Abstract
The opioid receptor system plays a major role in the regulation of mood, reward, and pain. The opioid receptors therefore make attractive targets for the treatment of many different conditions, including pain, depression, and addiction. However, stimulation or blockade of any one opioid receptor type often leads to...
on-target adverse effects that limit the clinical utility of a selective opioid agonist or antagonist. Literature precedent suggests that the opioid receptors do not act in isolation and that interactions among the opioid receptors and between the opioid receptors and other proteins may produce clinically useful targets. Multifunctional ligands have the potential to elicit desired outcomes with reduced adverse effects by allowing for the activation of specific receptor conformations and/or signaling pathways promoted as a result of receptor oligomerization or crosstalk. In this chapter, we describe several classes of multifunctional ligands that interact with at least one opioid receptor. These ligands have been designed for biochemical exploration and the treatment of a wide variety of conditions, including multiple kinds of pain, depression, anxiety, addiction, and gastrointestinal disorders. The structures, pharmacological utility, and therapeutic drawbacks of these classes of ligands are discussed.

Keywords
Anxiety · Bivalent · Depression · GPCR · Mixed efficacy · Mood · Multifunctional · Opioid · Pain · Reward

1 Introduction

Opioid agonists have long been used in the treatment of acute and chronic pain and are still widely used in the clinic today. After the discovery and cloning of the three classical opioid receptors – mu (MOR), delta (DOR), and kappa (KOR) – the search for additional and increasingly selective opioid ligands began, driven in part by the need for tools to characterize the opioid receptors. It was assumed that selective opioid agonists would be the future of opioid analgesics, and it seemed intuitive that a more specific ligand would have fewer off-target interactions and unintended effects.

Clinically relevant opioid therapeutics produce their analgesic effects through stimulation of MOR. Unfortunately, adverse effects associated with opioid analgesics such as constipation, respiratory depression, euphoria, tolerance to opioid-mediated analgesia, and physical dependence are mediated by MOR as well. Further, the development of tolerance to and dependence on opioid analgesics may contribute to the prevalence of opioid abuse (Ross and Peselow 2009; Bailey and Connor 2005; Johnston et al. 2009). The development of these undesirable side effects is problematic in many ways; not only does it complicate dosing regimens and decrease patient compliance, but it also limits the clinical utility of opioids and has been linked to increased addiction liability. As the desired analgesic effects and negative side effects are all mediated through MOR, the development of more selective MOR agonists will not address the problems associated with acute and chronic opioid analgesic use.

The stimulation of DOR or KOR has been shown to produce analgesic effects in vivo; however, there are also adverse effects associated with stimulation of each of these receptors. Stimulation of KOR produces aversive and dysphoric effects, while stimulation of DOR produces convulsions under some conditions (Lutz and Kieffer
As a result, pure DOR and KOR ligands have not been pursued as therapeutic tools. However, since it has been shown that opioid receptors do not act in isolation in vitro or in vivo, the simultaneous modulation of multiple targets may generate a more desirable drug profile (Morphy et al. 2004; Morphy and Rankovic 2009; Dietis et al. 2009; Balboni et al. 2002, 2007). This strategy may allow for the activation of specific receptor conformations and/or signaling pathways promoted as a result of receptor oligomerization or crosstalk.

Multifunctional ligands that interact with multiple receptors simultaneously or with unique receptor/signaling complexes possess considerable advantages over the traditional approach of using a combination of selective drugs. Combination therapies or drug cocktails contain multiple active components with differing pharmacokinetic properties. Different drugs often need to be taken on different schedules in order to be most effective (e.g., every 6 h vs. every 12 h) due to the unique absorption and clearance rates of each chemical entity; further, the optimal absorption conditions for each drug may be different (e.g., on a full stomach, on an empty stomach, with a full glass of water). The necessary complicated dosing regimens associated with administration of multiple drugs can reduce patient compliance. In addition to these complications, coadministration of multiple chemical entities often alters metabolism and clearance rates due to off-target drug effects in the liver and kidneys. This increases the risk of patient to patient variation in efficacy and adverse drug reactions.

The multifunctional ligands reported in the literature thus far fall into two main categories: (1) bivalent or bidentate ligands, in which two separate pharmacophores are linked by a flexible spacer, and (2) multifunctional or mixed efficacy ligands, which contain a single set of binding elements that interacts with multiple targets (Fig. 1). Strategies for simultaneous modulation of multiple receptors have been

![Fig. 1](image-url)
developed for the treatment of pain and other disorders, a selection of which are discussed in the sections below.

## 2 Multifunctional Opioid/Opioid Ligands

Opioid receptors are involved in the regulation of pain, mood, and reward; as a result, opioid ligands show great therapeutic potential for treating many different sensory and mood disorders. As the different opioid receptors often exhibit opposing effects, the simultaneous modulation of multiple receptors has been proposed as a strategy to balance these therapeutic and adverse effects to elicit desired pharmacological profiles. Specific, nonselective ligands for a variety of opioid receptor combinations have been developed for a variety of applications.

### 2.1 DOR/MOR

It has been reported in the literature that the coadministration of a DOR agonist with a MOR agonist lessens the development of tolerance to and dependence on MOR agonists without attenuating MOR-mediated analgesia (Li et al. 2012; Lowery et al. 2011; Rozenfeld et al. 2007). Coadministration of a DOR agonist with a MOR agonist also reduces the incidence of other unwanted side effects such as stimulation of forward locomotion, which is generally interpreted as an activation of dopamine systems and has been used as an early indicator of abuse liability (Li et al. 2012). It has also been reported that small doses of DOR agonists potentiate the affinity and antinociceptive potency of MOR agonists as well as potentiating the efficacy of MOR agonists (Lowery et al. 2011; Heyman et al. 1989a, b; Horan et al. 1992; Qi et al. 1990). This suggests that the combination of a DOR agonist with a traditional MOR agonist opioid analgesic may decrease the dose necessary to produce effective analgesia, widening the therapeutic index between analgesia and adverse events and adaptations. Further, coadministration of a MOR agonist with a DOR agonist may reduce the abuse liability associated with MOR agonist opioid analgesics.

