opioid Analgesics: Rise and Fall of Ligand Biased Signaling and Future Perspectives in the Quest for the Holy Grail

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Highlights:

- Oliceridine was the first FDA-approved specifically designed biased MOP agonist
- Low intrinsic efficacy at MOP, rather than biased signaling may contribute to improved side effect profiles
- Translational challenges remain for biased/low intrinsic efficacy ligand therapies
- Multimodal analgesia approaches emerge as alternatives to traditional MOP ligands

Abstract: Opioid analgesics have been used for more than 5,000 years and remain the principal pain medications prescribed today. Although morphine is considered the gold standard for pain relief, this μ-opioid receptor (MOP)-selective agonist provides only moderate relief for many chronic pain conditions and promotes several unwanted effects that can adversely affect patient quality-of-life, prevent adherence to treatment or generate addiction. In addition to the lack of progress in the development of better analgesics, there have been no significant breakthroughs to date that have addressed side effects mentioned above. Fortunately, a better understanding of opioid pharmacology has restored hope for the development of better and safer pain medications. In this review, we describe how clinically approved opioids were initially characterized as biased ligands and what impact this approach could have on clinical practice. We also looked at the preclinical and clinical development of MOP biased agonists, with an emphasis on the oliceridine story, as the first specifically designed biased painkiller. Moreover, we explore the discrepancies between ligands with low intrinsic efficacy and those with biased properties. Finally, we examine the rationale behind biased ligand development during the opioid crisis era.

Key Points: The concept of biased agonism, where a drug preferentially activates beneficial signaling pathways over those causing side effects, has generated excitement for developing improved opioid painkillers. While the first rationally designed biased opioid olicerdine recently gained FDA approval, its clinical profile is similar to existing opioids. Nevertheless, retrospective studies characterizing biased signaling of clinically used opioids like buprenorphine suggest that optimizing bias may still be a viable strategy for creating novel analgesics with better safety and effectiveness compared to morphine.

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Keywords: pain; opioids; morphine; biased signaling; functional selectivity; G protein; β-arrestin; G protein-coupled receptors; GPCRs

1. Introduction

Chronic pain constitutes a pervasive and debilitating condition that imposes a substantial burden on afflicted individuals, significantly compromising their quality of life and overall well-being. Despite its high prevalence, effective management of chronic pain remains a challenge [1]. Unsatisfying treatment negatively affects patients' health and well-being, interfering with essential daily activities [2]. To alleviate pain, extracts of *Papaver somniferum* (opium poppy) have been used for thousands of years [3]. It was only during the 19th century that morphine was isolated from the plant and characterized as its primary active pharmaceutical ingredient [4]. To date, opioids, especially morphine, remain the gold standard for the treatment of several types of pain ranging from acute to chronic pain. However, opiate-derived analgesics provide only modest and short-term pain relief in patients with chronic non-cancer pain such as neuropathic pain [5]. Moreover, they are associated with a wide range of adverse effects, such as constipation, respiratory depression, tolerance, hyperalgesia, and abuse potential, which can seriously affect patients' adherence to treatment [6]. Indeed, up to 30% of patients do not see the expected pain relief with morphine, and 70% experience debilitating adverse events [7]. Despite these drawbacks, morphine remains on the list of essential medicines of the World Health Organization, as no other effective analgesics are able to achieve comparable pain relief [8]. Over the last 50 years, more than 60 new analgesics have been introduced onto the market. However, most of these are simply new formulations of existing drugs [7]. In fact, 88% of these new pain medications showed very limited improvement in terms of efficacy or adverse effects compared with existing treatments. In the last decade, their increasing availability and abuse potential have led to the opioid crisis and to an epidemic of drug overdose deaths with serious social and economic consequences [9,10]. Modest pain relief, undesirable effects, and overuse or misuse of these addictive drugs are real dilemmas for medical professionals [11]. This has prompted researchers to further elucidate the mode of action of opioids and intensify their search for better versions of these analgesics.

2. Shaping Analgesic Drug Design by Understanding Opioid Pharmacology

Owing to the extensive use of morphine and opiate derivatives for their potent analgesic effects in acute pain, researchers have long been interested in understanding the mechanism of action of opioids, in parallel with the progress made in ligand and receptor pharmacology. The opioid receptor family comprises four members: δ (DOP), κ (KOP), μ (MOP), and the opioid-related nociceptin (NOP) receptors [12]. Morphine and its derivatives bind to the μ-opioid receptor (MOP) and trigger activation of a signaling cascade downstream of the receptor (**Figure 1**) [13]. It is now widely accepted that all opioid receptors activate pertussis toxin (PTX)-sensitive G proteins, including $G\alpha_{i/o}$, which inhibit adenylyl cyclase isoforms and thus reduce intracellular levels of cyclic AMP [14,15]. After dissociation from $G\alpha_i$, the $G\beta\gamma$ dimer can interact with inwardly rectifying potassium channels (Kir_3) and facilitate potassium efflux [16,17]. The $G\beta\gamma$ dimer can also directly interact with voltage-activated Ca^{++} channels to reduce calcium entry [18,19]. Overall, G protein activation by opioid receptors leads to membrane hyperpolarization and inhibition of neuronal activity [20]. These receptors are also known to mediate post G protein-dependent signaling events by inducing recruitment of GRK 2 and 3 and β-arrestins to the C-terminal tail of the receptor, attenuating G protein-signaling, and promoting the phosphorylation of c-jun N-terminal kinase (JNK) and other mitogen-activated protein kinases (MAPK) [21–25]. MOP agonist-mediated β-arrestin recruitment drives MOP receptor desensitization and internalization, and therefore fine-tunes analgesic responses mediated by MOP activation.

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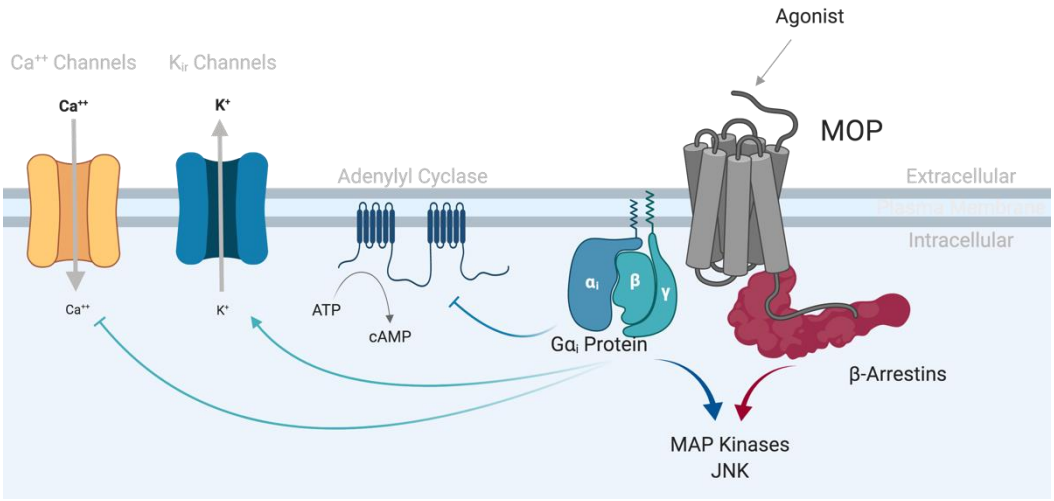

Figure 1. μ-opioid receptor signaling c. Upon activation of the μ-opioid receptor, the heterotrimeric G-protein dissociates into active Gα and Gβγ subunits. The $G\alpha_i$ subunit inhibits adenylyl cyclase, thereby decreasing intracellular cAMP levels. The Gβγ dimer activates inwardly rectifying potassium channels (K_{ir}), promoting K^+ efflux out of the cell and inhibits voltage-gated Ca^{++} channels, slowing down the entry of Ca^{++} into the cytosol, thus leading to cell hyperpolarization and reduction of neuronal excitability. The C-terminal tail of the opioid receptor is phosphorylated by GRK2 and GRK3, and β-arrestins are then recruited to the receptor, leading to desensitization and internalization of MOP. β-arrestins also serve as scaffolding proteins for the activation of secondary signaling cascades, such as ERK1/2 and JNK MAP kinases.

In the case of MOP, the link between intracellular signaling and the physiological effects of opioids was discovered during the 90s. First, Raffa, *et al.* [26] used siRNA targeting $G\alpha_i$ proteins *in vivo* and found that the knockdown of $G\alpha_{i2}$ blocked the analgesic action of morphine. Later, Bohn, *et al.* [27] showed that in mice lacking β-arrestin 2, the analgesic effect of morphine was enhanced and the adverse effects usually triggered by MOP activation were significantly reduced. They also concluded that the analgesic action of morphine was achieved via a $G\alpha_i$ protein-dependent mechanism, whereas constipation and respiratory depression were triggered by the recruitment of β-arrestin 2 [28]. Tolerance has also been linked to the recruitment of β-arrestin 2 in knockout mice, which showed no decrease in morphine analgesic potency after chronic treatment [28,29]. These findings, as confirmed by others, significantly shaped the development of opioid ligands towards $G\alpha_i$-biased agonists [30–33].

Nevertheless, some controversies remain regarding the signaling pathways leading to undesired effects; therefore, the perfect dichotomy between the effects triggered by G proteins and β-arrestin 2 may not be as simple as initially assumed. Indeed, in a recent international multisite study evaluating the effects of morphine and fentanyl, the authors showed similar levels of respiratory depression and constipation in β-arrestin 2 knockout mice compared to their wildtype littermates [34]. This study corroborates the observations of respiratory depression and constipation induced by morphine and fentanyl in different phosphorylation-deficient MOP knock-in mice (these mutant receptors showed a decreased recruitment of β-arrestin 2 upon activation assessed *in vitro*) [32,35,36]. Thus, these discrepancies highlight some of the limitations of whole animal knockouts and genetic strain differences used to decipher pharmacological contributions relevant to human patients, as compensatory mechanisms may interfere with the studied functional responses [37].

It is widely accepted that MOP-induced analgesia is dependent on G protein activation, and this effect is enhanced in β-arrestin 2 knockout mice [38]. However, other cellular mechanisms might be responsible for the μ-opioid agonist adverse effects that cannot be explained by the convenient but overly simple version of biased agonism characterized by a dichotomy between G protein versus β-arrestin signaling.

