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An updated assessment of the translational promise of G-protein-biased kappa opioid receptor agonists to treat pain and other indications without debilitating adverse effects

Alexander R. French^{1,2}, Richard M. van Rijn^{1,2,3,†}

¹Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, USA

²Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, IN 47907, USA

³Purdue Institute for Drug Discovery, Purdue University, West Lafayette, IN 47907, USA

Abstract

Kappa opioid receptor (κ OR) agonists lack the abuse liability and respiratory depression effects of clinically used mu opioid receptor (μ OR) analgesics and are hypothesized to be safer alternatives. However, κ OR agonists have limiting adverse effects of their own, including aversion, sedation, and mood effects, that have hampered their clinical translation. Studies performed over the last 15 years have suggested that these adverse effects could result from activation of distinct intracellular signaling pathways that are dependent on β -arrestin, whereas signaling downstream of G protein activation produces antinociception. This led to the hypothesis that agonists biased away from β -arrestin signaling would have improved therapeutic windows over traditional unbiased agonists and allow for clinical development of analgesic G-protein-biased κ OR agonists. Given a recent controversy regarding the benefits of G-protein-biased μ OR agonists, it is timely to reassess the therapeutic promise of G-protein-biased κ OR agonists. Here we review recent discoveries from preclinical κ OR studies and critically evaluate the therapeutic windows of G-protein-biased κ OR agonists in each of the adverse effects above. Overall, we find that G-protein-biased κ OR agonists generally have improved therapeutic window relative to unbiased agonists, although frequently study design limits strong conclusions in this regard. However, a steady flow of newly developed biased κ OR agonists paired with recently engineered behavioral and molecular tools puts the κ OR field in a prime position to make major advances in our understanding of κ OR function and fulfill the promise of translating a new generation of biased κ OR agonists to the clinic.

[†]**Corresponding author address:** Richard M. van Rijn, Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, Indiana 47907 (Phone: 765-494-6461; rvanrijn@purdue.edu).

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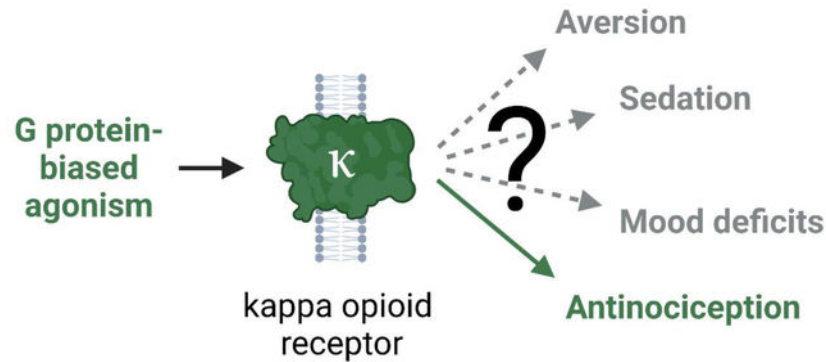
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Graphical Abstract



Keywords

kappa opioid receptor; biased agonism; antinociception; aversion; sedation; depression

1. Introduction to the kappa opioid receptor

The kappa opioid receptor (κ OR) is a G protein-coupled receptor expressed throughout the central nervous system as well as in some peripheral tissues [1,2] and is activated by the endogenous opioid agonist peptide, dynorphin A, which was discovered in 1981 [3]. Dynorphin A is a cleavage product of its precursor protein, preprodynorphin, and is released from neurons through the merging of its resident large dense core vesicles to the plasma membrane [4]. When dynorphin A binds to the κ OR, it initiates a signal transduction cascade involving the activation of the heterotrimeric G-protein complex, which for the κ OR generally includes the inhibitory $G_{\alpha i/o}$ -proteins. Following activation, the κ OR is rapidly phosphorylated which promotes recruitment of the β -arrestin class of scaffolding proteins [5]. Acutely, these events depress neuronal activity through G protein-mediated inhibition of voltage-gated Ca^{2+} channels, and the activation of hyperpolarizing channels such as the G protein-couple inwardly rectifying K^+ [6], κ OR desensitization, as well as β -arrestin-initiated trafficking of neurotransmitter re-uptake channels to the plasma membrane [7]. The longer-term effects of extended κ OR activation include the engagement of kinases that lead to a variety of transcriptional changes, the downstream mediators of which we are only beginning to identify [8]. It is these intracellular events that ultimately allow endogenous and exogenous κ OR agonists to impact behavior [6,9].

Like the mu opioid receptor (μ OR), the κ OR is appreciated for its role in nociception and its activation causes analgesia in mammals [10]. κ OR activation does not share the same considerable adverse effects, commonly observed with clinical μ OR agonists, like morphine and fentanyl, such as respiratory depression, constipation, and high abuse liability [6]. Yet, potent, efficacious κ OR agonists have not translated into humans as alternative analgesic options as they are noteworthy for inducing aversion, dysphoria, sedation, and/or depressive and anxiety-related effects [10,11]. Nevertheless, analgesic drugs that modulate the κ OR have been clinically approved, though often the drugs have low potency for κ OR (e.g. tramadol) [12,13], are partial agonists (e.g. nalbuphine, butorphanol) [10], or even

antagonists (buprenorphine) [14]. In the majority of these cases, the drug has strong(er) potency at μ OR from which it derives most of the analgesic efficacy.

However, the concept of biased signaling, i.e. the idea that certain agonists can stabilize κ OR conformations that preferentially engage one signaling pathway over another, has unveiled the opportunity for a fully efficacious κ OR therapeutic agonist. This idea was driven by early studies in the Chavkin lab, which produced multiple papers suggesting that κ OR agonist-induced dysphoria and aversion is dependent on β -arrestin-mediated p38 activation [7,15–17]. Thus, together with earlier *in vivo* studies that utilized the $G_{\alpha i}$ -protein inhibitor pertussis toxin to demonstrate that the analgesic effects of κ OR agonists depended on G protein signaling [18–20], biasing κ OR agonists towards G protein activation and away from β -arrestin signaling was predicted to be beneficial [5]. Later, the finding that κ OR agonists remain antinociceptive in mice lacking β -arrestin 2 further confirmed that G protein signaling was necessary, whereas β -arrestin signaling was dispensable for therapeutic efficacy [21]. These studies, and others, some of which will be reviewed here, have fueled efforts to develop and discover novel G protein-biased κ OR agonists for treatment of not only pain, but a variety of central and peripheral pathologies. In this review, we examine whether the initial promise of G-protein-biased κ OR agonist as a path towards clinical relevance still holds true, needs refining or should be discarded. This is particularly important in light of a recent, and ongoing reassessment of the value of G protein-biased μ OR agonists as safer analgesics [22–25].

2. Definition and calculation of bias

As discussed in Section 1, ligand “bias” refers to an agonist preferentially signaling through one pathway over another, relative to a reference agonist. Signaling bias at GPCRs can extend to cover any type of signal output produced following receptor activation, and can include, amongst others, different preferences in G protein isoform recruitment, receptor internalization, phosphorylation by GRK isoforms or recruitment of β -arrestin isoforms. Two pathways can be compared to generate a single scalar measure of bias, or “bias factor”, or several pathways compared simultaneously in spiderweb/radial plots [26] or forest plots [8]. Biased signaling at the κ OR has been reviewed recently [27–29] and for this review we mostly limit our discussion to studies investigating bias for G protein signaling over β -arrestin recruitment.

As stated above, bias factors are always calculated with respect to a reference agonist, which ideally taken to be the endogenous agonist of the receptor, or an unbiased synthetic compound that activates and recruits both G protein and β -arrestin pathways to the same degree as the endogenous ligand. The advantage of choosing the endogenous agonist as the reference is that a comparison is made to native biology. A disadvantage of using an endogenous agonist is that endogenous κ OR agonists are peptidic with poor central penetration, limiting ‘easy’ systemic administration in preclinical studies. Additional challenges for using dynorphins are that the endogenous opioid peptides have poor selectivity (relative to synthetic agonists designed to be more selective) [30,31] and that it is difficult to measure which endogenous peptide is the most relevant or dominant in a given circuit [32]. In contrast, synthetic small molecule κ OR agonists can enable studies where

the same reference compound is used to draw comparisons between cellular and behavioral pharmacology of the test compounds, without significant concerns over off-target signaling.