It is noteworthy that the coadministration of a DOR antagonist and a MOR agonist also decreases the adverse effects typically associated with MOR agonists (Dietis et al. 2009; Martin et al. 2000; Horan et al. 1993; Zielińska et al. 2016; Abbott and Romero 1996; Li et al. 2007). Similarly, it has been shown that the coadministration of a DOR antagonist with an addictive MOR agonist, such as heroin, can reduce self-administration of that MOR agonist (Martin et al. 2000). These data suggest that the coadministration of a DOR antagonist with a traditional MOR agonist analgesic may slow or prevent the emergence of adverse events and minimize the abuse potential associated with chronic MOR agonist use, providing a safer alternative to traditional opioid analgesics. At this time, it is unclear why administration of either a DOR agonist or DOR antagonist would have similar effects on MOR agonist effects.
2.1.1 Bifunctional DOR Agonist/MOR Agonist Ligands

Bifunctional DOR agonist/MOR agonist ligands have been developed to harness the beneficial aspects of DOR agonist/MOR agonist drug cocktails without the complicated pharmacokinetic profiles associated with administering two distinct chemical entities. These “selectively promiscuous” compounds combine two different pharmacophores in the same molecule.

The dimeric enkephalin peptide, biphalin, was one of the first reported potent DOR agonist/MOR agonist bifunctional compounds (Fig. 2). Biphalin consists of two tetrapeptides connected “tail-to-tail” by a hydrazide bridge. It produced dose-dependent antinociception in the warm water tail withdrawal assay after both peripheral administration and intracerebroventricular (icv) administration in mice. While it is expected that icv administration of a MOR agonist will produce antinociception, it is surprising that intraperitoneal (ip) administration of biphalin produced antinociception because unmodified peptides often do not cross the blood–brain barrier (BBB). As such, it is likely that the antinociceptive response produced by biphalin is peripherally mediated (Horan et al. 1993). Parenteral administration of biphalin significantly inhibits gastric transit (Zielińska et al. 2016). Further, when biphalin is infused directly into the brain into the lateral ventricles (icv), mice become physically dependent similar to that observed with morphine (icv), such that naloxone precipitates withdrawal signs (Abbott and Romero 1996). Overall, these findings suggest that biphalin is acting as a peripheral MOR agonist and that not all of the adverse effects associated with MOR agonists were ameliorated by this DOR agonist/MOR agonist profile (Horan et al. 1993).

More recently, a series of peptides based on endomorphin II (Tyr-Pro-Phe-Phe-NH₂), an endogenous MOR agonist, has been reported (Li et al. 2007); it was found that replacing the Tyr¹ of endomorphin II with 2',6'-dimethyltyrosine (Dmt) increased affinity for DOR and adding moderate bulk to the 2 and 6 position of the Phe³ produced compounds with both DOR and MOR agonist activity. A series of mixed DOR agonist/MOR agonist ligands were generated that were selective relative to KOR (Li et al. 2007). As this series is structurally similar to endogenous endomorphins, it is possible that these ligands will produce limited tolerance and dependence as compared to traditional opioid analgesics. To date, however, no in vivo data have been reported.

One of the best characterized DOR agonist/MOR agonist peptides is MMP-2200 (Tyr-D-Thr-Gly-Phe-Leu-Ser-(O-β-D-lactose)-NH₂), a glycosylated, bioavailable,
centrally active derivative of the opioid peptide DTLES (Fig. 3). The in vivo actions of MMP-2200 have been thoroughly investigated (Li et al. 2012; Lowery et al. 2011; Polt et al. 2005). Antinociceptive tolerance was examined in the mouse warm water tail withdrawal assay. Twice daily injections of an antinociceptive dose $A_{90}$ of MMP-2200 for 3 days produced approximately fivefold shift in $ED_{50}$; the same dosing regimen of morphine produced approximately 13-fold shift in $ED_{50}$. Mice treated twice daily with an $A_{90}$ dose of MMP-2200 displayed significantly fewer withdrawal signs after precipitated withdrawal than mice treated twice daily for 4 days with an $A_{90}$ dose of morphine (Lowery et al. 2011). The reinforcing effects of MMP-2200 have also been explored. Morphine produced robust self-administration in monkeys, while MMP-2200 did not. However, morphine was active in the warm water tail withdrawal assay in monkeys, while MMP-2200 did not produce antinociception in the same assay. MMP-2200 only showed antinociceptive effects in a capsaicin-induced model of allodynia in nonhuman primates (Do Carmo et al. 2008). These data suggest that the mixed efficacy DOR agonist/MOR agonist profile may reduce the negative neurochemical adaptations and addiction liability problems associated with pure MOR agonist analgesics.

There are currently very few reports of DOR/MOR dual agonist small molecules that are selective relative to KOR. One recent report describes a series of pyrrolo- and pyridomorphans that displayed full agonist activity at DOR and partial agonist activity at MOR (Kumar et al. 2014a). These compounds have not yet been explored in animal models.

While there are several DOR agonist/MOR agonist compounds that are promising leads for developing safer opioid analgesics, DOR/MOR agonist crosstalk has been pursued less vigorously than DOR antagonist/MOR agonist interactions. This may be due to the severe unwanted effects associated with DOR stimulation such as convulsions and seizures (Jutkiewicz 2006), which are significant drawbacks for any therapeutic.

### 2.1.2 Bivalent DOR Antagonist/MOR Agonist Ligands

The design of bivalent DOR/MOR ligands is predicated on the idea of DOR/MOR heterodimers. Literature reports suggest that using a flexible 21-atom linker between a DOR antagonist pharmacophore and a MOR agonist pharmacophore produced robust pain relief with limited development of adverse effects, while linkers of
shorter or longer length showed decreased binding and/or more adverse effects (Daniels et al. 2005a; Lenard et al. 2007; Aceto et al. 2012). The Portoghese lab proposes that this 21-atom linker is the appropriate length to reach from the orthosteric binding site of DOR into the orthosteric binding site of MOR in DOR/MOR heterodimers. Additionally, they suggest that these bivalent ligands stimulate DOR/MOR heterodimers, which may be unique signaling entities, to produce antinociception without adverse effects.