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3. GPCRs: From Simple Switches to Integrated Signaling Processors

The G protein-coupled receptor (GPCR) family is among the most important classes of druggable targets in the human proteome, with nearly 40% of prescribed drugs across various therapeutic fields acting through the modulation of these proteins [39]. Traditionally, GPCRs were considered as simple toggle switches, turning 'on' or 'off' pre-selected ensembles of signaling modalities [40]. GPCR activation often leads to the recruitment of multiple membrane and cytosolic proteins, enabling them to engage in multiple signaling pathways. Thus, the classical view of these receptors as simple heterotrimeric G protein activators was supplanted by their roles as complex modulators of a variety of intracellular signaling pathways [41]. In the late 1990s, a new concept called ligand-directed signaling, later renamed biased signaling or functional selectivity, emerged in the GPCR field [42]. This new model is based on the ability of a receptor ligand to modulate (activate or block) only a subset of signaling pathways that are normally activated by endogenous or balanced agonists (**Figure 2**). In other words, a biased ligand can induce a distinct conformation of the receptor that modulates only a specific subset of the signaling repertoire [43]. Of particular interest was the demonstration that ligands behaving as agonists for a given signaling pathway can act through the same receptor, as antagonists, or even inverse agonists on a different pathway in the same cell [44]. As a result, a lack of efficacy for a single pre-selected signaling pathway does not necessarily translate into a lack of receptor activation and concomitant cellular and physiological responses. The concept of biased signaling has brought new opportunities for the development of therapeutics and has rapidly changed the way drugs are screened. Therefore, researchers could design molecules that would only activate beneficial signaling pathways leading to the desired pharmacological effects, while avoiding undesirable effects [45]. To design such compounds, it is necessary to understand the receptor-ligand system in detail and decipher the relationship that exists between signaling pathways and physiological effects *in vivo* [46,47]. Unfortunately, such links are not always obvious, often remain unknown and neglected, and may even be distinct between different cell types, tissues, or species in which the same receptors are expressed. For instance, β-arrestin-biased dopamine D_2 receptor ligands have been shown to behave as agonists in cortical regions and antagonists in the striatum, these region-specific actions being related to differential expression levels of β-arrestins and G protein-coupled receptor kinases across brain structures [48]. In addition, differences between species can significantly influence clinical translation. In this respect, the G protein-biased agonist nalfurafine was reported to exhibit greater biased signaling towards human KOP than its rodent counterpart [49].

The translation of biased agonism from *in vitro* studies to *in vivo* efficacy to the market faces several challenges. These include potential differences in bias profiles between human and animal receptor orthologs and the need to thoroughly characterize the true efficacies and signaling profiles of biased candidate molecules beyond the simple comparison of G protein- and β-arrestin-dependent pathways. Failure to adequately address these factors during the drug discovery process may contribute to the high attrition rate of new opioid drug candidates in clinical development [50,51]. Interestingly, retrospective analyses of clinically approved opioid drugs for biased signaling can improve the understanding of relevant signaling pathways and help future drug development by identifying patterns and mechanisms that can be leveraged to design more effective and safer opioid analgesics.

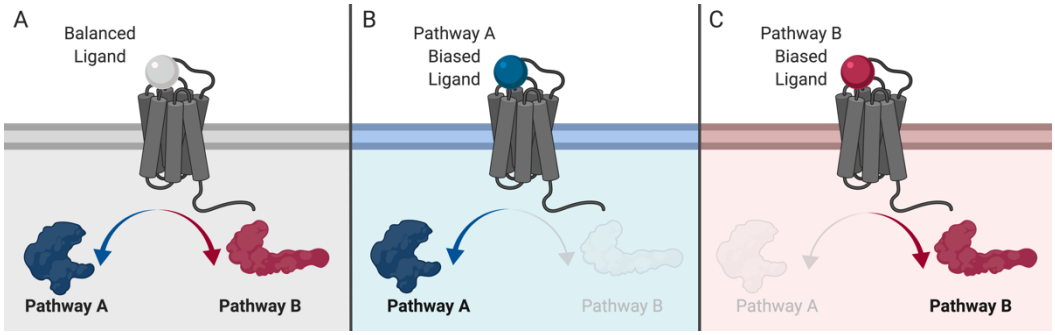

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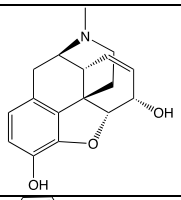
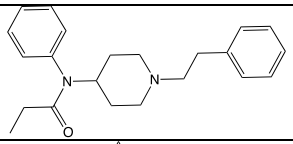
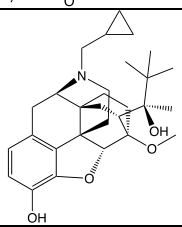
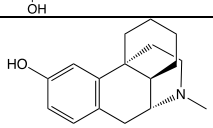
Figure 2. The concept of biased signaling. (**A**) A balanced ligand triggers the activation of all signaling proteins coupled to a given GPCR (Pathways A and B). (**B-C**) Biased agonists, however, act by activating only a subset of the receptor signaling repertoire. (**B**) A Pathway A-biased ligand favors the recruitment of pathway A signaling partners rather than the activation of pathway B proteins. (**C**) On the other hand, a Pathway B-biased ligand triggers the activation of pathway B signaling cascade rather than the recruitment of pathway A proteins.

4. Old Drugs, New Tricks: MOP-Biased Agonists in Clinically Approved Opioids

The concept of biased signaling was not yet known when the majority of current clinically used opioids appeared on the market. Nonetheless, deciphering the signaling effects of these clinically relevant drugs can be useful to better understand both the beneficial and adverse effects associated with opioids. One of the first reports of biased properties of clinically used opioids was the difference in MOP internalization observed between cells treated with Met-enkephalin and morphine [52]. Met-enkephalin was found to trigger a rapid internalization of MOP, while morphine did not promote receptor endocytosis. Since these first demonstrations, several studies have revealed the biased properties of MOP ligands [24,53]. McPherson et al. [54] compared the intrinsic efficacies for G protein activation and β-arrestin 2 recruitment of 22 preclinical and clinical MOP agonists. Although the authors did not calculate bias factors (relative efficacies of a ligand for these two signaling pathways), they described fentanyl and alfentanil, two opioids used in surgical analgesia with a rapid onset, and etorphine, a tranquilizer used in veterinary medicine, as MOP ligands that favor recruitment of β-arrestin 2 [54] (**Table 1**). Fentanyl and sufentanil were also assessed in a recent study that evaluated the functional selectivity of novel MOP ligands, and these two synthetic opioids showed a strong bias towards the recruitment of β-arrestin 2 when compared to DAMGO used here as the reference compound [55]. Interestingly, morphine has been described as either a balanced ligand or a slightly β-arrestin-biased agonist (**Table 1**).

Table 1. Opioid compounds used in clinical practice and under development. Clinically approved opioid agonists are in non-colored cells, while biased ligands under preclinical and clinical development are in grey-shaded cells.

Compound	Structure	Biased toward[1]	Contradictory literature
Morphine		β-arrestins [55,68,147]	No bias [101,148,149]
Fentanyl		β-arrestins [55,95]	No bias [96]
Buprenorphine		G protein [101,117,147,150]	
Levorphanol		G protein [64]	

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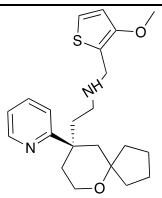
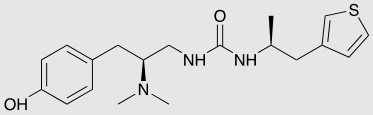
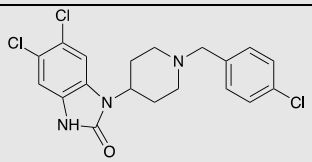
Oliceridine		G protein [67,68]	Tendency towards G protein [101]
PZM21		G protein [90,93]	Tendency towards G protein [101]
SR-17018		G protein [55]	Tendency towards G protein [101]

[1]Bias was calculated using DAMGO, a peptide agonist at the μ-opioid receptor, as a reference agonist.

Buprenorphine, is a drug used to treat opioid abuse disorder and has been recently exploited for its analgesic action [56]. Buprenorphine binds and activates all four opioid receptors, making its pharmacology even more complicated. It is an agonist at MOP, an antagonist at KOP and DOP, and a weak agonist at NOP [57]. Moreover, buprenorphine is a biased agonist at MOP as it activates $G\alpha_i$ but fails to recruit β-arrestin 2 [54]; thus, it is reasonable to assume that this prescribed drug will exert an analgesic efficacy similar to morphine and a lower risk of undesired effects [58] (**Table 1**). Furthermore, owing to its complex pharmacology at opioid receptors, buprenorphine is thought to have a lower potential for abuse [59]. Nevertheless, it must be noted that norbuprenorphine, an active degradation metabolite of buprenorphine, presents a very different signaling profile at MOP [57]. Norbuprenorphine is a potent activator of G proteins, with an efficacy similar to morphine and better potency than DAMGO. However, unlike buprenorphine, it is also a strong recruiter of β-arrestin 2, with a profile similar to that of DAMGO [54]. Thus, patient dosing with buprenorphine must be carefully monitored to avoid norbuprenorphine-induced adverse effects [60].

Finally, levorphanol is a non-selective opioid agonist with complex pharmacodynamic properties, synthesized in the late 1940s as an alternative to morphine [61]. This pan-opioid receptor agonist is also an N-methyl-d-aspartate (NMDA) antagonist that also acts as a norepinephrine and serotonin reuptake inhibitor (SNRI) [62]. Often referred to as the "forgotten opioid," levorphanol is neither known nor prescribed by most physicians [62,63]. It has been revisited, and an extended pharmacological characterization showed that levorphanol acts as a moderate μ-biased agonist favoring G protein activation at several MOP splice variants [64] (**Table 1**). In addition, less respiratory depression was observed with levorphanol than with morphine at a dose 5-fold greater than their analgesic ED_{50} [64]. This new classification, together with its safety profile, might give this forgotten opioid drug a new start [65,66].