One parameter frequently used to measure and rank signal bias is the bias factor, defined as the $10^{\log(\tau/K_A)}$, which compares the dissociation constant, K_A , and τ , which estimates the efficacy of the receptor-agonist complex, for a test compound relative to the reference compound between two assays [33]. A downside of this method is that it conflates potency and efficacy; this is problematic as some κ OR agonists are deemed G-protein-biased by nature of their potency to activate G proteins despite being able to recruit β -arrestin with 100% efficacy. These types of G-protein-biased agonists are sometimes referred to as affinity-dominant and are in contrast with efficacy-dominant agonists that are biased because the compound has minimal β -arrestin recruitment efficacy [27,34]. In this review, we discuss nalfurafine, the salvinorin A analogues RB-64, mesyl salvinorin B (Mesyl Sal B), ethoxymethyl ether salvinorin B (EOM Sal B), 16-Bromo salvinorin A (16-Bromo Sal A), and triazole 1.1 which are affinity-dominant G-protein-biased agonists (Table 1). We also discuss salvinorin A (Sal A) as a G-protein-biased agonist relative to the early-discovered U50,488 and U69,593 [35,36], though due to its weak bias this compound is often utilized as the unbiased reference compound [21]. In the efficacy-dominant category, we discuss BPHA, 6'-GNTI, HS665, HS666, as well as recently developed compounds LOR17, "compound 4a" and the new peptide agonist Helianorphan19 (Table 1).

3. G biased agonism in analgesia

The κ OR is expressed in dorsal root ganglia neurons and in the spinal cord, where they directly modulate nociception [37,38]. The κ OR is also expressed throughout the basal ganglia and the mesolimbic pathway, putting it in key positions to regulate reward, motivation, mood and general motor behavior [9,39,40]. Generally, the κ OR suppresses each of these behaviors. Thus, the κ OR system is commonly summarized as analgesic in the periphery, with the undesirable side effects of κ OR activation (aversion, sedation, anxiety, et cetera) in the central nervous system [6,41]. Supporting this, classic κ OR agonists that penetrate the brain such as U50,488 [42] and U69,593 [43] produce antinociception but then are hampered by small therapeutic windows due to their activation of these alternative processes, giving them a negative side effect profile.

However, there is evidence that some of the antinociceptive components of κ OR activation occur centrally [44,45]. Additionally, pain is multidimensional: though the detection of noxious stimuli occurs in the periphery, the aversive component of chronic pain is centrally modulated via κ ORs in the nucleus accumbens [46]. Thus, attempts to generate successful, peripherally restricted κ OR analgesics have so far failed, leaving the idea that biased agonism can biochemically separate analgesic from undesired effects as the most promising avenue for bringing κ OR analgesics into the clinic [47]. G-protein-biased κ OR agonists have been tested in a variety of pain models – both acute and chronic – including thermal (e.g. hot plate/tail flick), mechanical (e.g. formalin/Von Frey), visceral (e.g. acetic acid writhing test) and neuropathic (e.g. nerve constriction test). From these studies it can be concluded that both balanced and G-protein-biased κ OR agonists generally are

antinociceptive (Table 1), supporting the idea that analgesia is driven through G protein-associated pathways at the κ OR [6,27].

Though all G-protein-biased κ OR agonists tested to date cause some form of antinociception, certain agonists appear more effective against certain types of pain than others. For example, nalfurafine (also known as TRK-820) was nearly 20 times more potent in the acetic acid writhing test than the tail flick assay in ddY mice [48]. When evaluated in Wistar rats, nalfurafine was approximately seven times more potent in the formalin (aqueous formaldehyde) test than a paw pressure test [49]. Mesyl Sal B [35] and 16-Bromo Sal B [50] demonstrated an opposite pattern: each showed antinociception in the warm water tail withdrawal model of thermal pain but were much less effective in a formalin model of pain in mice. In contrast, the reference compounds in these studies, Sal A [35] and the novel balanced agonist 16-ethynyl salvinorin A [50] were effective in both pain assays. Likewise, the newly developed SLL039/SLL1206 exhibited similar potencies in the hot plate, formalin, and acetic acid writhing tests [51]. The efficacy-dominant G-protein-biased κ OR agonist LOR17 also displayed similar potencies in the hot plate and acetic acid writhing tests [52].

Thus, there is more nuance to antinociception by the κ OR than can be captured by merely G protein bias vs β -arrestin. The data also cannot be explained by relative selectivity for the κ OR. For example, though nalfurafine is only moderately selective for the κ OR over the μ OR [53], both Mesyl Sal B and Sal A are extremely selective for the κ OR [54], and yet show different patterns of potency in the nociceptive assays. Gaining pharmacokinetic data, including information on the relative tissue distribution of these compounds, may help explain the differences in their performance. Additionally, applying newer techniques such as phosphoproteomic approaches that evaluate the impact of an agonist on many signaling partners simultaneously may reveal specific biochemical pathways that are responsible [8]. As discussed in the following sections, these compounds also differ in their potencies in generating adverse effects, which can potentially influence behavior in antinociceptive assays.

4. Aversion

If “rewarding” is defined as the quality that makes an organism repeat a behavior or desire an outcome, then “punishment” is the quality that makes an organism avoid or dislike a behavioral outcome [55]. The rewarding or punishing nature of a drug, when paired with neutral environmental stimuli (e.g. different patterning in a testing chamber), will generate a preference or an aversion, respectively, of those environmental stimuli that is readily evaluated in a conditioned place preference test [56,57]. Aversion stands as a serious hurdle to patient compliance with κ OR-mediated drugs. Thus, it is a key effect that researchers have sought to engineer out of κ OR agonists by introducing G protein bias.

It was discovered early that classic, unbiased κ OR agonists such as U50,488 [42] and U69,593 [43] are aversive, significantly restricting their therapeutic window. For example, U50,488 has an ED₅₀ in the formalin test of 0.58 mg/kg in male CD-1 mice but causes aversion at doses as low as 0.25 mg/kg [58]. In C57BL/6J mice, U50,488 has an ED₅₀ of 5.0

mg/kg in the tail withdrawal assay [59] but is aversive at a 2.5 mg/kg dose [15]. The same holds true in Sprague-Dawley rats, which show a 12.7 mg/kg ED₅₀ for U50,488 in the hot plate assay [60], with aversion occurring at just 10 mg/kg [59].

Nalfurafine is a biased κ OR agonist that is affinity-dominant (Table 1) and has very strong potency in the formalin pain model; nalfurafine consistently has ED₅₀ values below 10 μ g/kg [49,58]. However, nalfurafine does not produce aversion up to a dose of 40 μ g/kg in Sprague-Dawley rats [61], 30 μ g/kg in male ddY mice [62], and up to 20 μ g/kg in CD-1 mice [58] and 15 μ g/kg in C57BL/6J mice [36]. Thus, nalfurafine has a clear therapeutic window in rodents, supporting its clinical approval in Japan for uremic pruritis [63,64].

Salvinorin A and its analogue RB-64, which is significantly more G-protein-biased than salvinorin A, were both aversive at antinociceptive doses tested (3 mg/kg) in the hot plate assay [21]. Like nalfurafine, RB-64 is an affinity-dominant G-protein-biased agonist that efficaciously recruits β -arrestin 2, yet lacks an apparent therapeutic window for aversion [21]. It may be that RB-64 is antinociceptive at lower doses than 3 mg/kg, and the data underestimates its potency. Another possible reason for this discrepancy is that nalfurafine was more potent in formalin and acetic acid pain tests than in thermal pain models (Table 1) [48,58], and perhaps RB-64 might similarly be more potent in those pain assays, resulting potentially in a more significant therapeutic window.