MDAN-21 is one such ligand comprised of a naltrindole derivative (DOR antagonist) linked to an oxymorphone derivative (MOR agonist) by a 21-atom linker (Aceto et al. 2012) (Fig. 4). Tolerance to MDAN-21 and related bivalent compounds was tested in the radiant tail flick assay; antinociceptive dose–response curves were established before and after 3-day treatment with continuous icv infusion of 12 times the ED50/h. Treatment with DOR antagonist/MOR agonist compounds produced less tolerance and dependence as compared with either morphine or a monovalent MOR agonist. Three-day chronic treatment with bivalent compounds with linker length in the 19–21-atom range showed no shift in ED50 in the radiant tail flick assay, while morphine and the monovalent MOR agonist showed approximately sixfold shift in ED50. Mice receiving 3 days of continuous icv infusion of bivalent ligands with linker lengths in the 19–21-atom range demonstrated eight- to tenfold fewer naloxone-precipitated jumps as compared with mice treated with chronic morphine or a monovalent MOR agonist (Daniels et al. 2005a). The rewarding properties of bivalent MDAN-21 were also compared to monovalent MOR agonists with and without a monovalent DOR antagonist present. Four-day training with a monovalent MOR agonist produced significant conditioned place preference (CPP) in both the presence and absence of a monovalent DOR antagonist. In contrast, the bivalent DOR antagonist/MOR agonist ligand MDAN-21 did not produce significant CPP (Lenard et al. 2007). The authors suggest that by linking a DOR antagonist pharmacophore to a MOR agonist pharmacophore with a 19–21-atom linker DOR/MOR heterodimers can be activated which results in unique signaling that confers pain relief with limited development of tolerance and dependence and limited reinforcing properties. However, MDAN-21 produced inconsistent antinociceptive effects when administered peripherally suggesting that the failure of MDAN-21 to produce CPP may be due to its variable effects following peripheral administration (Aceto et al. 2012). These inconsistent results following peripheral administration may indicate variable absorption or distribution of the drug due to
its large size or individual differences in metabolism, producing two distinct pharmacophores with their own variable pharmacokinetics.

2.1.3 Bifunctional DOR Antagonist/MOR Agonist Ligands
Small molecule DOR antagonist/MOR agonist compounds have been derived from known opioid alkaloids (Healy et al. 2013; Hiebel et al. 2007; Ananthan et al. 2012). The DOR antagonist/MOR agonist/KOR antagonist alkaloid UMB425 displayed antinociception in the mouse hot plate and tail flick assays (Fig. 5). One advantage of this small molecule scaffold is that it is active after peripheral administration, a desirable trait in a potential therapeutic. To assess the development of tolerance to the antinociceptive effects, mice were treated with an ED90 dose of either morphine or UMB425 twice daily for 5 days. Latencies in the hot plate and tail flick assays were determined after each dose of drug. Mice treated repeatedly with morphine developed tolerance to its antinociceptive effects on day 4 in both the hot plate and tail flick assays. Mice treated repeatedly with UMB425 developed tolerance in the hot plate assay on day 4 or day 5 and in the tail flick assay on day 5, though to a lesser degree than morphine (Healy et al. 2013); these results are consistent with a mixed efficacy DOR antagonist/MOR agonist. However, UMB425 displays selectivity for MOR (approximately 3 nM) and similar affinity for DOR and KOR (approximately 200 nM), so it is somewhat misleading to describe UMB425 as DOR antagonist/MOR agonist. What role, if any, the KOR antagonist activity of UMB425 plays in the development of tolerance to and dependence on MOR agonists is unclear and warrants further investigation.

There have also been reports of small molecule peptidomimetic or pseudo-peptide DOR antagonist/MOR agonist compounds which are generally larger than alkaloid opioid ligands but smaller than most opioid peptides (Balboni et al. 2002; Salvadori et al. 1999; Mosberg et al. 2013; Dietis et al. 2012). Some of these ligands are based on known DOR antagonist/MOR agonist peptides (Dietis et al. 2009; Balboni et al. 2002; Healy et al. 2013; Hiebel et al. 2007; Ananthan et al. 2012; Mosberg et al. 2013). Most contain at least one amide bond but are generally more “drug-like” in size and overall physiochemical properties than peptides. Several DOR antagonist/MOR agonist pseudo-peptide ligands containing the Dimethyltyrosine-Tetrahydroisoquinoline carboxylic acid (Dmt-Tic) pharmacophore have been reported (Balboni et al. 2002; Salvadori et al. 1999; Schiller 2010). The most notable of these, UFP-505 (Dmt-Tic-Gly-NH-Bzl), is reported to be a DOR antagonist/MOR agonist compound in vitro and ex vivo (Dietis et al. 2009, 2012) and has been shown to produce less tolerance in rats as compared with morphine when given via intrathecal (it) injection (Dietis 2012).

Fig. 5 Structure of UMB425, a small molecule DOR antagonist/MOR agonist/ KOR antagonist
(Fig. 6). However, there are no reports of any Dmt-Tic compounds which produce antinociception after peripheral administration.

A series of more constrained peptidomimetics, based on a tetrahydroquinoline scaffold and containing fewer amide bonds than the Dmt-Tic series, has also been reported (Mosberg et al. 2013; Anand et al. 2016; Bender et al. 2015; Harland et al. 2015). This series of compounds also displayed DOR antagonist/MOR agonist properties in vitro. Many of the compounds in this series displayed opioid-mediated dose-dependent antinociception after peripheral administration in mice (Mosberg et al. 2013; Anand et al. 2016; Bender et al. 2015; Harland et al. 2015), though there are no reports on the development of tolerance or physical dependence.