5. Oliceridine, the Prototype MOP-Biased Agonist

Oliceridine, also known as TRV130 or Olinvik™, its commercial name, is a MOP ligand developed by the biotech company Trevena, Inc. (NASDAQ: TRVN, Chesterbrook, PA, USA). This compound is a small MOP-selective molecule that was discovered after screening a compound library and a structure-activity relationship study, where it displayed a biased profile towards the activation of $G\alpha_i$ (**Table 1**) [67]. Oliceridine was then tested *in vivo* in mice and rats, and it was noted that oliceridine had an efficacy similar to that of morphine in acute and post-operative pain models. However, due to its lower efficacy in recruiting β-arrestin 2 following MOP activation, oliceridine displayed an increased therapeutic window (range of drug doses providing safe and effective therapy) compared to morphine for assays of constipation and respiratory suppression [68]. These encouraging results combined with the early phase III results prompted Trevena to move oliceridine forward in the drug discovery process and file a new drug application in November 2017 to the U.S. Food and Drug Administration (FDA), which was accepted in January 2018 [69,70]. Unfortunately,

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in October of that year, the FDA Anesthetic and Analgesic Drug Products Advisory Committee (AADPAC) voted against the approval of oliceridine [71]. The FDA recognized that oliceridine demonstrated statistically increased pain relief compared to a placebo, and that some doses showed fewer side effects than morphine. However, the doses showing fewer undesired effects were also less effective than morphine, and therefore, did not support the conclusion that it possesses a safer profile [72]. Nevertheless, the FDA agreed that the safety database of Trevena supports a maximum dose of 27 mg per 24 h of oliceridine. The company also agreed to conduct a study to collect requested QT interval data (time from the start of the Q wave to the end of the T wave on the electrocardiogram), as those administered during the new drug application were judged inadequate for the proposed dosing [73]. When reading the full meeting report, one can appreciate that the expert committee found much to interest them in oliceridine, but as it was an application for a new entity, the committee expected more safety data before approving the drug.

In 2019, Trevena announced the initiation of a healthy volunteer QT interval study for oliceridine with the aim of collecting additional data as requested by the FDA before the re-submission of the new drug application for this molecule [74]. Trevena also published a clinical safety study showing that, in contrast to morphine and hydromorphone, no dose adjustment of oliceridine is necessary in patients with renal dysfunction or mild-to-moderate hepatic impairment. However, dose reduction and careful monitoring should be performed in patients with severe hepatic dysfunction [75]. In early 2020, Trevena resubmitted a new drug application at the FDA, including data from a multi-dose healthy volunteer QT study, nonclinical data that confirmed levels of an inactive metabolite, and drug product validation reports [76,77]. These newly submitted data were able to convince the FDA about the safety of oliceridine with respect to the maximal dosage of 27 mg/day, leading to the approval of oliceridine in August 2020 [78]. The FDA's decision underscored the need for new opioid analgesics with improved safety profiles, especially in hospital settings where intravenous administration is required [79]. This was the first approval of a specifically developed MOP-biased agonist. Unfortunately, even if the rational design was initially highly elegant, oliceridine has a benefit-risk profile similar to that of other previously approved opioids, with some evidence for a slight improvement in the respiratory safety profile [80,81]. Following FDA approval, oliceridine was classified as a Schedule II controlled substance by the U.S. Drug Enforcement Administration (DEA). This classification reflects its potential for abuse, similar to other opioids, and necessitates careful monitoring and control in clinical use [82,83]. Since its approval in the U.S., oliceridine has been brought to the Chinese market in the first semester of 2023 by Jiangsu Nhwa Pharmaceutical, in partnership with Trevena [84].

Follow-up studies have shown that olicerdine induces less respiratory depression than morphine at equianalgesic doses, regardless of age and body mass index, two factors known to exacerbate morphine-induced respiratory depression [85,86]. Furthermore, oliceridine seems to be better tolerated than morphine in patients and allows for a reduction in the use of rescue antiemetics [87,88]. Ongoing research aims to further elucidate the efficacy and safety of olicerdine in broader clinical settings and explore its potential applications in other pain management paradigms such as acute pain after burn injury [89].

Overall, the approval of oliceridine marked an encouraging advancement in the field of pain management, offering a novel approach to opioid analgesia with a potentially improved safety profile. Its development and approval represent a significant milestone in addressing the challenges associated with traditional opioid therapies.

6. MOP-Biased Ligands in Preclinical Development

Although oliceridine was the first specifically designed MOP-biased ligand to be pushed into clinical trials, the search for other MOP ligands has not stopped. Indeed, in the past few years, many MOP-biased ligands have been reported to be effective analgesics in preclinical studies (**Figure 3**). Among the ligands developed are PZM21 [90] and SR-17018 [55] (**Table 1**).

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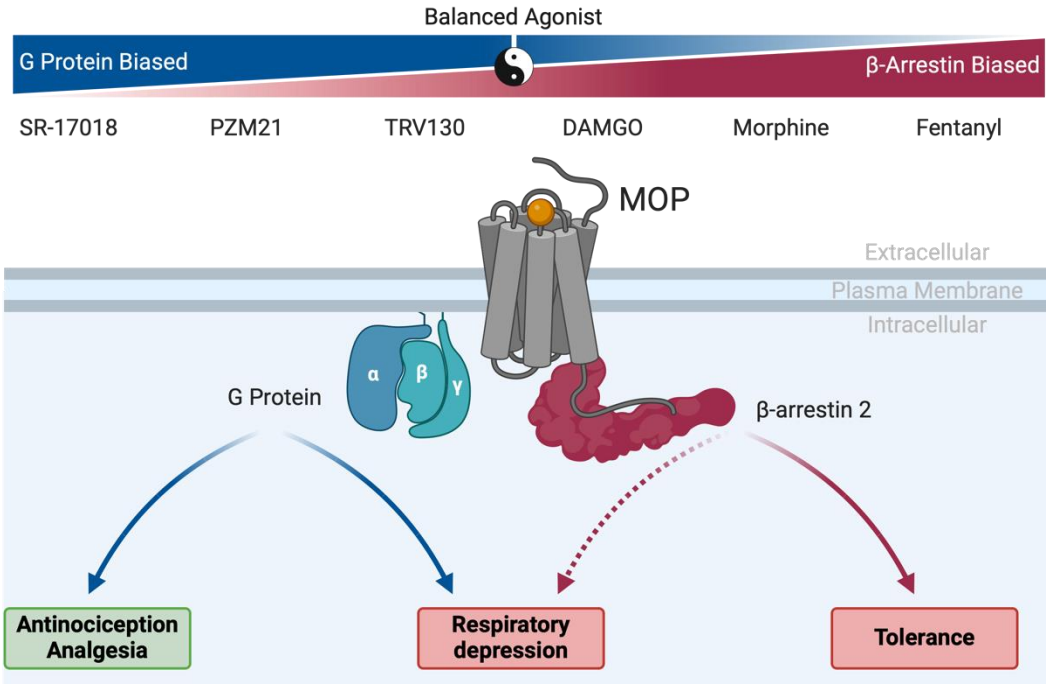

Figure 3. Biased agonism at μ-opioid receptor. MOP-induced Gαi activation is responsible for the antinociceptive and analgesic effects of μ-agonists, whereas unwanted effects are, in part, related to the recruitment of β-arrestin 2 following MOP activation. Therefore, MOP ligands favoring the recruitment of β-arrestin 2 over G protein activation, such as fentanyl, produce more undesirable effects than G protein-biased ligands, such as PZM21 or TRV130. These include tolerance and may include respiratory suppression. G protein-biased agonists are of greater therapeutic interest due to their increased analgesic profile but may also lead to respiratory depression.

PZM21 is a MOP-selective opioid agonist and a KOP antagonist developed after virtual library screening using the MOP crystal structure, followed by a structure-activity relationship study. Similar to oliceridine, this compound has a structure that is unrelated to other known opioids. Thus, as it has been recently suggested, completely new molecular entities can exhibit the same primary outcome (*i.e.*, analgesia), but can also come with their own range of unwanted effects, such as glial activation and interactions with cytokines, which can affect the immune system's ability to fight potential infections [91]. PZM21 was described as an antinociceptive compound in acute- and formalin-induced tonic pain tests. At the cellular level, PZM21 was initially reported to activate the Gαi pathway with a potency similar to that of morphine and to not trigger β-arrestin 2 recruitment. This new entity is as effective as morphine and possesses an extended length of action in both hot-plate and tail-flick acute thermal nociception studies [90,92]. Furthermore, it caused less constipation than morphine and presents with no respiratory suppression, nor rewarding effects at a dose lower than the maximal effective analgesic dose (equivalent to 10 mg/kg of morphine). In a subsequent study of PZM21 by another research team, it appeared that this compound was as potent as morphine to engage the Gαi pathway, but also seemed to promote recruitment of β-arrestin 2 [93]. These authors also examined the effects of PZM21 on respiratory suppression using whole-body plethysmography. Their results demonstrated that PZM21 was comparable to morphine in the induction of respiratory depression at an equianalgesic dose [93]. The authors even tested a separately synthesized batch of PZM21 to confirm their results. Thus, PZM21 appears to be more like morphine than a pure G protein-biased agonist. However, a recent study showed that, in contrast to clinically relevant opioids, PZM21 was not able to induce reward-associated behaviors in both conditioned place preference and locomotor sensitization experiments in mice, and in intravenous self-administration in rats [92].