Another salvinorin A analogue, EOM Sal B, did not produce aversion at 0.1 mg/kg, a dose at which it reduced cocaine-primed reinstatement in Sprague-Dawley rats [59]. EOM Sal B displayed some antinociceptive potency (EC₅₀ = 0.8 mg/kg in the warm water tail withdrawal assay), but aversion was not tested at higher doses so it is unclear if there is a therapeutic window for analgesia. Another study found that a 1 mg/kg dose of EOM Sal B was aversive in C57BL/6J mice [36]. Similar gaps are present for Mesyl Sal B, which has an antinociceptive ED₅₀ of 3 mg/kg in the tail withdrawal assay and is at least not aversive at a dose of 0.3 mg/kg, but higher doses were not evaluated [35].

With regard to efficacy-dominant G-protein-biased κ OR agonists, studies suggest that limited β -arrestin 2 recruitment efficacy correlates with limited conditioned place aversion. Intriguingly, the diphenylethylamines HS665 and HS666 both displayed potent single digit nanomole ED₅₀ values in the tail withdrawal assay, however HS665 which recruits β -arrestin 2 recruitment more efficaciously than HS666, displayed conditioned place aversion at 30 nmol (i.c.v.) whereas HS666 did not, even at 150 nmol [65]. Similarly, the efficacy-dominant G protein-biased agonist 6'-GNTI, which is antinociceptive at 10 nmol i.c.v. in the tail flick assay in ICR-CD1 mice [66] was not aversive in C57BL/6N mice up to 30 nmol i.c.v. [67].

Thus, nearly all explored affinity-dominant G-protein-biased agonists produce aversion at a large enough dose, and agonists biased in efficacy or both efficacy and affinity may have higher therapeutic windows for aversion. Still, a limitation for reaching such a conclusion is that many studies utilized different species, strains and pain assays, making proper comparisons difficult. Moreover, despite early studies linking aversion to arrestin-dependent signaling in cells [7,15–17], RB-64 and salvinorin A remained aversive in β -arrestin 2 knockout mice, suggesting this response was not dependent on β -arrestin 2 [21]. Given the

diverse phosphorylation patterns in κ OR agonists, it may be that reducing κ OR signaling to simply “G protein” or “arrestin” may not be sufficient for dissecting the plethora of behavioral effects of the κ OR [8].

5. Sedation

Beyond aversion, the promise of G-protein-biased κ OR agonism comes from studies suggesting that avoiding β -arrestin may reduce the sedative or hypolocomotive properties of κ OR agonists. Sedation is typically measured through either measuring an animal’s ambulatory locomotor behavior (hypolocomotion), sometimes after the “novel stimulus” of entering the testing chamber, or through a rotarod experiment that measures the length of time a rodent is able to maintain walking on a rotating beam [68]. These two assays have some nuances in that the locomotion is motivated differently (exploring environment versus fear of falling from rod) and in that the rotarod is also a measure of muscle coordination. These nuances can sometimes be detected by κ OR agonists. For example, whereas 20 μ g/kg nalfurafine does not cause hypolocomotion it does show impairment on the rotarod at that dose [58].

Generally, nalfurafine has an ED₅₀ of 27 μ g/kg or higher on the rotarod [69,70], which is above its antinociceptive ED₅₀ in several pain assays (Table 1). Similarly, nalfurafine’s therapeutic antipruritic effects require a 10-fold lower dose (0.1 μ g/kg) than the 1 μ g/kg nalfurafine in rhesus monkeys that reduces overall activity [71]. Thus, nalfurafine has a therapeutic window both for antinociception and antipruritic activity over sedative adverse effects. The importance of bias with regards to improving therapeutic window is exemplified by comparing nalfurafine with its analog nalfurafine 42B. Nalfurafine 42B showed significant hypolocomotor effects at just two times its antinociceptive midpoints and produced muscle incoordination on the rotarod at sub-antinociceptive doses [72]. The difference between nalfurafine and nalfurafine 42B is that the former has a G protein bias factor of 4.49, whereas the latter’s is 2.85 [72]. Similarly, the κ OR agonist U50,488, which is generally considered to have limited bias relative to dynorphin, also has poor resolution between doses causing antinociception and doses causing sedation. For example, 5 mg/kg U50,488 produced only modest antinociception in a tail flick assay in C57BL/6 mice, a dose that caused significant deficits in novelty-induced locomotion [73]. Another study reported ED₅₀ values for hot plate and acetic acid writhes to be 4.42 and 0.89 mg/kg, respectively, with an ED₅₀ of 3.32 mg/kg for U50,488 in the rotarod test, confirming a negligible therapeutic window for U50,488 [74].

Similarly to U50,488, salvinorin A produces hypolocomotive effects in rodents [21,50] and non-human primates [71,75]. However, derivatives of salvinorin A with greater G protein-bias can have higher thresholds for sedation. For example, 10 and 3 mg/kg RB-64 did not have sedative effects in either the rotarod or novelty-induced locomotion tests, respectively, compared to a 3 mg/kg dose producing antinociception in a hot plate assay in C57BL/6J mice [21].

Unfortunately, the locomotor effects of the salvinorin analogue Mesyl Sal B were evaluated at doses lower than those for antinociception, making it difficult to draw conclusions with

regard to its therapeutic. Specifically, two studies found Mesyl Sal B to be effective in the 1 – 3 mg/kg range in the tail withdrawal assay but tested for locomotor deficits at 0.3 – 2 mg/kg range in rodents [35,54]. Since no hypolocomotive effects were seen, this data neither rules out nor demonstrates a therapeutic window for Mesyl Sal B.

While Mesyl Sal B may yet have its therapeutic window demonstrated, two other salvinorin A analogues, EOM Sal B and 16-Bromo Sal A, look less promising. For example, one study measured the ED₅₀ of EOM Sal B in the warm water tail withdrawal test to be 0.83 mg/kg, and found that 0.3 mg/kg EOM Sal B did not show decreased ambulatory activity in Sprague-Dawley rats [59]. However, another study found that a 1 mg/kg dose of EOM Sal B that was antinociceptive in a tail flick assay in C57BL/6 mice also inhibited novelty-induced locomotor activity, arguing against the pursuit of EOM Sal B as a clinical candidate [36]. Likewise, 16-Bromo Sal A showed mild muscle incoordination on the rotarod assay at 1 mg/kg, compared to an ED₅₀ of 2.1 mg/kg in the tail withdrawal test [50]. Though 16-Bromo Sal A did not impact ambulatory locomotor activity at the 1 mg/kg dose [76], 16-Bromo Sal A is unlikely to have a strong therapeutic window for sedation. This data is another example of the rotarod assay being more sensitive to κ OR effects than ambulatory locomotion.

The affinity-dominant G-protein-biased κ OR agonist Triazole 1.1 did not show alterations in locomotor activity in male C57BL/6 mice up to 30 mg/kg but elicited antipruritic and antinociceptive responses at doses as low as 1 and 15 mg/kg, respectively [73]. This held true across species as doses of triazole 1.1 up to 0.32 mg/kg i.v. did not promote sedative behaviors in rhesus monkeys but blocked oxycodone-induced scratching [71]. Two κ OR agonists biased in both efficacy and affinity, HS665 and HS666, also showed no motor impairment in the rotarod assay when injected at 30 nmol i.c.v., much higher than the minimal antinociceptive dose of 3 nmol in C57BL/6 mice [65]. This result repeated when the agonists were injected subcutaneously in CD-1 mice, with HS665 and HS666 not causing muscle incoordination at up to 10 and 20 mg/kg, respectively, compared with ED₅₀ values of 1.91 and 3.23 mg/kg for antinociception in an acetic acid writhing test [77]. Thirdly, 6'-GNTI, an efficacy-dominant agonist, did not modulate ambulatory locomotor activity of C57BL/6N mice at doses up to 30 nmol (i.c.v.) [67].