While traditionally thought to be less “drug-like” than small molecules, peptide ligands do possess some advantages over alkaloid and peptidomimetic opioid compounds. The larger size of peptide ligands provides many contact points between ligand and receptor. By making multiple points of contact, peptides can interact with more elements in the binding pockets of receptors and form favorable or unfavorable interactions, allowing for fine tuning of binding and efficacy profiles of multiple targets. This is a key advantage in multifunctional opioid ligands as the structural homology in the binding sites of MOR, DOR, and KOR is high; thus, it is difficult to maintain high MOR and DOR affinity without significant KOR affinity and to simultaneously produce MOR agonist activity without residual DOR or KOR agonist activity.

The Schiller peptide DIPP$\psi$NH$_2$ (Dmt-Tic-$\psi$[CH$_2$NH]-Phe-Phe-NH$_2$) is a well-characterized DOR antagonist/MOR agonist peptide (Fig. 7); it was the first reported DOR antagonist/MOR agonist compound with balanced affinity at DOR and MOR. DIPP$\psi$NH$_2$ produces robust antinociception in the rat tail flick assay after icv administration. Rats treated continuously for 4 days with a small dose of DIPP$\psi$NH$_2$ (icv) developed less tolerance in the tail flick assay than rats treated with morphine at comparable doses; however, treatment with larger doses of morphine and DIPP$\psi$NH$_2$ produced similar degrees of tolerance. Rats treated twice daily with DIPP$\psi$NH$_2$ showed fewer signs of withdrawal after treatment with antagonist than rats treated with repeated morphine, suggesting that chronic treatment with DIPP$\psi$NH$_2$ produces less physical dependence (Schiller et al. 1999). Unfortunately, the therapeutic potential of DIPP$\psi$NH$_2$ is limited by its poor BBB penetration, as expected for an unmodified peptide, and by the seizures produced after chronic administration of large doses (Schiller 2010).

The problem of BBB penetration is addressed by the cyclic glycosylated peptide, VRP26 (Dmt-c(SeiS)-[D-Cys-Aic-D-Pen]-Ser(O-β-D-glucose)-NH$_2$) (Mosberg et al. 2014) (Fig. 8). This ligand is reported to be a DOR antagonist/
MOR agonist in vitro and produced opioid-mediated antinociception in mice after peripheral administration. Further, a single bolus dose of VRP26 produced no acute tolerance in the mouse warm water tail withdrawal assay, making it a promising lead for the development of mixed efficacy opioid analgesics (Mosberg et al. 2014). Continuous infusion of VRP26 subcutaneously for 7 days did not shift the dose–response curve in the mouse warm water tail withdrawal assay, while a similar treatment with fentanyl produced a significant rightward shift in the dose–response curve, suggesting that tolerance developed to fentanyl but not VRP26. Mice treated for 7 days with a continuous infusion of fentanyl exhibited significantly more withdrawal signs after injection with naloxone than mice treated chronically with VRP26, suggesting that under these conditions physical dependence on VRP26 does not develop. In the CPP assay, fentanyl produced robust, dose-dependent increases in time spent on the drug-paired side of the apparatus as expected. While VRP26 did produce slight increases in time spent on the drug-paired side of the apparatus, the increases were not significant at any of the doses tested, which suggests that VRP26 is less rewarding than fentanyl (Anand et al. 2016). These data provide proof of concept that mixed efficacy DOR antagonist/MOR agonist compounds provide a better alternative to traditional opioid analgesics in rodent behavioral models.

2.1.4 DOR/MOR Conclusions

The mechanism(s) by which DOR ligands modulate MOR-mediated signaling are not clear. It has been suggested that DOR and MOR form functionally distinct heterodimers that signal differently than their monomeric or homomeric counterparts. In the case of DOR agonist/MOR agonist ligands, it has been proposed that these DOR/MOR heterodimers can be simultaneously occupied by both a DOR agonist and a MOR agonist and that these activated heterodimers couple to different downstream effectors, thereby producing effects different from DOR or MOR agonist stimulation alone (Gomes et al. 2000, 2011; Rios et al. 2001). Alternatively, DOR/MOR heterodimers may be desensitized, recycled, or resensitized at different rates or under different conditions than DOR or MOR alone. The desirable profile
produced by multifunctional DOR/MOR ligands could also be explained without invoking heterodimers; DOR and MOR may be occupied by agonists in distinct cell populations or brain regions, in which case the confluence of these signals potentiates analgesic activity without stimulating the development of tolerance, dependence, or drug seeking behavior. Alternatively, these multifunctional ligands could simply stabilize conformations of the receptor which promote different signaling pathways which do not produce the same adverse effects as traditional opioid ligands.

Several theories have been proposed to explain how a DOR antagonist could decrease the development of tolerance to MOR agonists, many of which also involve DOR/MOR heterodimers. One theory proposes that upon DOR antagonist treatment, DOR surface expression is increased, either through blockade of basal DOR signaling such that cells traffic more DOR to the surface from intracellular stores to maintain enkephalinergic tone or through molecular chaperoning, which stabilizes the receptor and enhances trafficking to the surface of the cell from the endoplasmic reticulum (Cahill et al. 2007; Dunham and Hall 2009). MOR is co-trafficked to the plasma membrane in the form of a DOR/MOR heterodimer from the endoplasmic reticulum or vesicular stores, thereby increasing the number of active MOR binding sites available on the plasma membrane and preventing the development of tolerance through retention/increase of cell surface binding (Cvejic and Devi 1997; George et al. 2000). Another hypothesis proposes that DOR/MOR heterodimers form at the plasma membrane and that antagonist-bound DOR will remain on the cell surface and prevent internalization of agonist-bound MOR through receptor/receptor dimerization; the proximity of DOR may prevent phosphorylation of MOR, thereby maintaining surface expression of active MOR (Law et al. 2005). These theories are supported by evidence which shows that DOR and MOR co-localize in the same cell in the dorsal root ganglion (Wang et al. 2010; Peng et al. 2012; Liu et al. 1995), a brain region associated with pain signaling. There also exists another set of possibilities which do not involve the dimerization of DOR and MOR. It is possible that the confluence of signals from both DOR and MOR attenuates the development of tolerance and dependence. These signals may alter the trafficking pattern of the receptors but do not necessarily do so through a direct physical interaction between DOR and MOR.