SR-17018 is a MOP agonist from a series of MOP-targeting compounds recently reported to display a wide array of bias factors, ranging from molecules favoring the recruitment of β-arrestins

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to highly G protein-biased ligands. Here, the authors compared the dose-response curves of their new compounds, including SR-17018, and clinically relevant opioids, such as morphine, fentanyl, and sufentanil, on respiratory depression and analgesia. They found a significant correlation between the therapeutic window and bias factor, meaning that the more a ligand was biased towards G protein activation, the safer the compound was with respect to respiratory events [55]. In this study, it was not only the fact that the ligand was a biased agonist, but also the extent to which the ligand was biased, which was relevant for compound safety. SR-17018 showed a preference for G proteins over β-arrestin of >100-fold for murine MOP, as well as sustained antinociceptive action in acute thermal pain paradigms in mice. However, in sharp contrast to morphine and fentanyl, SR-17018 did not induce signs of respiratory depression at the analgesic dose, thus drastically increasing its therapeutic window to nearly 30-fold the ED_{50} for analgesia (for reference, the therapeutic windows of morphine and fentanyl are at 5- and 2-fold the ED_{50}, respectively). In a follow-up study, the authors showed that a 6-day pretreatment with SR-17018 did not impair its own analgesic potency nor that of morphine in an acute thermal pain test, indicating that SR-17018 does not seem to induce tolerance at MOP [94]. This result was confirmed as [^{35}S]-GTPγS binding was not reduced in membranes from the periaqueductal grey of animals subjected to chronic treatment with SR-17018 (whereas a significant decrease in [^{35}S]-GTPγS binding was observed in morphine-treated animals) [94].

Finally, a recent report of fentanyl, carfentanyl, and lofentanyl poses in molecular dynamics simulations revealed that the residue M153$^{3.36}$ of MOP is crucial for inducing recruitment of β-arrestin 2 following stimulation with fentanyl [95]. Moreover, they also showed that the n-aniline group of fentanyl and its derivatives were drivers of β-arrestin 2 recruitment at MOP. Accordingly, the newly synthesized analogs without the n-aniline ring exhibited a biased profile towards the activation of $G\alpha_i$. Interestingly, a second study revealed that carfentanil, in contrast with fentanyl, was able to interact with Y128$^{2.64}$ or I296$^{6.51}$ residues available via its 4-carbomethoxy moiety [96]. In parallel, they calculated the bias factor of car-fentanil and other fentanyl derivatives from bioluminescence resonance energy transfer assays, with carfentanil being the only one that exhibited significant bias towards β-arrestin 2 recruitment. These new important structural insights may open the way for the development of potent MOP-biased ligands based on fentanyl chemical scaffold in the near future. Moreover, the recent resolution of the MOP bound to different ligands has paved the way for receptor-based virtual screening and possibly AI-assisted drug discovery [12,97,98].

7. MOP-Biased Ligands Versus Low Intrinsic Efficacy Ligands at the μ-Opioid Receptor

Following multiple studies conducted in the past five years, biased ligands at the MOP as an avenue for pain therapies, was less exciting than initially thought [99]. Herein, work previously done to establish a strong correlation between β-arrestin 2 recruitment and debilitating MOP-related side effects were revisited by a consortium of three different laboratories [34]. The main findings from these experiments led to a discrepancy between the original work and current understanding [27]. The role of β-arrestin 2 in side effects was reconsidered with respect to respiratory depression and that is now thought to be dependent on G protein activation [34]. However, G protein activation is still considered the primary signaling pathway to relevant to antinociceptive action, and the development of tolerance towards analgesic compounds is related to receptor desensitization via the recruitment of β-arrestin 2 following GRK phosphorylation [32,100]. Hence, the development of biased MOP ligands remains relevant.

In 2020, Gillis *et al.* [101] positied that low intrinsic efficacy component (*i.e.*, partial agonism at all signaling pathways) of opioid agonists was correlated with an improved analgesic effect/side effects profile. In fact, they hypothesized that the improved safety profiles of biased-opioid ligands were due to their low intrinsic efficacy, rather than bias *per se* (**Figure 4**). They suggested that these molecules were previously interpreted as biased ligands since the original assessment of bias was performed using assays where G protein responses were amplified (*i.e.*, cAMP accumulation) but β-arrestin recruitment responses were not. They used assays with minimal amplification (recruitment of Nb33 (a biosensor for active-state MOP [102]), mGsi, GRK2 and β-arrestin 2) which may be more relevant to reflect efficacy. Their findings suggested that TRV130, PZM21, buprenorphine, and SR-

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17018 were low intrinsic efficacy agonists rather than biased ligands and that there is a strong correlation between the efficacy of receptor activation, G protein coupling, and β-arrestin recruitment and the improved side effect profile. Interestingly, the latter study was re-analyzed by Stahl and Bohn [103], and their analysis showed that bias was the main driving force to predict desirable pharmacological properties. Thus, partial agonism in all signaling pathways may be a key determinant in the development of safer opioid analgesics, but it is certainly not the only one, nor should it eclipse all previous research on biased compounds.

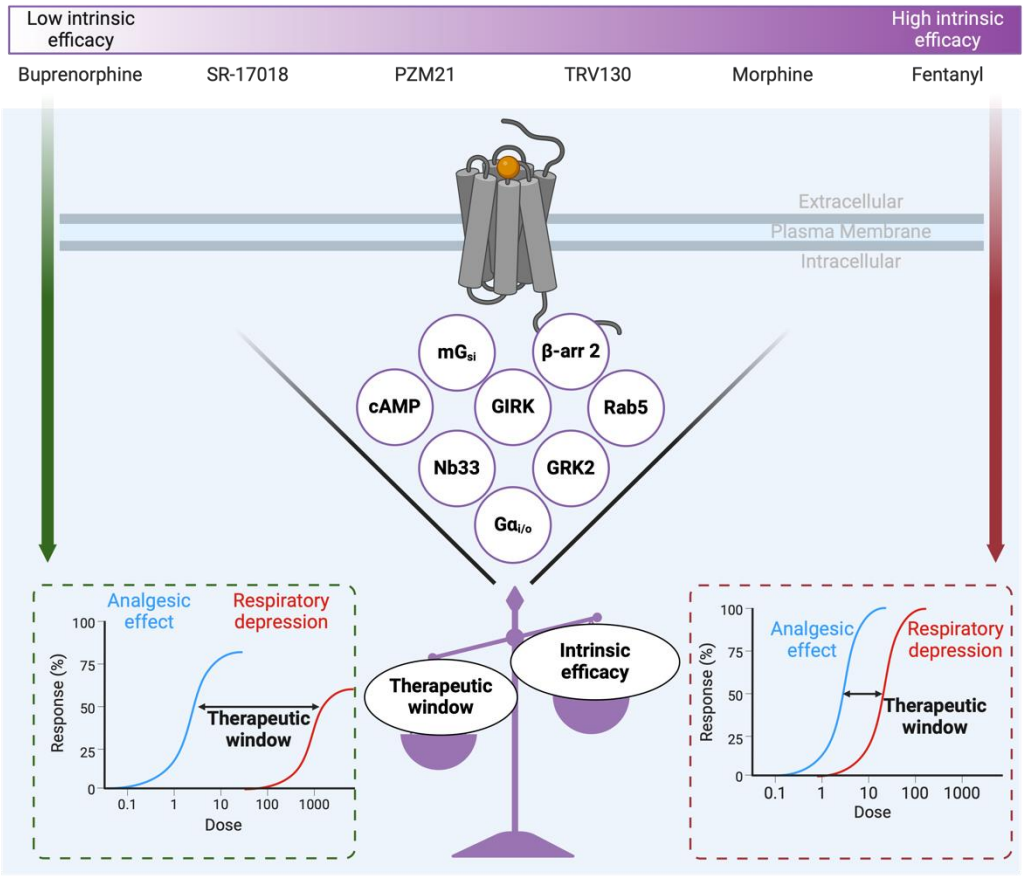

Figure 4. Low intrinsic efficacy at μ-opioid receptor. Different signaling pathways were evaluated following MOP stimulation. These include receptor activation and G protein signaling, Nb33 recruitment as surrogate of active-state MOP, mG_{si} recruitment, $G\alpha_{i/o}$ activation, cAMP inhibition, GIRK activation and recruitment of regulatory proteins and internalization, GRK2 recruitment, β-arrestin 2 recruitment, and Rab5 trafficking as reported by Gillis, Gondin [101]. The close correlation of efficacy across each assay led to the quantification of intrinsic efficacy. Buprenorphine was the lowest, followed by SR-17018, PZM21, TRV130, morphine and fentanyl. Efficacy is inversely correlated with the therapeutic window (*i.e.*, buprenorphine exhibits a wider therapeutic window than fentanyl).

8. Novel MOP Ligands in the Current Opioid Crisis Era

The current opioid epidemic is largely related to an increase in opioid prescriptions, misuse, overuse, and abuse. The development of synthetic illicit opioids, which are more potent than morphine (*e.g.*, carfentanil is 10,000 times stronger than morphine or nitazene compounds that often require multiple doses of naloxone to reverse severe side effects), has also contributed to the higher risk of overdose fatalities [104–108]. The opioid crisis is particularly visible in North America (predominantly in the U.S. and Canada) and Australia, whereas European and low-income countries seem less affected [109]. In the past 20 years, the use of opioids (both legal and illegal) in the U.S. has increased nearly 15-fold, leading to more than 33,000 opioid-related deaths in 2015 [10,110]. Canada is the second country after the U.S. with the highest prescription opioid consumption per capita in

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the world; however, when considering the morphine equivalents dispensed, Canada ranks first [111]. The opioid crisis has prompted the search for analgesics with a reduced potential for abuse, and it is legitimate to think that biased MOP ligands could be a possible solution to this growing epidemic.

Analgesia has been extensively linked to the activation of G proteins by MOP, and some of the adverse effects, such as tolerance, have been linked to the recruitment of β-arrestin 2. Although opioid abuse can result in addiction and physical dependence, activation of the reward circuitry by opioids has not yet been positively correlated with a specific cellular signaling pathway. However, there is some evidence indicating that β-arrestin 2 KO mice display drug addiction withdrawal symptoms similar to those of their wild-type littermates [29]. Accordingly, the same research group showed that dopamine, considered a key player in addiction, was released to a greater extent in the striatal region of β-arrestin 2 knockout mice following morphine treatment compared to WT mice. Furthermore, β-arrestin 2 knockout mice spent more time in the drug chamber in a conditioned place preference assay [112]. Altogether, these results strongly suggest that morphine-induced rewarding effects are not triggered by β-arrestin 2 signaling, but rather by G protein engagement. Thus, the potential for abuse of MOP G protein-biased agonists is expected to be as high as or even higher than that of other opioids.