Lastly, two new efficacy-dominant G-protein-biased κ OR agonists show promise for antinociception without sedation. LOR17 did not alter rotarod performance at 10 mg/kg s.c., double its ED₅₀ (5.74 mg/kg) in the acetic acid writhing test and equal to its ED₅₀ in the hot plate test (10.07 mg/kg) [52]. Similarly, a novel beta-carboline (compound 4a) was antinociceptive in the hot plate assay in C57BL/6J mice at 5 mg/kg i.p. but did not decline rotarod performance at 10 mg/kg (although this dose was administered orally) [78].

Overall, G-protein-biased κ OR agonists show a wide range of abilities to avoid sedation. Nalfurafine, RB-64, triazole 1.1, the diphenethylamines HS665 and HS666, and the efficacy-dominant agonists 6'-GNTI, LOR17, and compound 4a tend to not cause sedation at antinociceptive doses. In contrast, salvinorin A, EOM Sal B, and to a lesser extent 16-Bromo Sal A do cause hypolocomotion at antinociceptive doses. The results for Mesyl Sal B are promising but ambiguous until doses above the antinociceptive range are tested. Of note

is that drugs tested in the rotarod tend to have lower therapeutic windows for sedation than those tested by ambulatory locomotor activity (e.g. nalfurafine, [58]; 16-Bromo Sal A, [50,76]). Thus, muscle coordination may be more sensitive to κ OR activation than general sedation and could therefore be more important to assess in order to establish safety and maximal tolerable doses.

The above results would be meaningfully extended by studies evaluating the effects of biased κ OR agonists on electrocortical activity. Large scale changes in brain activity patterns between sleep and wakefulness produce unique signatures that can be readily detected by electroencephalographic methods. These techniques can therefore give a more direct measure of whether a novel κ OR agonist is altering movement due to sedation as opposed to other psychological effects [79]. These measurements can be made concurrently with electromyographs to give additional insights on whether immobility is due to sedation or incoordination. Though the invasive nature of these methods makes them less amenable to initial drug screening, they offer a strong complement to behavioral methods for establishing the sedative profile of candidate drugs.

6. Pro-anxiety and pro-depressive behaviors

A major hurdle for the translational development of κ OR agonists has been their ability to induce anxiety-like (often measured by the elevated plus maze test [80]), depression-like (forced-swim test [81]) behaviors as well as anhedonia (intracranial self-stimulation (ICSS) [82]) and dysphoria. The classic and relatively unbiased/balanced κ OR agonists U50,488 and U69,693 cause these undesirable effects at doses close to antinociceptive doses. For example, U69,593 increased immobility time in the forced swim test, i.e. increased depression-like behavior, at doses as low as 0.3 mg/kg i.p. in Sprague-Dawley rats [83]. As little as 0.25 mg/kg U69,593 can cause anhedonia in an ICSS test in Sprague-Dawley rats [84,85]. U50,488 promotes anhedonia in the ICSS test in Fisher 344 rats at a 6 mg/kg dose [73] and decreases sucrose responding (an alternative measure for anhedonia to ICSS) at 5 and 10 mg/kg in Long-Evans [86] and Sprague-Dawley [59] rats, respectively. In mice, doses as low as 0.5 and 1 mg/kg of U50,488 and U69,593, respectively, were sufficient to raise ICSS thresholds [21,58]. In contrast, the G-protein-biased nalfurafine did not alter ICSS in CD-1 mice up to a dose of 20 μ g/kg, three times higher than the ED₅₀ for reducing pain in the formalin test in this study [58].

Inconsistency in doses selected for assessing anxiety, depression, and anhedonia relative to the agonist's therapeutic effect hampers evaluating the therapeutic window of several G-protein-biased agonists that have been studied in rodent models. For example, RB-64 is antinociceptive at a 3 mg/kg dose, but was tested in ICSS at 1 mg/kg [21]. Though RB-64 did not alter ICSS thresholds, a core measure of anhedonia, it did create a rightward shift in how mice responded to different frequencies of stimulation, an alternative measure of anhedonia. Thus, the results are ambiguous for RB-64 [21]. Mesyl Sal B produced depression-like effects at 0.3 mg/kg (well below the antinociceptive dose, Table 1), but was not anxiogenic at this dose in male Sprague-Dawley rats, nor did a 0.3 and 1 mg/kg Mesyl Sal B dose alter sucrose self-administration [35]. EOM Sal B and salvinorin A did not induce anhedonia in male Sprague-Dawley rats at 0.1 and 0.3 mg/kg doses, respectively, as

measured by decreases in responding to sucrose [59]. Additionally, while 0.1 and 0.3 mg/kg EOM Sal B did not alter time spent in the open arm in the elevated maze test, 0.3 mg/kg salvinorin A decreased open arm time (promoted anxiety) [59]. These doses of EOM Sal B also did not alter mobility in the forced swim test [59]. However, similarly to the studies on RB-64 and Mesyl Sal B [21,35], the tested doses of EOM Sal B and salvinorin A are lower than their warm water tail flick ED₅₀ values of 0.83 mg/kg and 1.4 mg/kg, respectively [59]. Thus, the no observed adverse effect level (NOAEL) of salvinorin A is lower than its antinociceptive dose, whereas the results for EOM Sal B are ambiguous as it lacks a Maximum Tolerated Dose, relative to its respective tail-flick ED₅₀.

In contrast to the above studies, 16-Bromo Sal A was tested for anxiety measures at antinociceptive doses [50]. 16-Bromo Sal A up to 2 mg/kg did not alter time in open arm or arm entries in the elevated zero maze test, nor did it alter marble burying (a measure of anxiety/compulsivity) in C57BL/6J mice, though 1 and 2 mg/kg was sufficient for thermal antinociception in B6-SJL mice [50]. Triazole 1.1 also did not affect ICSS in Fisher 344 rats at doses up to 24 mg/kg, compared with antinociceptive responses at doses as low as 15 mg/kg in C57BL/6J [73]. the 24 mg/kg dose of Triazole 1.1 muted the ability of 1.8% lactic acid to reduce ICSS in the ventral tegmental area in C57BL/6J mice [73]. Thus, not only is triazole 1.1 not anhedonic on its own, but it can relieve anhedonia brought on by painful stimuli [11].

LOR17, a recently discovered G-protein-biased κ OR agonist, is the only efficacy-dominant agonist in this review to have been tested for depression-like behavior. LOR17 did not alter mobility time in the forced swim paradigm at 10 mg/kg, double its ED₅₀ (5.74 mg/kg) in the acetic acid writhing test and equal to its ED₅₀ (10.07 mg/kg) in the hot plate test [52]. More work is needed to identify the therapeutic window in this assay and whether this extends to other efficacy-dominant G-protein-biased agonists at the κ OR.

Despite the limitations of interpreting therapeutic windows for G-biased κ OR agonists relative to mood-related adverse effects, it appears that multiple biased agonists, including nalfurafine, triazole 1.1, 16-Bromo Sal A, and LOR17 can avoid negative mood behavior at antinociceptive doses, and thus can differentiate themselves from unbiased κ OR agonists.