The role of DOR itself, as opposed to DOR ligands, has also been explored in the development of MOR-mediated tolerance and dependence. It has been shown that the knockdown or knockout of DOR in mice slows the development of tolerance to a MOR agonist (Kest et al. 1996; Zhu et al. 1999; Chefer and Shippenberg 2009); as DOR has a high basal signaling rate, this suggests that prevention of DOR-mediated signaling slows the development of MOR-mediated tolerance and dependence. When these data are considered with the findings from research on DOR agonist/MOR agonist and DOR antagonist/MOR agonist interactions, it becomes clear that there is a clinically significant interaction between the two receptor types. Taking advantage of this interaction by developing mixed efficacy DOR agonist/MOR agonist or DOR antagonist/MOR agonist compounds may be the key to developing a new generation of safer opioid analgesics.
2.2 DOR/KOR

The interactions of DOR and KOR have been investigated for the treatment of depression. Simultaneous administration of DOR agonist ADL5859 and KOR antagonist LY2444296 in mice resulted in synergistic antidepressant-like effects in a forced swim test, demonstrating promise for therapeutic use of a DOR agonist/KOR antagonist (Huang et al. 2016). DOR activation can also alter the effects of a KOR agonist. Initial studies showed that pretreatment with DOR agonist SNC80 blocked the KOR agonist-mediated antinociception of U50,488H in MOR knockout mice (Taylor et al. 2015). It has been proposed that DOR activation may also have the potential to reverse stress-induced addictive and depressive behaviors that result from KOR activation. Despite the demonstrated promise of these pharmacological profiles, to our knowledge, there have been no reported specific DOR/KOR bifunctional ligands at this time.

Two series of bivalent DOR/KOR ligands have been developed and used to study interactions between the two receptor types and to identify putative DOR/KOR heterodimers in vitro and in vivo. The KDAN series links the DOR antagonist naltrindole and KOR agonist ICI-199,441. KDAN-18, which joins these pharmacophores with an 18-atom spacer, exhibited antinociceptive activity in the mouse tail flick assay (Fig. 9). Based on the absence of an allosteric effect between DOR and KOR receptors that bind this ligand, the authors suggest that this compound does not interact with DOR/KOR heterodimers in which DOR and KOR are allosterically coupled but rather interacts via a bridging mechanism with DOR and KOR receptor homodimers (δ2 and κ1 subtypes) which are associated through a passive interface (Daniels et al. 2005b). The KDN series, on the other hand, is reported to demonstrate ligand selectivity for DOR/KOR heterodimers. KDN-21 links naltrindole and KOR antagonist 5′-guanidinonaltrindole with a 21-atom spacer (Fig. 10). This ligand displays no antinociceptive activity in the mouse tail flick assay. Based on binding studies in HEK293 cells and pharmacological studies in mice via it injection, the authors suggest that it bridges the two orthosteric binding sites in DOR/KOR heterodimers (Xie et al. 2005; Bhushan et al. 2004).

The localization of DOR/KOR receptor complexes to specific tissues suggests promise for the development of ligands selective for these entities for use as spinally
selective analgesics. 6'-guanidinonaltrindole has been reported to selectively activate DOR/KOR heterodimers but not DOR or KOR homomers and results in analgesia in the mouse tail flick assay only when the compound is administered in the spinal cord but not in the brain (Waldhoer et al. 2005) (Fig. 11).

2.3 MOR/KOR

The primary application being explored for MOR/KOR ligands is treatment of addiction to cocaine and other drugs of abuse. It has been demonstrated that KOR agonists have the potential to reduce cocaine self-administration in nonhuman primates due to their reward-modulating properties (Mello and Negus 1998; Negus et al. 1997). It has been suggested that the inhibitory effects of KOR agonists on abuse-related behaviors are a result of inhibition of dopamine release from dopaminergic neurons (Di Chiara and Imperato 1988; Maisonneuve et al. 1994). However, highly selective KOR agonists also produce severe undesirable effects such as salivation, emesis, sedation, and intense hallucinations in nonhuman primates (Mello and Negus 1998; Negus et al. 1997) and in humans (Cruz et al. 2017). It has been suggested that euphoric effects associated with weak MOR agonism may be able to balance dysphoria associated with KOR agonism, increasing the therapeutic potential of a KOR agonist. Thus, mixed MOR/KOR ligands offer potential advantages over selective KOR agonists for the treatment of drug abuse.

Orvinols are known for high affinity binding to MOR and KOR with varying efficacy and have been proposed as potential treatment for cocaine and other psychostimulant abuse, though little in vivo work has been reported on this series (Greedy et al. 2013). Nalbuphine, a mixed MOR/KOR agonist, has been shown to produce a modest attenuation of cocaine’s abuse-related effects in humans
(Mello et al. 2005) (Fig. 12a). Exploration of 3-benzylaminomorphinan derivatives with full KOR agonist and partial MOR agonist properties (Neumeyer et al. 2013) led to development of MCL-101 (butorphan), a MOR agonist/KOR agonist (Neumeyer et al. 2000), which decreased the rewarding effects of cocaine in intracranial self-stimulation studies in rats (Provencher et al. 2013) and dose-dependently decreased cocaine self-administration with minimal side effects in rhesus monkeys (Bowen et al. 2003) (Fig. 12b). A novel quinoline derivative with MOR/KOR agonist activity, S4, was shown to inhibit naloxone-precipitated withdrawal symptoms (Deb et al. 2009) (Fig. 12c). Aminothiazolomorphinans have also been explored as mixed MOR/KOR agonists (Zhang et al. 2011a), leading to the development of (−)-3-Amino-thiazolo[5,4-b]-N-cyclopropylmethylmorphinan hydrochloride (ATPM), a MOR agonist/antagonist and KOR agonist, which was shown to attenuate heroin self-administration in rats (Wang et al. 2009) (Fig. 13). ATPM has also been shown to produce KOR- and MOR-mediated, but not DOR-mediated, antinociception in the mouse hot plate assay, to inhibit morphine-induced antinociception, and to dose-dependently attenuate tolerance to the antinociceptive effects of morphine when coadministered with morphine (Wang et al. 2009; Zhang et al. 2004). Other mixed MOR/KOR ligands have also shown some potential to elicit antinociceptive effects with limited adverse events. Endomorphin II based cyclic pentapeptides exhibiting weak MOR/KOR agonism have been reported to result in antinociceptive effects after both central and peripheral administration in mice (Perlikowska et al. 2016).