The MOP-biased ligand oliceridine was assessed during its preclinical development for morphine-like reward effects. Self-administration in rats showed that oliceridine triggers the same rewarding behaviors as oxycodone, another opioid analgesic with a high abuse potential [113]. Intracranial self-stimulation in rats and place conditioning experiments conducted with mice showed that oliceridine has the same potential for abuse as other opioid drugs.[114,115] This was also assessed in a randomized clinical trial involving healthy volunteers who reported a 'high' in the drug effects questionnaire, thus confirming the potential for abuse liability of oliceridine [116].

On the other hand, PZM21 was also assessed for its abuse potential in a conditioned place preference test using mice. In the first report of PZM21, the authors showed that chronic injections of this compound at submaximal doses did not induce place preference. The authors also reported that the use of oliceridine failed to promote any place preference [90]. Thus, knowing that oliceridine promotes reward-seeking behavior, the potential for abuse of PZM21 could not be established. In another independent study on PZM21, failure to induce place preference was demonstrated using increasing doses of PZM21. This confirmed the lack of abuse potential of this new entity [92]. However, the chronic administration of PZM21 resulted in signs of physical withdrawal in the naloxone-precipitated jump paradigm [92]. It has not been established whether the lack of reward effects of PZM21 is due to its unique biased signaling profile at the MOP or if this can be ascribed to its antagonist properties at the KOP or its atypical structure.

Finally, it has recently been reported that the G protein-biased MOP agonist SR-17018 fails to induce analgesic tolerance, but also displays fewer signs of physical dependence than morphine after chronic administration [117]. The authors also showed that administration of SR-17018 reversed signs of withdrawal upon morphine pump removal, with an efficacy comparable to that of buprenorphine [117]. Sustained analgesic potency and low dependence liability give SR-17018 highly desirable properties, prompting further characterization of this promising compound. Importantly, to date, no clinical trials have been conducted to investigate SR-17018 nor PZM21 analgesic properties or safety profiles.

9. Alternatives to Biased Signaling at the MOP

Despite decades of research on pain, we are faced with the realization that the initial approaches taken were undoubtedly incorrect. While the focus of research has been primarily on designing high-affinity MOP ligands, the emergence of other novel avenues has begun to prove some clinical interest. Targeting other members of the opioid receptor family could be an attractive avenue since most of the debilitating side effects associated with today's analgesics are related to MOP activation. Among these, DOP agonists have shown sustained analgesia; however, their ability to produce seizures has limited their clinical development [118]. However, further studies that are carefully designed to address why certain DOP agonists are seizurogenic may expedite development of novel DOP clinical

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candidates in the near future [119]. Some evidence has shown that G protein-biased ligands at the DOP are preferred. For instance, the biased and selective DOP small molecule, PN6047, which exhibits chronic pain relief without tolerance or seizures in rodents, has recently completed a phase I clinical trial with encouraging results [120,121].

In contrast, selective KOP agonists were initially forgotten because of severe side effects such as sedation, dysphoria, and hallucination [122,123]. To date, there are no selective KOP agonists clinically approved for pain management, but preclinical studies have revealed in the past few years that G protein activation is responsible for antinociceptive action, whereas β-arrestin recruitment has been linked to dysphoric effects [124–126]. Utilizing this strategy may allow the development of a KOP agonist with a safer profile.

Another alternative to classical opioids is the inhibition of scavenger receptors for opioid peptides [127]. The rationale here is that endogenous opioid peptides such as endorphins, enkephalins, and dynorphins can modulate pain on their own, but their action is negatively affected by scavenger receptors such as the atypical chemokine receptor 3 (ACKR3, previously known as CXCR7), reducing their availability for canonical opioid receptors [128,129]. Hence, competitive ligands for ACKR3, such as LIH383, could increase the availability of endogenous ligands and permit extended analgesic action [127].

With respect to the complexity of chronic pain development and nociceptive transmission, it is unlikely that a single mechanism of action can provide sustained and effective pain relief. For instance, nociceptive transmission involves several endogenous systems that are modulated and fine-tuned by receptors, hormones, and neuromodulators. To this end, multimodal therapies have emerged in the past few years as a way to address chronic pain treatment on a much broader spectrum [130,131]. The concurrent use of two or more drugs (polypharmacy) is common [132,133]. Therefore, a growing number of fixed-dose combinations (two medicines incorporated into a single formulation), such as oxycodone/ibuprofen or tramadol/acetaminophen, are being developed [134,135]. Its advantage over polypharmacy lies in improving patient compliance by reducing the pill load. However, many drawbacks such as drug interactions and solubility incompatibility limit some of these combinations [136]. Hence, development of a single entity that can incorporate both drugs has emerged with co-crystals, such as Seglentis (a co-crystal of both enantiomers of tramadol with celecoxib), to resolve pharmacokinetic and formulation issues [137]. Interestingly, multiple single agents exerting pain-relief action through a combination of mechanisms have recently been clinically approved. These include tapentadol (combining opioid and noradrenergic mechanisms) [138], tramadol (combining opioid and monoaminergic mechanisms) [139], buprenorphine, and cebranopadol (dual MOP/NOP actions) [140–142]. As a more selective approach, bifunctional compounds, including an opioid and a non-opioid moiety, have been proposed to be of high interest [143]. However, to date, these compounds remain in academic development, and none has yet reached the market.

10. The Remaining Unknown Ingredients for an Ideal Analgesic

Studies using knockout mice have revealed the basic ingredients needed for a highly desirable opioid analgesic, a μ-selective G protein-biased ligand. This type of molecule has been hypothesized to promote analgesia without major unwanted effects such as respiratory depression and tolerance. However, some ingredients are still missing. Discrepancies between studies highlight the translational gaps and challenges that still remain when translating *in vitro* bias to *in vivo* physiology (*e.g.*, variation in stoichiometry between receptors and signaling components or between GRKs and β-arrestin, varying temporal differentiation of agonist signals in cells, location bias, and implication of intrinsic pharmacokinetic properties; *for an extensive and comprehensive review, see* Kenakin (2024) [50]). We need to keep in mind that as we move forward from *in vitro* to *in vivo*, we will have additional factors to consider as interactions and interconnections between co-dependent systems increase [144]. Filling in the translational gap that exists between *in vitro* and *in vivo* may require making radical changes to the process of developing biased molecules in order to refine the choice of molecules and to make better-informed decisions. The use of primary cells from animal models of

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diseases is often cited as an intermediate approach, but the use of more complex readouts, such as transcriptome analysis, might yield more relevant and textured data for the development of biased ligands [145,146]. This could help explain why some G protein-biased agonists with similar profiles display no rewarding effects and no dependence, whereas others have the same abuse liability as clinically used opioids.

Although oliceridine is the first prospectively designed MOP-biased agonist to receive clinical approval, it has not yet demonstrated clear superiority over standard opioid treatments in terms of safety and efficacy. Nevertheless, retrospective analyses of known drugs for bias signaling, such as buprenorphine and levorphanol, suggest that biased agonism and/or low intrinsic efficacy agonists remain a promising approach to pursue in the quest for new and improved painkillers.

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References

1. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet. 2021 May 29;397(10289):2082-97.
2. Volkow ND, Blanco C. The changing opioid crisis: development, challenges and opportunities. Mol Psychiatry. 2021 Jan;26(1):218-33.
3. Duarte DF. [Opium and opioids: a brief history.]. Rev Bras Anestesiol. 2005 Feb;55(1):135-46.
4. Santoro D, Bellinghieri G, Savica V. Development of the concept of pain in history. J Nephrol. 2011 May-Jun;24 Suppl 17:S133-6.
5. Abdel Shaheed C, Maher CG, Williams KA, Day R, McLachlan AJ. Efficacy, Tolerability, and Dose-Dependent Effects of Opioid Analgesics for Low Back Pain: A Systematic Review and Meta-analysis. JAMA Intern Med. 2016 Jul 1;176(7):958-68.
6. de Leon-Casasola OA. Opioids for chronic pain: new evidence, new strategies, safe prescribing. Am J Med. 2013 Mar;126(3 Suppl 1):S3-11.
7. Kissin I. The development of new analgesics over the past 50 years: a lack of real breakthrough drugs. Anesth Analg. 2010 Mar 1;110(3):780-9.
8. WHO Expert Committee. The selection and use of essential medicines: report of the WHO Expert Committee, 2017 (including the 20th WHO Model List of Essential Medicines and the 6th WHO Model List of essential Medicines for Children). WHO technical report series ; no 1006. Geneva: World Health Organization; 2017. p. 604.