7.1 Utility for G-protein-biased κ OR agonists to reduce substance use disorders

The therapeutic potential of κ OR agonists is not restricted to their use as alternatives to μ OR analgesics. Two indications actively being explored are substance use disorders and pruritus (discussed in the following subsection). Agonism of the κ OR can block the rewarding aspects of μ OR agonists, supporting the idea that κ OR agonists may serve as adjuvants to analgesic drugs to mute their abuse liability [36,87]. This idea remains true for G-protein-biased κ OR agonists. For example, 15 μ g/kg nalfurafine and 1 mg/kg EOM Sal B each reduced morphine-induced conditioned place preference in C57BL/6J mice [36]. Additionally, nalfurafine disrupted self-administration of fentanyl over food reward in male and female Sprague-Dawley rats [88]. This effect was specific to nalfurafine co-administered with fentanyl, as pretreatment with nalfurafine, even at doses that decreased overall responding (presumably through sedation), had no effect [88]. Likewise, nalfurafine and triazole 1.1 decreased oxycodone (56 μ g/kg/injection) self-administration in Sprague-

Dawley rats when co-administered at doses as low as 3.2 and 1800 $\mu\text{g}/\text{kg}/\text{injection}$, respectively [60]. Notably, nalfurafine and the relatively unbiased agonist salvinorin A also prevented increased responding for oxycodone (25 – 100 $\mu\text{g}/\text{kg}/\text{injection}$) in male rhesus monkeys when co-administered at doses as low as 0.18 and 6 $\mu\text{g}/\text{kg}/\text{injection}$, respectively [89].

Thus, G-protein-biased agonists can block the place preference of μOR analgesics and limit their self-administration, suggesting co-administration of these drugs can limit clinical abuse liability. In addition, when co-administered, κOR agonists often potentiate the analgesia of traditional pharmaceuticals, which could help lower the amount used and prevent the development of a substance use disorder. For example, 15 $\mu\text{g}/\text{kg}$ nalfurafine potentiated the antinociceptive effect of 5 mg/kg morphine in both the tail immersion and hot plate assays [36]. Additionally, both nalfurafine and triazole 1.1 co-administered with oxycodone significantly increased the antinociceptive potency of oxycodone in Sprague-Dawley rats [60]. 10–15 $\mu\text{g}/\text{kg}$ nalfurafine can also block morphine-induced hyperlocomotion in rodents, an effect that is blocked by the κOR antagonist norBNI [36,62]. Note that it is important to assess whether κOR agonists can block μOR agonist reward and hyperlocomotion at doses where the κOR agonists are not sedative on their own. For example, one study found mild hypolocomotive effects at mid-range doses of nalfurafine (15 $\mu\text{g}/\text{kg}$), an effect that passed within 30 minutes [36]. 1 mg/kg EOM Sal B showed more pronounced hypolocomotive effects in C57BL/6J mice [36].

Note that many of the findings on κOR agonists blocking morphine reward also generalize to other substances of abuse. For example, doses of 3–20 $\mu\text{g}/\text{kg}$ nalfurafine can block cocaine place preference [61,70]. Thus, the utility of biased κOR agonists may extend beyond preventing development of opioid use disorders to reducing relapse/reinstatement following abstinence of other substances of abuse. Historically, κOR antagonists have been pursued to block drug reinstatement and prevent relapse [90,91]. A number of studies have suggested that part of the mechanism by which unbiased κOR agonists can promote reinstatement involves β -arrestin signaling [7,92,93]. As such it can be hypothesized that G-protein-biased agonism may be able to inhibit relapse as it could serve as a functional antagonist of dynorphin-mediated β -arrestin signaling.

Supporting this, pre-injection of either EOM Sal B (0.1 or 0.3 mg/kg), salvinorin A (0.3 mg/kg) [59], or Mesyl Sal A (0.3 or 1.0, but not 0.1 mg/kg) [54] prevented cocaine-induced cocaine self-administration in Sprague-Dawley rats, a model of re-instatement. 16-Bromo Sal A at 0.3 and 1 mg/kg attenuated cocaine reinstatement primed by a 20 mg/kg injection of cocaine in Sprague-Dawley rats [76]. 0.3 mg/kg Mesyl Sal B also blocked sensitization to cocaine-induced hyperlocomotion in Sprague-Dawley rats previously receiving cocaine [35]. Similarly, 0.1 mg/kg EOM Sal B [59] and 0.3 mg/kg Mesyl Sal B [35] reduced cocaine-induced hyperlocomotion in Sprague-Dawley rats. 0.3 and 1 mg/kg Mesyl Sal B co-administered with 1 mg/kg of the FDA-approved alcohol use disorder treatment naltrexone also decreased ethanol consumption in an intermittent access drinking model in male and female C57BL/6J mice [94]. A 10x higher dose of Mesyl Sal B was required to lower ethanol consumption when administered without naltrexone. These effects didn't repeat in

a lower-consumption drinking-in-the-dark model, suggesting this result may be specific to animals with a history of high ethanol consumption [94].

However, pretreatment with 10 µg/kg, but not 3, nalfurafine increased self-administration of cocaine in C57BL/6 mice, an effect that was blocked by the reversible κOR agonist LY2444296 [70]. Indeed, studies with the unbiased κOR agonist U50,488 find that effects on drug reward and self-administration are time and dose-dependent, with U50,488 able to either block or potentiate both cocaine/ethanol place preference [95–97] and cocaine self-administration [98]. Therefore, since the field is new enough that few results have been published with multiple time points for G-protein-biased agonists, caution should be used when interpreting the above findings.

Overall, co-administration of G-protein-biased κOR agonists consistently lowers the place preference and self-administration of µOR analgesics while improving their antinociceptive potency. However, especially considering the potential confounding effects of timing and dose seen with unbiased κOR agonists, this field is still in its infancy and it is too soon to ascertain a therapeutic window relative to hypolocomotion or aversion for the presented biased κOR agonists. With regard to treating substance use disorders that are already developed, the data is even more complex. Nonetheless, triazole 1.1 and, to a lesser extent, nalfurafine, show promise in this area. Note that of the fourteen G-protein-biased agonists in Table 1, only six have data in the substance use disorder field. Diversifying these studies to include a wider array of G-protein-biased agonists will also help develop our understanding of the potential of these agonists in this area.

7.2. Utility for G-protein-biased κOR agonists to treat itch and inflammation

κORs are expressed in human mast cells and κOR stimulation reduces mast cell numbers [99] which may attribute to the established antipruritic efficacy of κOR agonists [100,101]. As our understanding of pruritus and the role of κOR therein continues to expand, G-protein-biased κOR agonists continue to show promise in this area. An early study looking at orally administered nalfurafine found that 100 µg/kg nalfurafine reduced scratching in substance P-induced itch in ICR mice, an effect that was blocked by norBNI [102]. This same study found that both 30 and 100 µg/kg nalfurafine p.o. inhibited scratching in histamine-induced (10 µg/site) itch [102]. These early studies helped nalfurafine to become the first κOR agonist approved for use in humans in Japan for uremic pruritus [63,64]. Nalfurafine continues to be a standard bearer in testing in this area. Nalfurafine decreased scratching behavior induced by the MRGPRX2 agonist compound 48/80 with an ED₅₀ of 8.0 µg/kg [58]. Nalfurafine also reduced scratching behavior in an ethynylestradiol (2 mg/kg/day for 14 days) cholestatic liver disease model in Sprague-Dawley rats with an ED₅₀ of 13 µg/kg [103]. Recently, doses as low as 0.1 µg/kg nalfurafine were found to reduce spontaneous scratching behavior in rhesus monkeys [71]. Pretreatment with 50 µg/kg nalfurafine also inhibited scratching induced by the κOR antagonist 5'-GNTI in C57BL/6 mice (30 µg/kg) [104].

The affinity-dominant G-protein-biased triazole 1.1 inhibited chloroquine phosphate (40 mg/kg)-induced scratching at doses as low as 1 mg/kg in C57BL/6J mice [73]. Additionally,

doses up to 0.32 mg/kg i.v. did not promote sedative behaviors in rhesus monkeys but blocked scratching induced by 0.1 mg/kg oxycodone [71].