Finally, MOR/KOR ligands show promise as a treatment for gastrointestinal disorders such as irritable bowel syndrome. Quaternization with benzyl bromide of the pyridyl ring of NAP, a peripherally selective MOR ligand, resulted in BNAP, a peripherally active MOR antagonist/KOR partial agonist which resulted in inhibition
of abdominal stretching and showed analgesic activity in the acetic acid induced stretch assay in mice (Williams et al. 2016) (Fig. 14).

In addition, dimeric and bivalent MOR/KOR ligands have been developed to study receptor oligomerization. These include cyclorphan-, butorphan-, ATPM-, and other morphinan-based dimeric ligands with subnanomolar affinity for MOR and KOR which function either as MOR partial agonist/KOR full agonist or as MOR partial agonist/KOR partial agonist (Neumeyer et al. 2003; Decker et al. 2009; Zhang et al. 2011b; Peng et al. 2006). The most notable of these chemical tools is KMN-21, an antagonist of MOR/KOR heterodimers, which links MOR antagonist β-naltrexamine and KOR antagonist 5'-guanidononaltrindole with a 21-atom spacer (Zhang et al. 2009) (Fig. 15).

### 3 Multifunctional Opioid/Nociceptin Receptor Ligands

The nociceptin receptor (NOP) has high sequence homology with the three classical opioid receptors (MOR, DOR, and KOR) but has low affinity for standard opioid ligands, such as naloxone, due to a unique configuration of its binding site residues compared to the classical opioid receptors. As such, NOP is variably considered the fourth opioid receptor or an opioid-like receptor. NOP receptors are highly expressed in the spinal cord and many brain regions including those involved in pain, reward, drug abuse, and motor control. Its endogenous ligand, nociceptin (also known as orphanin FQ), blocks or mediates analgesic effects of opioids depending on the exposure to endogenous opioids, and NOP agonists attenuate reward properties of opioids and other drugs of abuse. However, there remain many discrepancies

Co-immunoprecipitation studies suggest that heterodimerization may occur between NOP and members of the classical opioid receptor family (Evans et al. 2010), and nonselective opioids have been explored for NOP activity (Butour et al. 1997). While etorphine shows only moderate NOP affinity, its derivative TH-030418 shows high affinity and full agonism at NOP and all three canonical opioid receptors (Fig. 16a). TH-030418 showed potent, naloxone-reversible antinociception when administered subcutaneously in mice (Yu et al. 2011); however, chronic treatment resulted in dramatic tolerance development (Wen et al. 2011). Administration of buprenorphine, a mixed agonist/antagonist at classical opioid receptors with partial agonist activity at NOP, in opioid receptor knockout mice has shown that its MOR-mediated analgesia is attenuated by its NOP activation (Lufty and Cowan 2004) (Fig. 16b). Structural analogues of buprenorphine have been synthesized with the aims of increasing affinity for NOP in order to investigate the role of NOP activation in the behavioral profile of this series (Cami-Kobeci et al. 2011) and developing potential agents for relapse prevention for multiple drugs of abuse (Kumar et al. 2014b). Most notably, BU08028, a universally high affinity opioid ligand which shows full agonism at MOR, DOR, and KOR and partial agonism at NOP (Khroyan et al. 2011a), demonstrated long-lasting analgesia with reduced side effects in nonhuman primates (Ding et al. 2016) (Fig. 16c). Intravenous administration of MOR/DOR/KOR agonist/NOP antagonist peptides in mice resulted in antinociception without respiratory depression (Guillemyn et al. 2016). Cebranopadol (also known as GRT 6005) showed agonism at MOR, DOR, KOR, and NOP and demonstrated antinociceptive and antihypersensitive effects in rats after iv and oral administration with a favorable side effect profile (Linz et al. 2014) and is currently in Phase III clinical trials for several indications including cancer pain (Lambert et al. 2015) (Fig. 16d).

Fig. 16 Structures of (a) TH-030418, (b) buprenorphine, (c) BU0828, and (d) cebranopadol (GRT 6005), nonselective small molecule opioid agonists
3.1 DOR/NOP

DOR is also considered a therapeutic target in neuropsychiatric disorders including Parkinson’s disease (Chu Sin Chung and Kieffer 2013; Pradhan et al. 2011). In rat models, DOR agonists have demonstrated antiparkinsonian changes in motor effects which are attributed to regulation of nigro-thalamic GABA neurons (Mabrouk et al. 2009). However, high doses of nonpeptidic DOR agonists have low clinical utility due to undesired side effects such as convulsions, and chronic treatment may result in tolerance to therapeutic effects (Mabrouk et al. 2014). Coadministration of DOR agonist SNC80 and NOP antagonist J-113397 in mice and rats produced synergistic antiparkinsonian effects. This observation suggests that NOP antagonism allows for reduction of dosage of DOR agonists in the treatment of Parkinson’s disease with retention of full therapeutic efficacy and limited undesired effects (Mabrouk et al. 2014). However, to our knowledge, no specific DOR/NOP ligands have been reported at this time.

3.2 KOR/NOP

It has been suggested that a KOR antagonist/NOP agonist could be beneficial for preventing relapse to a variety of abused drugs (Toll et al. 2013). Recently, aryl ring analogues of buprenorphine with KOR antagonist/NOP partial agonist activity were reported and are being evaluated in vivo for this and other potential applications (Cueva et al. 2015). Nalfurafine (also known as TRK-820), a KOR agonist/NOP antagonist with MOR partial agonist activity, demonstrated antinociception without the development of dependence and adverse effects when administered subcutaneously in mice (Seki et al. 1999; Mizoguchi et al. 2003) and is currently in development as an antipruritic (Mustazza and Bastanzio 2011) (Fig. 17). Additionally, a series of KOR/NOP chimeric peptides, structurally based on nociceptin and dynorphin A, were prepared to delineate the functional domain of each endogenous ligand (Lapalu et al. 1997; Reinscheid et al. 1998).