9. Shipton EA, Shipton EE, Shipton AJ. A Review of the Opioid Epidemic: What Do We Do About It? Pain Ther. 2018 Jun;7(1):23-36.
10. Manchikanti L, Helm S, 2nd, Fellows B, Janata JW, Pampati V, Grider JS, et al. Opioid epidemic in the United States. Pain Physician. 2012 Jul;15(3 Suppl):ES9-38.
11. Webster L, Schmidt WK. Dilemma of Addiction and Respiratory Depression in the Treatment of Pain: A Prototypical Endomorphin as a New Approach. Pain Med. 2019 Jun 5.
12. Wang Y, Zhuang Y, DiBerto JF, Zhou XE, Schmitz GP, Yuan Q, et al. Structures of the entire human opioid receptor family. Cell. 2023 Jan 19;186(2):413-27 e17.
13. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature. 1996 Oct 31;383(6603):819-23.
14. Hsia JA, Moss J, Hewlett EL, Vaughan M. ADP-ribosylation of adenylate cyclase by pertussis toxin. Effects on inhibitory agonist binding. J Biol Chem. 1984 Jan 25;259(2):1086-90.
15. Taussig R, Iniguez-Lluhi JA, Gilman AG. Inhibition of adenylyl cyclase by Gi alpha. Science. 1993 Jul 9;261(5118):218-21.
16. Ippolito DL, Temkin PA, Rogalski SL, Chavkin C. N-terminal tyrosine residues within the potassium channel Kir3 modulate GTPase activity of Galphai. J Biol Chem. 2002 Sep 6;277(36):32692-6.
17. Sadja R, Alagem N, Reuveny E. Gating of GIRK Channels. Neuron. 2003;39(1):9-12.
18. Díaz A, Flórez J, Pazos A, Hurlé MA. Opioid tolerance and supersensitivity induce regional changes in the autoradiographic density of dihydropyridine-sensitive calcium channels in the rat central nervous system. Pain. 2000;86(3):227-35.
19. Diaz A, Ruiz F, Florez J, Pazos A, Hurle MA. Regulation of dihydropyridine-sensitive Ca++ channels during opioid tolerance and supersensitivity in rats. J Pharmacol Exp Ther. 1995 Sep;274(3):1538-44.
20. Wickman K, Clapham DE. Ion channel regulation by G proteins. Physiol Rev. 1995 Oct;75(4):865-85.
21. Dang VC, Christie MJ. Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons. Br J Pharmacol. 2012 Mar;165(6):1704-16.
22. Groer CE, Schmid CL, Jaeger AM, Bohn LM. Agonist-directed interactions with specific beta-arrestins determine mu-opioid receptor trafficking, ubiquitination, and dephosphorylation. J Biol Chem. 2011 Sep 9;286(36):31731-41.
23. Melief EJ, Miyatake M, Bruchas MR, Chavkin C. Ligand-directed c-Jun N-terminal kinase activation disrupts opioid receptor signaling. Proc Natl Acad Sci U S A. 2010 Jun 22;107(25):11608-13.
24. Whistler JL, Chuang H-h, Chu P, Jan LY, von Zastrow M. Functional Dissociation of μ Opioid Receptor Signaling and Endocytosis. Neuron. 1999;23(4):737-46.
25. Gondin AB, Halls ML, Canals M, Briddon SJ. GRK Mediates mu-Opioid Receptor Plasma Membrane Reorganization. Front Mol Neurosci. 2019;12:104.
26. Raffa RB, Martinez RP, Connelly CD. G-protein antisense oligodeoxyribonucleotides and μ-opioid supraspinal antinociception. European Journal of Pharmacology. 1994;258(1-2):R5-R7.
27. Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science. 1999 Dec 24;286(5449):2495-8.
28. Raehal KM, Walker JK, Bohn LM. Morphine Side Effects in β -Arrestin 2 Knockout Mice. J Pharmacol Exp Ther. 2005 Sep;314(3):1195-201.
29. Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ, Caron MG. Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. Nature. 2000 Dec 7;408(6813):720-3.
30. Gaveriaux-Ruff C, Kieffer BL. Opioid receptor genes inactivated in mice: the highlights. Neuropeptides. 2002 Apr-Jun;36(2-3):62-71.
31. Yang CH, Huang HW, Chen KH, Chen YS, Sheen-Chen SM, Lin CR. Antinociceptive potentiation and attenuation of tolerance by intrathecal beta-arrestin 2 small interfering RNA in rats. Br J Anaesth. 2011 Nov;107(5):774-81.
32. Kliewer A, Schmiedel F, Sianati S, Bailey A, Bateman JT, Levitt ES, et al. Phosphorylation-deficient G-protein-biased mu-opioid receptors improve analgesia and diminish tolerance but worsen opioid side effects. Nat Commun. 2019 Jan 21;10(1):367.
33. Bull FA, Baptista-Hon DT, Sneddon C, Wright L, Walwyn W, Hales TG. Src Kinase Inhibition Attenuates Morphine Tolerance without Affecting Reinforcement or Psychomotor Stimulation. Anesthesiology. 2017 Nov;127(5):878-89.
34. Kliewer A, Gillis A, Hill R, Schmiedel F, Bailey C, Kelly E, et al. Morphine-induced respiratory depression is independent of beta-arrestin2 signalling. Br J Pharmacol. 2020 Jul;177(13):2923-31.
35. Chen YJ, Oldfield S, Butcher AJ, Tobin AB, Saxena K, Gurevich VV, et al. Identification of phosphorylation sites in the COOH-terminal tail of the mu-opioid receptor. J Neurochem. 2013 Jan;124(2):189-99.
36. Miess E, Gondin AB, Yousuf A, Steinborn R, Mosslein N, Yang Y, et al. Multisite phosphorylation is required for sustained interaction with GRKs and arrestins during rapid mu-opioid receptor desensitization. Sci Signal. 2018 Jul 17;11(539).

37. Gregory KJ. Commentary on "Morphine-induced respiratory depression is independent of beta-arrestin2 signalling". Br J Pharmacol. 2020 Jul;177(13):2904-5.
38. Lam H, Maga M, Pradhan A, Evans CJ, Maidment NT, Hales TG, et al. Analgesic tone conferred by constitutively active mu opioid receptors in mice lacking beta-arrestin 2. Mol Pain. 2011 Apr 12;7:24.
39. Stone LS, Molliver DC. In search of analgesia: emerging roles of GPCRs in pain. Mol Interv. 2009 Oct;9(5):234-51.
40. Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. Nat Rev Drug Discov. 2010 May;9(5):373-86.
41. Audet M, Bouvier M. Restructuring G-protein- coupled receptor activation. Cell. 2012 Sep 28;151(1):14-23.
42. Kenakin T. Functional selectivity and biased receptor signaling. J Pharmacol Exp Ther. 2011 Feb;336(2):296-302.
43. Wootten D, Christopoulos A, Marti-Solano M, Babu MM, Sexton PM. Mechanisms of signalling and biased agonism in G protein-coupled receptors. Nat Rev Mol Cell Biol. 2018 Oct;19(10):638-53.
44. van der Westhuizen ET, Breton B, Christopoulos A, Bouvier M. Quantification of ligand bias for clinically relevant beta2-adrenergic receptor ligands: implications for drug taxonomy. Mol Pharmacol. 2014 Mar;85(3):492-509.
45. Kenakin T, Christopoulos A. Signalling bias in new drug discovery: detection, quantification and therapeutic impact. Nat Rev Drug Discov. 2013 Mar;12(3):205-16.
46. Besserer-Offroy E, Sarret P. Sending out Biased Signals: an Appropriate Proposition for Pain? Douleur et Analgésie. 2019;32(2):108-10.
47. Michel MC, Charlton SJ. Biased Agonism in Drug Discovery-Is It Too Soon to Choose a Path? Mol Pharmacol. 2018 Apr;93(4):259-65.
48. Urs NM, Gee SM, Pack TF, McCorvy JD, Evron T, Snyder JC, et al. Distinct cortical and striatal actions of a beta-arrestin-biased dopamine D2 receptor ligand reveal unique antipsychotic-like properties. Proc Natl Acad Sci U S A. 2016 Dec 13;113(50):E8178-E86.
49. Schattauer SS, Kuhar JR, Song A, Chavkin C. Nalfurafine is a G-protein biased agonist having significantly greater bias at the human than rodent form of the kappa opioid receptor. Cell Signal. 2017 Apr;32:59-65.
50. Kenakin T. Bias translation: The final frontier? Br J Pharmacol. 2024 May;181(9):1345-60.
51. Kenakin T. Know your molecule: pharmacological characterization of drug candidates to enhance efficacy and reduce late-stage attrition. Nat Rev Drug Discov. 2024 Aug;23(8):626-44.
52. Keith DE, Murray SR, Zaki PA, Chu PC, Lissin DV, Kang L, et al. Morphine activates opioid receptors without causing their rapid internalization. J Biol Chem. 1996 Aug 9;271(32):19021-4.
53. Ehrlich AT, Kieffer BL, Darcq E. Current strategies toward safer mu opioid receptor drugs for pain management. Expert Opin Ther Targets. 2019 Feb 25:1-12.
54. McPherson J, Rivero G, Baptist M, Llorente J, Al-Sabah S, Krasel C, et al. mu-opioid receptors: correlation of agonist efficacy for signalling with ability to activate internalization. Mol Pharmacol. 2010 Oct;78(4):756-66.
55. Schmid CL, Kennedy NM, Ross NC, Lovell KM, Yue Z, Morgenweck J, et al. Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics. Cell. 2017 Nov 16;171(5):1165-75 e13.
56. Butler S. Buprenorphine-Clinically useful but often misunderstood. Scand J Pain. 2013 Jul 1;4(3):148-52.
57. Lutfy K, Cowan A. Buprenorphine: a unique drug with complex pharmacology. Curr Neuropharmacol. 2004 Oct;2(4):395-402.
58. Davis MP, Pasternak G, Behm B. Treating Chronic Pain: An Overview of Clinical Studies Centered on the Buprenorphine Option. Drugs. 2018 Aug;78(12):1211-28.
59. Ehrlich AT, Darcq E. Recommending buprenorphine for pain management. Pain Manag. 2019 Jan 1;9(1):13-6.
60. Vlok R, An GH, Binks M, Melhuish T, White L. Sublingual buprenorphine versus intravenous or intramuscular morphine in acute pain: A systematic review and meta-analysis of randomized control trials. Am J Emerg Med. 2019 Mar;37(3):381-6.
61. Gudin J, Fudin J, Nalamachu S. Levorphanol use: past, present and future. Postgrad Med. 2016 Jan;128(1):46-53.
62. Prommer E. Levorphanol: the forgotten opioid. Support Care Cancer. 2007 Mar;15(3):259-64.
63. Prommer E. Levorphanol: revisiting an underutilized analgesic. Palliat Care. 2014;8:7-10.
64. Le Rouzic V, Narayan A, Hunkle A, Marrone GF, Lu Z, Majumdar S, et al. Pharmacological Characterization of Levorphanol, a G-Protein Biased Opioid Analgesic. Anesth Analg. 2019 Feb;128(2):365-73.
65. Nair AS, Upputuri O, Mantha SSP, Rayani BK. Levorphanol: Rewinding an Old, Bygone Multimodal Opioid Analgesic! Indian J Palliat Care. 2019 Jul-Sep;25(3):483-4.
66. Pham TC, Fudin J, Raffa RB. Is levorphanol a better option than methadone? Pain Med. 2015 Sep;16(9):1673-9.