Overall, the G-protein-biased κ OR agonists nalfurafine and triazole 1.1. consistently mitigate scratching behaviors in animal models. In contrast, 6'-GNTI was found to induce scratching behavior, though it should be noted that 6'-GNTI-induced scratching behavior in C57BL/6J mice was sustained in κ OR KO mice, suggesting this is a unique off-target effect [105]. The peripherally restricted κ OR agonist CR845 (difelikefalin) recently received approval in the United States for treatment of pruritus associated with chronic kidney disease in hemodialysis patients [106]. With both CR845 and nalfurafine approved in humans for pruritus, it is likely work in this area will continue to expand. In particular, as the mechanisms behind pruritus continue to be elucidated, it will be interesting to see whether some of the benefits of G-protein-biased agonists can be generalized to other inflammatory diseases, such as multiple sclerosis [107]. Indeed, the κ OR is expressed on numerous immune cells and its full therapeutic potential in this area has not yet been realized [108].

8.1. Overview of recently developed κ OR agonists with known G protein bias

Only a handful of biased κ OR agonists have been investigated extensively across multiple studies and in some cases multiple research teams. Yet there are numerous signal-biased κ OR agonists that have been characterized solely in the paper where they were first described. In the following section we review some of the more recently developed biased κ OR agonists and other agonists recently shown to be biased in order to highlight the structural diversity that exists amongst signal-biased κ OR agonists. Additionally, this section may promote follow-up studies that could aid in understanding the promise and limitations associated with affinity- and efficacy-dominant G-protein-biased κ OR agonists.

Extracts from the kratom plant *Mitragyna speciosa* have been found to have opioidergic activities via a collection of β -carboline compounds [109]. These compounds are generally non-specific between the opioid receptors, but in a recent collaboration between the Yadav and Batra labs researchers developed a series of fused ring β -carbolines that were highly specific for the κ OR [78]. Compound 4a was the most potent (46 nM, Glosensor) and showed bias toward G protein signaling (cAMP) and away from β -arrestin 2 (Tango) and ERK1/2 signaling (SRE-luc). A dose of 5 mg/kg compound 4a was antinociceptive in the tail-flick and hot plate tests – an effect that was blocked by norBNI – while no locomotor deficits were observed even at 10 mg/kg [78].

Two other agonists, N-n-butyl- N-phenylethyl-N-3-hydroxyphenylethyl-amine (BPHA [110], or compound 5 in [111]) and 16-Bromo Sal A [50,76] are derived from other G-protein-biased scaffolds. BPHA is a structural derivative of HS665 and HS666 with 10-fold lower affinity than HS665 [111]. BPHA has strong G protein activation with almost zero observed efficacy for β -arrestin recruitment and did not show sedation at doses up to 30 mg/kg in C57BL/6 mice [110]. 16-Bromo Sal A is a derivative of salvinorin A and has 7.7x bias for G protein signaling over arrestin signaling at the κ OR [50]. 16-Bromo Sal A was significantly antinociceptive in a warm water tail withdrawal assay and to a lesser extent in a formalin model of pain in B6-SJL mice [50]. 16-Bromo Sal A was not anxiogenic in the elevated zero maze or the marble burying tests in C57Bl/6J mice, and only had mild

incoordination in the rotarod test in B6-SJL mice. The specificity of 16-Bromo Sal A for the κ OR over other opioid receptors is unknown.

While biased signaling may be one way to ameliorate the adverse effects associated with κ OR agonism, another approach is to avoid engaging centrally expressed κ ORs in the first place. This is a potentially relevant strategy given that peripheral κ ORs are of therapeutic interest, as can be gleaned from difelikefalin. Difelikefalin is a synthetic κ OR agonist that is peripherally restricted by nature of its peptidic structure and is devoid of aversion and dysphoria [112,113]. Unfortunately, we have been unable to find any reports on the signal-bias of difelikefalin or characterization of the tetrapeptide in an arrestin-recruitment assay, limiting our ability to discuss it in light of biased signaling. Regardless, one does not need to rely on exogenous peptides to discover signal-biased κ OR peptides; one recent study characterized 20 endogenous opioid peptides at μ OR, δ OR and κ OR and identified intriguing profiles for several of them at the κ OR [31]. Using GTP γ S and β -arrestin 2 pathHunter assays to calculate bias factors, the study found that DynorphinA₁₋₁₃ and metorphamide are β -arrestin-biased peptides at the κ OR. Additionally, Stefanucci et al computationally screened 6 million tripeptides and found two sequences, H-D-Tyr-Val-Val-O-(3-Br)-Bz and H-D-Tyr-Val-Trp-OBz, that they then tested *in vivo* [114]. Both peptides show antinociception in the tail flick (i.c.v) and formalin (i.p.) tests in CD-1 mice, with the effect in the tail flick experiment lasting for up to 90 minutes. More work is needed to understand the level of central penetration of these peptides, as well as their signaling mechanisms and *in vivo* effects.

Cyclized peptides can overcome some of the metabolic liabilities of natural peptides and have been successfully produced for μ OR and δ OR. Building on previous work looking at cyclized δ OR and μ OR tetrapeptides [115], a novel cyclotetrapeptide (c[Phe-Gly-(b-Ala)-D-Trp]) named LOR17 that has 10⁵-fold selectivity for κ OR over μ OR or δ OR was recently discovered [52]. Additionally, the authors calculated a bias factor for G protein (cAMP EIA kit) over β -arrestin 2 (PathHunter) signaling of 853 for LOR17. The study also utilized two human cell lines, U87-MG (ATCC HTB-14) and normal human astrocytes (Lonza CC-2565) that endogenously express κ ORs to cross-validate their *in vitro* results. LOR17 was found to be antinociceptive in a panel of assays, including tests of thermal (warm water tail withdrawal), visceral (acetic acid writhing), and neuropathic pain (oxaliplatin cold plate test). The same dose (10 mg/kg) was found to have no sedative effects in a small battery of locomotor and coordination tests [52].

Another group, inspired by the antinociceptive cyclopeptides in sunflower, cyclized dynorphin and added two cysteine residues to bridge the ring across the middle of the sequence [116]. Their top hit, named helianorphin-19 after the species name for sunflower, *Helianthus annuus*, has ~200-fold selectivity for κ OR over μ OR and δ OR. Helianorphin-19 is a full agonist in the cAMP assay (CisBio HTRF) but only a partial agonist for β -arrestin 2 in a BRET assay, with similar shifts in potency between helianorphin-19 and dynorphin. Thus, it likely has some degree of bias toward G protein signaling over β -arrestin 2. Helianorphin-19 ablated hypersensitivity in a model of chronic visceral pain (dinitrobenzene sulfonic acid – induced colitis). This effect seems to be due to a lowered response in nociceptor firing rate to mechanical stimuli, an effect that was blocked by norBNI.

Moreover, a 5 mg/kg dose of helianorphan-19 did not cause incoordination in a rotarod assay. As the peptide was delivered in different routes, it is difficult to infer a therapeutic window from these tests. Nonetheless, the relative dosing suggests helianorphan-19 is a promising candidate for visceral pain.

8.2. Summary of newer κ OR agonists with unknown signaling properties

A number of recent studies have described preclinical efficacy of novel κ OR agonists for which bias is unknown but may be inferred based on the known profile of the compounds they are modelled after. For example, The Shao lab has recently found a series of 6,14-endoethanotetrahydronorthebaines, chemical structures related to the G-protein-biased nalfurafine, to be potent, κ OR-selective agonists [117,118]. Both SLL-039 [117] and SLL-1206 [118] showed antinociception in hot plate, abdominal constriction, and formalin tests [51,117,118]. These two compounds have long-lasting antinociception, each peaking at 4–6 hours post-injection. Moreover, each have 3–10x therapeutic windows for analgesia over hypolocomotor and aversion. However, the hypolocomotor and aversive effects of SLL-039 and SLL-1206 were evaluated at 2 to 3 hours post-injection, which matches the timing for the antinociception assays but misses early timepoints where κ OR agonists can have acute effects (e.g. [21,35]). Further investigating these compounds in this acute time range will help establish their clinical potential.