3.3 MOR/NOP

MOR and NOP co-localize in many brain regions, and co-immunoprecipitation and immunofluorescence microscopy studies have shown that the two receptor types
may heterodimerize. NOP agonism has the potential to suppress opioid-induced rewarding effects without decreasing antinociceptive effects (Zaveri et al. 2013; Journigan et al. 2014). It has been suggested that a MOR/NOP agonist may have therapeutic potential as an analgesic with a wider therapeutic window and lowered addiction liability due to reduced reward and tolerance development compared to classical opioid analgesics (Spagnolo et al. 2008). It has also been proposed that a compound with sufficient NOP agonism could be used as a treatment for drug abuse (Toll et al. 2013, 2016; Kiguchi et al. 2016). Naloxone benzoylhydrazone, a MOR antagonist/KOR agonist, was shown to act as an antagonist at NOP (Fig. 18). This ligand induced antinociception without affecting locomotor activity in wild-type mice, but this effect was lost in NOP knockout mice, suggesting that NOP plays a role key in the antinociceptive effects of naloxone benzoylhydrazone (Noda et al. 1998). KGNOP1 (H-Dmt-D-Arg-Aba-β-Ala-Arg-Tyr-Tyr-Arg-Ile-Lys-NH₂), a MOR agonist/NOP antagonist pseudo-peptide, was recently reported as a candidate for dual treatment of nociceptive and neuropathic pain (Lagard et al. 2017). SR16435, a high affinity, mixed MOR/NOP partial agonist, produced antinociception with reduced development of tolerance as compared to morphine in mice (Fig. 19a). However, SR16435 also induced CPP, suggesting that partial NOP agonism is not enough to attenuate the rewarding properties associated with MOR activation (Khroyan et al. 2007; Sukhtankar et al. 2013). As a result, additional bifunctional MOR/NOP ligands with varying ratios of MOR/NOP agonist potency were developed from this scaffold in search of a ligand with a nonaddicting analgesic profile (Zaveri et al. 2013; Journigan et al. 2014). SR14150 (also known as AT-200), a MOR/NOP partial agonist, showed MOR-mediated antinociceptive and antiallodynic effects in mice (Khroyan et al. 2011b), did not induce CPP or attenuate morphine-induced CPP (Toll et al. 2009), and is a promising candidate for treatment of pain in sickle cell anemia (Vang et al. 2015) (Fig. 19b). These results suggest that a MOR/NOP partial agonist may have potential as a nonaddictive analgesic while NOP full agonism may be used to modulate opioid-induced reward (Toll et al. 2009).

Chimeric MOR/NOP ligands linking a dermorphin peptide (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH₂ or Tyr-D-Arg-Phe-β-Ala-NH₂) and a NOP peptide (Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH₂) were developed as tools to study putative MOR/NOP

---

Fig. 18  Structure of naloxone benzoylhydrazone, a small molecule MOR antagonist/KOR agonist/NOP antagonist

Fig. 19  Structures of (a) SR16435 and (b) SR14150 (AT-200), small molecule MOR/NOP partial agonists
heterodimers (Kawano et al. 2006) and demonstrated potent antinociceptive activity in the mouse tail flick assay following \textit{it} administration but low activity following \textit{icv} administration (Kawano et al. 2007). The bivalent MOR/NOP agonist, DeNo, is a combination of dermorphin and nociceptin which displays only weak antinociceptive properties but may be useful as a tool for investigating simultaneous activation of MOR and NOP (Bird et al. 2016).

4 Multifunctional Opioid/Non-opioid Ligands

Opioid receptors are known to interact with other GPCRs, and the existence of various heterodimers remains controversial (Rios et al. 2001). Ligands have been developed to explore the existence of constitutive heterodimers and to selectively target these complexes. In addition, induction of non-endogenous opioid receptor heteromers through the use of bivalent ligands has been proposed as a way to promote unique pharmacology (Portoghese et al. 2017). Many bivalent ligands have been developed to explore crosstalk between opioid receptors and other systems, and these compounds are being explored for a variety of therapeutic uses.

4.1 Opioid/Cannabinoid

Cannabinoid receptors are found primarily in brain and neuronal tissue, and agonists of these receptors have been linked to many behavioral effects including analgesia and regulation of mood and appetite. Several endogenous agonists for the cannabinoid receptors have been identified, including eicosanoids; however, the primary endogenous agonists of the cannabinoid receptors are uncertain. There are two known cannabinoid receptor types (CB\textsubscript{1} and CB\textsubscript{2}), and some evidence suggests the existence of additional types or subtypes (Howlett et al. 2002). Activation of cannabinoid and opioid receptors results in similar behavioral effects including antinociception and regulation of mood, and both types of receptor are expressed in brain regions associated with antinociception. There is evidence which suggests that the cannabinoid 1 receptor (CB\textsubscript{1}) heterodimerizes with each of the classical opioid receptors (Bushlin et al. 2010; Rios et al. 2009). Rimonabant (also known as SR141716), a CB\textsubscript{1} antagonist, has been shown to bind to MOR and inhibit signaling in mouse cortex and MOR-CHO membranes (Cinar and Szucs 2009) and to inhibit DOR function at micromolar concentrations (Zádor et al. 2014). It has also been shown that coadministration of opioid and cannabinoid receptor agonists may have a synergistic antinociceptive effect (Grenald et al. 2017), and simultaneous activation of both opioid and cannabinoid receptors results in highly effective analgesia in neuropathic pain animal models (Kleczkowska et al. 2013). Preclinical coadministration studies suggest promise for the development of multifunctional opioid/cannabinoid ligands as analgesics which can be dosed at lower concentrations than opioids alone (Nielsen et al. 2017).