 doi:10.20944/preprints202410.2014.v1

67. Chen X-T, Pitis P, Liu G, Yuan C, Gotchev D, Cowan CL, et al. Structure–Activity Relationships and Discovery of a G Protein Biased μ Opioid Receptor Ligand, [(3-Methoxythiophen-2-yl)methyl]({2-[(9R)-9-(pyridin-2-yl)-6-oxaspiro-[4.5]decan-9-yl]ethyl})amine (TRV130), for the Treatment of Acute Severe Pain. Journal of Medicinal Chemistry. 2013;56(20):8019-31.
68. DeWire SM, Yamashita DS, Rominger DH, Liu G, Cowan CL, Graczyk TM, et al. A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. J Pharmacol Exp Ther. 2013 Mar;344(3):708-17.
69. Trevena Inc. Trevena Announces Submission of New Drug Application to U.S. FDA for Oliceridine Injection. 2017 [cited 2020-03-24]; Available from: https://www.trevena.com/investors/press-releases/detail/127/trevena-announces-submission-of-new-drug-application-to
70. Trevena Inc. Trevena Announces FDA Acceptance for Review of New Drug Application for Oliceridine Injection. 2018 [cited 2020-03-24]; Available from: https://www.trevena.com/investors/press-releases/detail/129/trevena-announces-fda-acceptance-for-review-of-new-drug
71. Trevena Inc. Trevena Announces Oliceridine FDA Advisory Committee Meeting Outcome. 2018 [cited 2020-03-24]; Available from: https://www.trevena.com/investors/press-releases/detail/154/trevena-announces-oliceridine-fda-advisory-committee
72. FDA AADPAC. Transcript for the October 11, 2018 Meeting of the Anesthetic and Analgesic Drug Products Advisory Committee (AADPAC). 2018 [cited 2020-03-24]; Available from: https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AnestheticAndAnalgesicDrugProductsAdvisoryCommittee/UCM629602.pdf
73. Trevena Inc. Trevena Announces Receipt of Type A Meeting Minutes and Provides Regulatory Update for Oliceridine. 2019 [cited 2020-03-14]; Available from: https://www.trevena.com/investors/press-releases/detail/160/trevena-announces-receipt-of-type-a-meeting-minutes-and
74. Trevena Inc. Trevena Announces Initiation of Healthy Volunteer Study for Oliceridine. 2019 [cited 2020-05-12]; Available from: https://www.trevena.com/investors/press-releases/detail/5/trevena-announces-initiation-of-healthy-volunteer-study-for
75. Nafziger AN, Arscott KA, Cochrane K, Skobieranda F, Burt DA, Fossler MJ. The Influence of Renal or Hepatic Impairment on the Pharmacokinetics, Safety, and Tolerability of Oliceridine. Clin Pharmacol Drug Dev. 2019 Nov 7.
76. Trevena Inc. Trevena Resubmits New Drug Application for Oliceridine. 2020 [cited 2020-06-16]; Available from: https://www.trevena.com/investors/press-releases/detail/224/trevena-resubmits-new-drug-application-for-oliceridine
77. Gan TJ, Wase L. Oliceridine, a G protein-selective ligand at the mu-opioid receptor, for the management of moderate to severe acute pain. Drugs Today (Barc). 2020 Apr;56(4):269-86.
78. Trevena Inc. Trevena Announces FDA Approval of OLINVYK™ (oliceridine) injection. 2020 [cited 2024-08-18]; Available from: https://www.trevena.com/investors/press-releases/detail/234/trevena-announces-fda-approval-of-olinvyk-oliceridine-injection
79. U.S. Food & Drug Administration. FDA Approves New Opioid for Intravenous Use in Hospitals, Other Controlled Clinical Settings. 2020 [cited; Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-new-opioid-intravenous-use-hospitals-other-controlled-clinical-settings
80. Shah A, Shah R, Fahim G, Brust-Sisti LA. A Dive Into Oliceridine and Its Novel Mechanism of Action. Cureus. 2021 Oct;13(10):e19076.
81. Azzam AAH, Lambert DG. Preclinical discovery and development of oliceridine (Olinvyk(R)) for the treatment of post-operative pain. Expert Opin Drug Discov. 2022 Mar;17(3):215-23.
82. Trevena Inc. Trevena, Inc. Announces DEA Scheduling of OLINVYK™ (oliceridine) injection. 2020 [cited; Available from: https://www.trevena.com/investors/press-releases/detail/244/trevena-inc-announces-dea-scheduling-of-olinvyk-oliceridine-injection
83. U.S. Food & Drug Administration. Second Cycle Review And Summary Basis for Approval - Oliceridine injection (NDA 210-730). 2020 2020-08-07 [cited 210730Orig1s000]; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/210730Orig1s000MultidisciplineR.pdf
84. Trevena Inc. Trevena Announces Approval of OLINVYK in China. 2023 [cited 2024-08-18]; Available from: https://www.trevena.com/investors/press-releases/detail/320/trevena-announces-approval-of-olinvyk-in-china
85. Ayad S, Demitrack MA, Burt DA, Michalsky C, Wase L, Fossler MJ, et al. Evaluating the Incidence of Opioid-Induced Respiratory Depression Associated with Oliceridine and Morphine as Measured by the Frequency and Average Cumulative Duration of Dosing Interruption in Patients Treated for Acute Postoperative Pain. Clin Drug Investig. 2020 Aug;40(8):755-64.
86. Brzezinski M, Hammer GB, Candiotti KA, Bergese SD, Pan PH, Bourne MH, et al. Low Incidence of Opioid-Induced Respiratory Depression Observed with Oliceridine Regardless of Age or Body Mass Index: Exploratory Analysis from a Phase 3 Open-Label Trial in Postsurgical Pain. Pain Ther. 2021 Jun;10(1):457-73.

87. Hammer GB, Khanna AK, Michalsky C, Wase L, Demitrack MA, Little R, et al. Oliceridine Exhibits Improved Tolerability Compared to Morphine at Equianalgesic Conditions: Exploratory Analysis from Two Phase 3 Randomized Placebo and Active Controlled Trials. Pain Ther. 2021 Dec;10(2):1343-53.
88. Beard TL, Michalsky C, Candiotti KA, Rider P, Wase L, Habib AS, et al. Oliceridine is Associated with Reduced Risk of Vomiting and Need for Rescue Antiemetics Compared to Morphine: Exploratory Analysis from Two Phase 3 Randomized Placebo and Active Controlled Trials. Pain Ther. 2021 Jun;10(1):401-13.
89. Hill DM, DeBoer E. State and Future Science of Opioids and Potential of Biased-ligand Technology in the Management of Acute Pain After Burn Injury. J Burn Care Res. 2023 May 2;44(3):524-34.
90. Manglik A, Lin H, Aryal DK, McCorvy JD, Dengler D, Corder G, et al. Structure-based discovery of opioid analgesics with reduced side effects. Nature. 2016 Sep 8;537(7619):185-90.
91. Emery MA, Eitan S. Members of the same pharmacological family are not alike: Different opioids, different consequences, hope for the opioid crisis? Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2019;92:428-49.
92. Kudla L, Bugno R, Skupio U, Wiktorowska L, Solecki W, Wojtas A, et al. Functional characterization of a novel opioid, PZM21, and its influence on behavioural responses to morphine. Br J Pharmacol. 2019 Jul 26.
93. Hill R, Disney A, Conibear A, Sutcliffe K, Dewey W, Husbands S, et al. The novel mu-opioid receptor agonist PZM21 depresses respiration and induces tolerance to antinociception. Br J Pharmacol. 2018 Jul;175(13):2653-61.
94. Grim TW, Acevedo-Canabal A, Bohn LM. Toward Directing Opioid Receptor Signaling to Refine Opioid Therapeutics. Biol Psychiatry. 2020 Jan 1;87(1):15-21.
95. de Waal PW, Shi J, You E, Wang X, Melcher K, Jiang Y, et al. Molecular mechanisms of fentanyl mediated beta-arrestin biased signaling. PLoS Comput Biol. 2020 Apr;16(4):e1007394.
96. Ramos-Gonzalez N, Groom S, Sutcliffe KJ, Bancroft S, Bailey CP, Sessions RB, et al. Carfentanil is a beta-arrestin-biased agonist at the mu opioid receptor. Br J Pharmacol. 2023 Sep;180(18):2341-60.
97. Huang W, Manglik A, Venkatakrishnan AJ, Laeremans T, Feinberg EN, Sanborn AL, et al. Structural insights into micro-opioid receptor activation. Nature. 2015 Aug 20;524(7565):315-21.
98. Zhuang Y, Wang Y, He B, He X, Zhou XE, Guo S, et al. Molecular recognition of morphine and fentanyl by the human mu-opioid receptor. Cell. 2022 Nov 10;185(23):4361-75 e19.
99. Kelly E, Conibear A, Henderson G. Biased Agonism: Lessons from Studies of Opioid Receptor Agonists. Annu Rev Pharmacol Toxicol. 2023 Jan 20;63:491-515.
100. Kise R, Inoue A. GPCR signaling bias: an emerging framework for opioid drug development. J Biochem. 2024 Mar 25;175(4):367-76.
101. Gillis A, Gondin AB, Kliewer A, Sanchez J, Lim HD, Alamein C, et al. Low intrinsic efficacy for G protein activation can explain the improved side effect profiles of new opioid agonists. Sci Signal. 2020 Mar 31;13(625).
102. Stoeber M, Jullie D, Lobingier BT, Laeremans T, Steyaert J, Schiller PW, et al. A Genetically Encoded Biosensor Reveals Location Bias of Opioid Drug Action. Neuron. 2018 Jun 6;98(5):963-76 e5.
103. Stahl EL, Bohn LM. Low Intrinsic Efficacy Alone Cannot Explain the Improved Side Effect Profiles of New Opioid Agonists. Biochemistry. 2022 Sep 20;61(18):1923-35.
104. Clark DJ, Schumacher MA. America's Opioid Epidemic: Supply and Demand Considerations. Anesth Analg. 2017 Nov;125(5):1667-74.
105. Killeen N, Lakes R, Webster M, Killoran S, McNamara S, Kavanagh P, et al. The emergence of nitazenes on the Irish heroin market and national preparation for possible future outbreaks. Addiction. 2024 Sep;119(9):1657-8.
106. Kozell LB, Eshleman AJ, Wolfrum KM, Swanson TL, Bloom SH, Benware S, et al. Pharmacologic Characterization of Substituted Nitazenes at mu, kappa, and Delta Opioid Receptors Suggests High Potential for Toxicity. J Pharmacol Exp Ther. 2024 Apr 18;389(2):219-28.
107. Bendjilali-Sabiani JJ, Eiden C, Lestienne M, Cherki S, Gautre D, Van den Broek T, et al. Isotonitazene, a synthetic opioid from an emerging family: The nitazenes. Therapie. 2024 May 28.
108. Amaducci A, Aldy K, Campleman SL, Li S, Meyn A, Abston S, et al. Naloxone Use in Novel Potent Opioid and Fentanyl Overdoses in Emergency Department Patients. JAMA Netw Open. 2023 Aug 1;6(8):e2331264.
109. Hauser W, Schug S, Furlan AD. The opioid epidemic and national guidelines for opioid therapy for chronic noncancer pain: a perspective from different continents. Pain Rep. 2017 May;2(3):e599.
110. Rudd RA, Seth P, David F, Scholl L. Increases in Drug and Opioid-Involved Overdose Deaths - United States, 2010-2015. MMWR Morb Mortal Wkly Rep. 2016 Dec 30;65(50-51):1445-52.
111. Busse JW, Craigie S, Juurlink DN, Buckley DN, Wang L, Couban RJ, et al. Guideline for opioid therapy and chronic noncancer pain. CMAJ. 2017 May 8;189(18):E659-E66.
112. Bohn LM, Gainetdinov RR, Sotnikova TD, Medvedev IO, Lefkowitz RJ, Dykstra LA, et al. Enhanced Rewarding Properties of Morphine, but not Cocaine, in βarrestin-2 Knock-Out Mice. The Journal of Neuroscience. 2003;23(32):10265-73.