The Abels lab recently synthesized two new series of perhydroquinoxalines with excellent potency and selectivity for the κ OR [119,120]. They selected molecules for restriction to the periphery and found candidates with anti-inflammatory activity. When tested in human skin organ culture, they found that compound 5a [119] decreased the number of mast cells, supporting its use as an antipruritic [99]. While the bias of the agonists in their 2017 series has not yet been evaluated, the agonists in the 2019 series are arrestin biased relative to their reference compound salvinorin A. Since salvinorin A has itself been found to have G protein bias relative to dynorphin [35,36], these calculations should be followed up on. Nonetheless, it would be interesting to see these compounds characterized in models of pain and pruritus.

The Wunsch lab recently engineered novel potent κ OR agonists building off earlier work [121] that investigated compounds wherein the N-atom of the dichlorophenylacetamide group of the κ OR agonist U50,488 is incorporated into a piperidine ring [122]. Their approach was to further modify the piperidine ring into bicyclic moieties. Their top hits have excellent selectivity for κ OR over μ OR, δ OR, and σ_2 receptors, with moderate (8 – 22x) selectivity over σ_1 receptors.

The Tao group recently designed a series of peptides modifying the termini of the peripheral κ OR agonist difelikefalin/CR845 [123]. Their top hit, SHR0687, is peripherally restricted, highly selective κ OR agonist showing antinociception in a carrageenan-induced allodynia model (paw withdrawal threshold). Notably, in this model SHR0687 produced antinociception with similar potency as difelikefalin.

9. Conclusions, recommendations, and future directions

Despite being proposed over a decade ago [5] G-protein-biased agonism at the κ OR is still in its infancy with regard to understanding the pharmacological basis of its adverse effect profile (Table 2). Given the availability of a diverse array of efficacy- and affinity-dominant G-protein-biased agonists, this is an excellent time to begin evaluating the pharmacology of these compounds in more stringent preclinical models for pain and substance use disorders, as well as testing their adverse effects in multiple models, at multiple time points, and at multiple doses. Additionally, assessment of tachyphylaxis upon repeated administration of efficacy- and affinity-dominant G protein-biased agonists to either the analgesic or adverse effects is not typically performed, but would be of great value for drug lead development [124]. It is also critical to carry out pharmacokinetic evaluations and perform absorption, distribution, metabolism, and excretion (ADME) screening to determine how much of the biased κ OR agonists enter the brain, and for how long. Additionally, the use of female subjects should be part of the study design as sex-differences have been reported for κ OR ligands [125–127].

In particular, no efficacy-dominant κ OR agonist has been fully characterized in all three of the adverse effect areas in this review. Moreover, among the affinity-dominant agonists, the evaluation of the therapeutic window for the salvinorin A derivative RB-64 is based only on single- and two-point tests. For certain κ OR agonists, such as EOM Sal B and Mesyl Sal B, adverse effects were measured at doses that were lower than the antinociceptive doses, making these results ambiguous [35,59]. We recommend testing for adverse effects at least at 1x and 3x (roughly a half log unit above) the antinociceptive dose tested (or the ED₅₀ value). Among the adverse effects, we recommend evaluating sedation first, since this can potentially confound nearly every other behavioral assay (including antinociception itself). Even if rotarod/ambulatory activity cannot be quantified, having some qualitative measure of locomotor effects adds significant confidence to the interpretation of any other behavioral tests performed. After screening in rodents, the establishment of a non-sedative profile in nonhuman primates for promising κ OR agonists can be performed using qualitative measurements of behavior [71], and is particularly important for motivating future translational studies of the drug.

The evaluation of many of the agonists are also based on only one type of nociception, most commonly thermal pain, which κ OR agonists are commonly not as potent for as chemical (formalin) and visceral pain (Section 3) [48,52]. In addition to more broadly characterizing agonists with the pain models discussed above, a host of new pain models have been developed that have been validated with traditional analgesics [47]. These include the evaluation of restoration of depressed behaviors following pain stimuli as well as operant measures, such as the restoration of ICSS responding following a painful stimulus [47,73]. Classic κ OR agonists typically fail in these antinociceptive assays, making them a more stringent test of effectiveness for new G-protein-biased κ OR agonists [11,47]. It should be noted that although the G-protein-biased agonist nalfurafine failed to progress in clinical trials for analgesia, it is still debated whether nalfurafine is G protein- or arrestin-biased (Table 1) [11]. Broader characterization of κ OR agonists with a diverse range in bias will help better prune candidates and define the profile of a successful κ OR analgesic [11,47].

Despite challenges in translating κ OR analgesics to humans, κ OR agonists have had some success in the clinic. Thus far, one of the most promising areas of translation of κ OR agonists is in treating pruritus. Nalfurafine was the first κ OR-selective agonist to receive clinical authorization, for drug-resistant itch in uremic patients in Japan [64]. Peripherally restricted κ OR agonists, which are designed with the mindset that most unwanted side-effects are centrally mediated, have also failed to translate to the clinic as analgesic option [47]. Nonetheless, the peripherally restricted κ OR agonist CR845 (difelikefalin) was recently approved in the United States under the brand name KORSUVA for pruritus in chronic kidney disease [106]. It is also currently in clinical trials for pruritus in patients with primary biliary cholangitis, notalgia paresthetica, and atopic dermatitis [128]. As discussed above in Section 7.2, G-protein-biased agonists tend to mitigate scratching behavior in animal models. Despite the clinical success of κ OR agonists in treating pruritus, of the sixteen studies summarized in Section 8 on new and understudied κ OR agonists, eleven of them used some measure of pain, while only one [51] used a measure of pruritus. Incorporating antipruritic behavioral tests would greatly enhance evaluations the clinical potential of new κ OR agonists.

Advances in tool development may further aid in probing *in vivo* effects of G-protein-biased κ OR agonists. For example, optodialysis probes [129] and the fluorescent, genetically encoded dynorphin sensor kLight[130] can be used to study to what degree G-protein-biased κ OR agonists compete for dynorphin binding or modulate dynorphin release. Efforts could be expended to create G-protein-biased agonists for chemogenetic tools such as κ OR Δ i [131], a κ OR that has been engineered to respond to Sal B but not to κ OR ligands. Similarly, G-protein-biased photoactivable dynorphin analogues [132] could be developed to help study biased κ OR signaling *in vivo*. Advanced mass spectrometry methods are also useful for characterizing divergent downstream signaling cascades, for example those that include p38 [15] and mTOR [58]. Finally, the use of specific nanobodies that stabilize signal-biased conformations will be useful both in cellular assays and in generating Cryo-EM or X-ray crystallographic structural information of κ OR bound to biased agonists [133–136]. These efforts together will help refine what the ideal signaling profile of a κ OR agonist looks like, in an iterative process that will improve translational efforts at this receptor.

While much of the above has focused on G protein signaling, further tool development is needed to understand the role of β -arrestins at the κ OR. Though early studies suggested a link between β -arrestin 2-dependent p38 signaling and adverse effects at the κ OR [7,15–17], new data in β -arrestin 2 KO mice suggests this protein is not essential for aversive components of κ OR signaling [21]. Following up with conditional β -arrestin 2 KO mice [137,138] could reduce any confounding compensatory changes in congenic β -arrestin 2 KO mice. Additionally, nearly all of the work summarized to date uses β -arrestin 2 recruitment as a proxy for arrestin signaling at the κ OR. However, when exploring biased signaling, β -arrestin 1 should not be discounted, as increasing evidence suggests the two β -arrestin isoforms fulfill unique and even opposing roles [139–143]. New tools to study these isoforms in detail will further our understanding of how β -arrestins contribute to the diverse behavioral profiles of κ OR agonists.

In summary, despite a decade of effort, there is much work to do in understanding the potential of G protein-biased κ OR agonists for clinical use in humans (Table 2), however we would argue their potential remains. With two κ OR agonists now approved for pruritus [64,106], testing in this area should be a major consideration when demonstrating initial therapeutic potential of new ligands, even among research teams searching for novel κ OR analgesics. Lastly, the plethora of new tools developed for studying the κ OR, including the ligands reviewed here, offer exciting opportunities to expand our understanding of how biased agonism translates into altered brain function, and the multifaceted roles of the κ OR in health and disease.

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Abbreviations:

κOR	kappa opioid receptor
μOR	mu opioid receptor
δOR	delta opioid receptor
6'-GNTI	6'-guanidinonaltrindol
16-Bromo Sal A	16-Bromo salvinorin A
BRET	bioluminescence resonance energy transfer
cAMP	cyclic adenosine monophosphate
EOM Sal B	ethoxymethyl ether salvinorin B
GPCR	G protein-coupled receptor
HS665	3-[2-(Cyclobutylmethyl-phenethyl-amino)-ethyl]-phenol Hydrochloride
HS666	3-(2-((Cyclopropylmethyl)(phenethyl)amino)ethyl)phenol hydrochloride
KO	knockout
LOR17	c[Phe-Gly-(b-Ala)-D-Trp]
Mesyl Sal B	mesyl salvinorin B
nalfurafine	(2E)-N-[(5 α ,6 β)-17-(cyclopropylmethyl)- 3,14-dihydroxy-4,5-epoxymorphinan- 6-yl]- 3-(3-furyl)-N-methylacrylamide hydrochloride
norBNI	norbinaltorphimine

U50,488	2-(3,4-dichlorophenyl)-N-methyl-N-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide
U69,593	a,7a,8b)-N-methyl-N-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)-benzenacetamide

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Table 1.
Signaling bias and antinociceptive potency of κ OR agonists.

Overview of the bias profile (unbiased, G-protein-biased or arrestin-biased as well as affinity- or efficacy-dominant) for what are generally considered G-protein-biased κ OR agonists. Antinociceptive ED₅₀ values are provided where possible, otherwise the reported dose or dose range in mg/kg that was significantly antinociceptive in the study is provided in parentheses. Listed are the pain model and animal species/strain used in the study. Doses are systemic unless otherwise indicated. Select studies for the classic agonist U50,488 are included at the top as reference.

Agonist name	Bias Direction	Bias Type	Bias Reference	Antinociceptive ED ₅₀ , mg/kg	Antinociception Assay	Organism	In vivo Reference
U50,488	Unbiased	NA	[144–146]	7.2 nmol i.c.v.	Tail withdrawal	C57BL/6 mice	[65]
	Arrestin (m κ OR)	Affinity	[145]	6.3–6.7	Tail withdrawal	B6-SJL mice	[50,147]
				5.2	Tail flick	ddY mice	[48]
				8.7	Low temperature hot plate	ddY mice	[69]
				1.2	Acetic acid writhe	ddY mice	[48]
				1.5	Acetic acid writhe	CD-1 mice	[111]
				0.58	Formalin	CD-1 mice	[58]
				11	Paw pressure	Wistar rats	[49]
			(5 – 30)	Tail flick	C57BL/6J mice	[73]	
Nalfurafine	G protein	Affinity	[36,72,104]	(0.05 – 0.15)	Tail withdrawal	C57BL/6 mice	[104]
	Unbiased	NA	[58]	(0.015 – 0.06)	Tail withdrawal	C57BL/6J mice	[36]
	Arrestin	Affinity	[144]	0.062	Tail flick	ddY mice	[48]
				0.129	Low temperature hot plate	ddY mice	[69]
				0.0033	Acetic acid	ddY mice	[48]
				0.0058	Formalin	CD-1 mice	[58]
				0.0096	Formalin	Wistar rats	[49]
				0.009	Tail pressure	ddY mice	[69]
				0.035	Tail pinch	ddY mice	[69]
			0.064	Paw pressure	Wistar rats	[49]	
Salvinorin A	G protein	Affinity	[35,36]	1.4	Tail withdrawal	Sprague-Dawley rats	[59]
	Unbiased	NA	[144,148]	2.1	Tail withdrawal	B6-SJL/ptprca mice	[35]
				(1 – 2)	Formalin	B6-SJL/ptprca mice	[35]
				(2)	Hot plate	B6-SJL mice	[50]
				(2)	Formalin	B6-SJL mice	[50]

Agonist name	Bias Direction	Bias Type	Bias Reference	Antinociceptive ED ₅₀ , mg/kg	Antinociception Assay	Organism	In vivo Reference
				(1)	Tail withdrawal	B6-SJL mice	[54]
				2.1	Tail withdrawal	B6-SJL mice	[147]
RB-64	G protein	Affinity	[21,148]	(3)	Hot plate	C57BL/6 mice	[21]
Mesyl Sal B	G protein	Affinity	[35,144]	3.0	Tail withdrawal	B6-SJL/ptprca mice	[35]
				(1 – 2)	Formalin	B6-SJL/ptprca mice	[35]
				(1)	Tail withdrawal	B6-SJL mice	[54]
EOM Sal B	G protein	Affinity	[36]	0.83	Tail withdrawal	Sprague-Dawley rats	[59]
				(1)	Tail withdrawal	C57BL/6J mice	[36]
16-Bromo Sal A	G protein	Affinity	[50]	2.1	Tail withdrawal	B6-SJL mice	[50]
				(2)	Hot plate	B6-SJL mice	[50]
				(1 – 2)	Formalin	B6-SJL mice	[50]
Triazole 1.1	G protein	Affinity	[36,73]	(15 – 30)	Tail flick	C57BL/6J mice	[73]
HS665	G protein	Both	[65,144]	3.7 nmol i.c.v.	Tail withdrawal	C57BL/6J mice	[65]
				1.9	Acetic acid writhe	CD-1 mice	[111]
HS666	G protein	Both	[65]	6 nmol i.c.v.	Tail withdrawal	C57BL/6J mice	[65]
	G protein	Efficacy	[144]	3.2	Acetic acid writhe	CD-1 mice	[77]
BPHA	G protein	Efficacy	[110,144]	Unknown			
6'-GNTI	G protein	Efficacy (may prefer heterodimers)	[66,149]	(10 nmol i.c.v)	Tail flick	ICR-CD1 mice	[66]
				0.36 nmol i.t.	Tail flick	129S6 mice	[150]
LOR17	G protein	Efficacy	[52]	10	Tail withdrawal	CD-1 mice	[52]
				5.7	Acetic acid writhe	CD-1 mice	[52]
				(1 – 20)	Oxaliplatin cold plate	CD-1 mice	[52]
Compound 4a	G protein	Efficacy	[78]	(5)	Tail flick	C57BL/6J mice	[78]
				(5)	Hot plate	C57BL/6J mice	[78]
Helianorphan 19	G protein	Both	[116]	(1 nmol i.c.)	Colorectal distension	C57BL/6J mice	[116]

Table 2.
Therapeutic windows of G protein-biased κ OR agonists in antinociception versus various adverse effects.

Green indicates that the minimum dose producing an adverse effect is higher than the antinociceptive dose or the antinociceptive ED₅₀. Red indicates that the adverse effect occurs at or below the antinociceptive dose/ED₅₀. Yellow indicates that the doses tested were below the antinociceptive dose/ED₅₀ and the results are therefore ambiguous. A “u” means this adverse effect is untested for this agonist. Design credit: [47].

	Aversion	Sedation	Pro-anxiety/ depression
Nalfurafine			
Sal A			
RB-64			
EOM Sal B			
Mesyl Sal B			
16-Bromo Sal A	u		
Triazole 1.1	u		
HS665	u		u
HS666			u
BPHA	u	u [#]	u
6'-GNTI			u
LOR17	u		
Compound 4a	u		u
Helianorphan19	u	u	u

[#]This property was studied but there is no antinociceptive data for BPHA