113. Zamarripa CA, Edwards SR, Qureshi HN, Yi JN, Blough BE, Freeman KB. The G-protein biased mu-opioid agonist, TRV130, produces reinforcing and antinociceptive effects that are comparable to oxycodone in rats. Drug Alcohol Depend. 2018 Nov 1;192:158-62.
114. Altarifi AA, David B, Muchhala KH, Blough BE, Akbarali H, Negus SS. 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. 2017 Jun;31(6):730-9.
115. Liang DY, Li WW, Nwaneshiudu C, Irvine KA, Clark JD. Pharmacological Characters of Oliceridine, a mu-Opioid Receptor G-Protein[FIGURE DASH]Biased Ligand in Mice. Anesth Analg. 2018 Jul 21.
116. Soergel DG, Subach RA, Burnham N, Lark MW, James IE, Sadler BM, et al. Biased agonism of the mu-opioid receptor by TRV130 increases analgesia and reduces on-target adverse effects versus morphine: A randomized, double-blind, placebo-controlled, crossover study in healthy volunteers. Pain. 2014 Sep;155(9):1829-35.
117. Grim TW, Schmid CL, Stahl EL, Pantouli F, Ho JH, Acevedo-Canabal A, et al. A G protein signaling-biased agonist at the mu-opioid receptor reverses morphine tolerance while preventing morphine withdrawal. Neuropsychopharmacology. 2020 Jan;45(2):416-25.
118. Quirion B, Bergeron F, Blais V, Gendron L. The Delta-Opioid Receptor; a Target for the Treatment of Pain. Front Mol Neurosci. 2020;13:52.
119. Blaine AT, van Rijn RM. Receptor expression and signaling properties in the brain, and structural ligand motifs that contribute to delta opioid receptor agonist-induced seizures. Neuropharmacology. 2023 Jul 1;232:109526.
120. Conibear AE, Asghar J, Hill R, Henderson G, Borbely E, Tekus V, et al. A Novel G Protein-Biased Agonist at the delta Opioid Receptor with Analgesic Efficacy in Models of Chronic Pain. J Pharmacol Exp Ther. 2020 Feb;372(2):224-36.
121. PharmNovo A. PharmNovo announces positive Phase I results for neuropathic pain management drug PN6047. 2024 [cited 2024-08-18]; Available from: https://www.mediconvillage.se/pharmnovo-announces-positive-phase-i-results-for-neuropathic-pain-management-drug-pn6047/
122. Cayir S, Zhornitsky S, Barzegary A, Sotomayor-Carreno E, Sarfo-Ansah W, Funaro MC, et al. A review of the kappa opioid receptor system in opioid use. Neurosci Biobehav Rev. 2024 Jul;162:105713.
123. Santino F, Gentilucci L. Design of kappa-Opioid Receptor Agonists for the Development of Potential Treatments of Pain with Reduced Side Effects. Molecules. 2023 Jan 1;28(1).
124. Dalefield ML, Scouller B, Bibi R, Kivell BM. The Kappa Opioid Receptor: A Promising Therapeutic Target for Multiple Pathologies. Front Pharmacol. 2022;13:837671.
125. Mores KL, Cummins BR, Cassell RJ, van Rijn RM. A Review of the Therapeutic Potential of Recently Developed G Protein-Biased Kappa Agonists. Front Pharmacol. 2019;10:407.
126. Valentino RJ, Volkow ND. Untangling the complexity of opioid receptor function. Neuropsychopharmacology. 2018 Dec;43(13):2514-20.
127. Meyrath M, Szpakowska M, Zeiner J, Massotte L, Merz MP, Benkel T, et al. The atypical chemokine receptor ACKR3/CXCR7 is a broad-spectrum scavenger for opioid peptides. Nat Commun. 2020 Jun 19;11(1):3033.
128. Gach-Janczak K, Biernat M, Kuczer M, Adamska-Bartlomiejczyk A, Kluczyk A. Analgesic Peptides: From Natural Diversity to Rational Design. Molecules. 2024 Mar 29;29(7).
129. Palmer CB, Meyrath M, Canals M, Kostenis E, Chevigne A, Szpakowska M. Atypical opioid receptors: unconventional biology and therapeutic opportunities. Pharmacol Ther. 2022 May;233:108014.
130. O'Neill A, Lirk P. Multimodal Analgesia. Anesthesiol Clin. 2022 Sep;40(3):455-68.
131. Young A, Buvanendran A. Recent advances in multimodal analgesia. Anesthesiol Clin. 2012 Mar;30(1):91-100.
132. Giummarra MJ, Gibson SJ, Allen AR, Pichler AS, Arnold CA. Polypharmacy and chronic pain: harm exposure is not all about the opioids. Pain Med. 2015 Mar;16(3):472-9.
133. Arumugam S, Lau CS, Chamberlain RS. Use of preoperative gabapentin significantly reduces postoperative opioid consumption: a meta-analysis. J Pain Res. 2016;9:631-40.
134. Pergolizzi JV, Jr., van de Laar M, Langford R, Mellinghoff HU, Merchante IM, Nalamachu S, et al. Tramadol/paracetamol fixed-dose combination in the treatment of moderate to severe pain. J Pain Res. 2012;5:327-46.
135. Pergolizzi J, Varrassi G, LeQuang JAK, Breve F, Magnusson P. Fixed Dose Versus Loose Dose: Analgesic Combinations. Cureus. 2023 Jan;15(1):e33320.
136. Frantz S. The trouble with making combination drugs. Nat Rev Drug Discov. 2006 Nov;5(11):881-2.
137. Gascon N, Almansa C, Merlos M, Miguel Vela J, Encina G, Morte A, et al. Co-crystal of tramadol-celecoxib: preclinical and clinical evaluation of a novel analgesic. Expert Opin Investig Drugs. 2019 May;28(5):399-409.
138. Caputi FF, Nicora M, Simeone R, Candeletti S, Romualdi P. Tapentadol: an analgesic that differs from classic opioids due to its noradrenergic mechanism of action. Minerva Med. 2019 Feb;110(1):62-78.

139. Barakat A. Revisiting Tramadol: A Multi-Modal Agent for Pain Management. CNS Drugs. 2019 May;33(5):481-501.
140. Cannella N, Lunerti V, Shen Q, Li H, Benvenuti F, Soverchia L, et al. Cebranopadol, a novel long-acting opioid agonist with low abuse liability, to treat opioid use disorder: Preclinical evidence of efficacy. Neuropharmacology. 2024 Oct 1;257:110048.
141. Kiguchi N, Ding H, Ko MC. Therapeutic potentials of NOP and MOP receptor coactivation for the treatment of pain and opioid abuse. J Neurosci Res. 2022 Jan;100(1):191-202.
142. Sanam M, Ashraf S, Saeed M, Khalid A, Abdalla AN, Qureshi U, et al. Cebranopadol: An Assessment for Its Biased Activation Potential at the Mu Opioid Receptor by DFT, Molecular Docking and Molecular Dynamic Simulation Studies. ChemistrySelect. 2023;8(37).
143. Kleczkowska P, Lipkowski AW, Tourwe D, Ballet S. Hybrid opioid/non-opioid ligands in pain research. Curr Pharm Des. 2013;19(42):7435-50.
144. Kenakin T. Biased Receptor Signaling in Drug Discovery. Pharmacol Rev. 2019 Apr;71(2):267-315.
145. Luttrell LM, Maudsley S, Gesty-Palmer D. Translating in vitro ligand bias into in vivo efficacy. Cell Signal. 2018 Jan;41:46-55.
146. Walker CS, Sundrum T, Hay DL. PACAP receptor pharmacology and agonist bias: analysis in primary neurons and glia from the trigeminal ganglia and transfected cells. Br J Pharmacol. 2014 Mar;171(6):1521-33.
147. Ehrlich AT, Semache M, Gross F, Da Fonte DF, Runtz L, Colley C, et al. Biased Signaling of the Mu Opioid Receptor Revealed in Native Neurons. iScience. 2019 Apr 26;14:47-57.
148. Rivero G, Llorente J, McPherson J, Cooke A, Mundell SJ, McArdle CA, et al. Endomorphin-2: a biased agonist at the mu-opioid receptor. Mol Pharmacol. 2012 Aug;82(2):178-88.
149. Kelly E. Efficacy and ligand bias at the mu-opioid receptor. Br J Pharmacol. 2013 Aug;169(7):1430-46.
150. Pedersen MF, Wrobel TM, Marcher-Rorsted E, Pedersen DS, Moller TC, Gabriele F, et al. Biased agonism of clinically approved mu-opioid receptor agonists and TRV130 is not controlled by binding and signaling kinetics. Neuropharmacology. 2019 Jul 24:107718.