Bivalent opioid/CB\textsubscript{1} ligands were also developed from high affinity DOR/MOR peptide Tyr-D-Ala-Gly-Phe-NH\textsubscript{2} and rimonabant as tools for investigating crosstalk
and synergistic effects (Mollica et al. 2017). It has been proposed that a bivalent MOR/CB₁ ligand which activates one receptor and blocks activity at the other may be useful as an analgesic since association between the two receptors leads to an antagonistic response (Bushlin et al. 2010). However, bivalent ligands based on MOR agonist α-oxymorphamine and rimonabant showed no reduced tolerance development compared to coadministration of the monovalent ligands, suggesting that MOR/CB₁ is not an important target for reduction of opioid tolerance (Le Naour et al. 2013). MOR antagonist/CB₁ antagonist bivalent ligands were developed from the opioid agonist fentanyl and rimonabant (Fernández-Fernández et al. 2014). Coadministration studies have shown such ligands to have potential therapeutic applications including reduction of pruritic response induced by rimonabant and regulation of alcohol intake and feeding behavior (Rowland et al. 2002; Tallett et al. 2009; Wright and Rodgers 2013).

4.2 Opioid/Neurokinin-1

Neurokinin-1 receptors (NK1R) are widely expressed throughout the central nervous system and often co-localize with the three classical opioid receptors (Pinto et al. 2008). The endogenous agonist for NK1R, Substance P (SP), is released in primary afferents in response to pain and other noxious stimuli (Besson 1999). The resulting stimulation of NK1R produces inflammation and signals of stress and pain (Xiao et al. 2016). In other words, NK1R functions to oppose the opioid receptors; stimulation of NK1R is nociceptive, while stimulation of the opioid receptors is antinociceptive. Interestingly, stimulation of the opioid receptors can inhibit SP release, and conversely, stimulation of NK1R modulates opioid receptor function and the development of adverse effects associated with chronic opioid analgesic use (Xiao et al. 2016).

Due to this intertwined relationship, a series of multifunctional opioid agonist/NK1R antagonist peptides/peptidomimetics that combine opioid and NK1R peptide sequences has been developed (Yamamoto et al. 2007; Nair et al. 2013, 2015). While many of these compounds produced antinociception in vivo, sometimes more potently than morphine, repeated administration produced tolerance to the antinociceptive effects of these compounds. Other mixed opioid agonist/NK1R antagonist peptides have also been developed (Betti et al. 2015; Dyniewicz et al. 2017). Surprisingly, some of these compounds do not produce cross-tolerance with morphine (Betti et al. 2015) and therefore may provide a novel class of compounds for treating pain.

4.3 MOR/CCR5

Opioid agonists have been shown to induce the expression of C-C chemokine receptor type 5 (CCR5), which assists in the entry of the AIDS virus into immune cells. Opioid receptors and CCR5 are closely situated on the cell membrane (Suzuki
et al. 2002), and heterodimerization between MOR and CCR5 has been proposed (Chen et al. 2004). A bivalent MOR agonist/CCR5 antagonist, MCC22, produced potent antinociception in the mouse tail flick assay and is a candidate for use both in treatment of chronic pain and in blocking penetration of HIV into the central nervous system (Akgun et al. 2015) (Fig. 20). Bivalent MOR antagonist/CCR5 antagonist ligands have been developed from MOR antagonist naltrexone and CCR5 antagonist maraviroc as probes to study putative MOR/CCR5 heterodimerization during progression of opioid-enhanced NeuroAIDS. Maraviroc alone does not effectively inhibit HIV infection in primary human astrocytes in the presence of morphine while a bivalent MOR/CCR5 antagonist ligand inhibits HIV invasion in both the presence and absence of morphine (Yuan et al. 2012, 2013; Arnatt et al. 2016).

4.4 MOR/mGluR5

The metabotropic glutamate-5 receptor (mGluR5) is widely distributed in the central nervous system, including in the dorsal horn and the glia of the spinal cord. This receptor modulates synaptic transmission, neuronal excitability, and plasticity (Akgun et al. 2013). Allosteric modulation of mGluR5 by antagonist MPEP has been shown to enhance MOR-mediated antinociception and suppress the development of morphine tolerance and dependence (Schröder et al. 2009). Heteromerization of MOR and mGluR5 has also been proposed; to target these putative heteromers, bivalent ligand MMG22, containing MOR agonist oxymorphone and mGluR5 antagonist m-methoxy-MPEP pharmacophores, was developed (Fig. 21). This ligand showed potent, long-lasting antinociception in a mouse model of bone cancer pain and is an

Fig. 20 Structure of MCC22, a bivalent MOR agonist/CCR5 antagonist ligand with a 22-atom linker

Fig. 21 Structure of MMG22, a bivalent MOR agonist/mGluR5 antagonist ligand with oxymorphone linked to an MPEP derivative by a 22-atom linker

5 Conclusions

Opioid receptors play a major role in the regulation of pain, mood, and reward; however, selective ligands for MOR, DOR, and KOR all have on-target adverse effects which complicate their use in treating pain, mood disorders, and addiction (Lutz and Kieffer 2013). As a result, the development of multifunctional ligands which display activity at multiple opioid receptors or activity at both opioid receptors and other receptors has been the focus of a great deal of research. The move away from selective ligands has been seen within and outside the opioid field, and many researchers in the GPCR community are developing multifunctional ligands as both tools and therapeutics.

Some of the ligands described in the sections above have been used as tools to elucidate the mechanisms of crosstalk between receptors as well as the organization of receptors into oligomers, which may provide a more nuanced view of the physiological role of receptor/receptor interactions and signaling. The information gathered from these studies is an important step in the rational design of novel therapeutics that display multifunctional activity. In fact, many of the ligands discussed in this chapter show improvement over selective opioid ligands with regard to therapeutic efficacy or reduction in the development of adverse effects in animal models and show promise as novel therapeutics for the treatment of pain, addiction, and mood disorders.

References

Multifunctional Opioid Ligands


Bender AM, Griggs NW, Anand JP, Traynor JR, Jutkiewicz EM, Mosberg HI (2015) Asymmetric synthesis and in vitro and in vivo activity of tetrahydroquinolines featuring a diverse set of polar substitutions at the 6 position as mixed-ef
ciency mu opioid receptor/delta opioid receptor ligands. ACS Chem Neurosci 6(8):1428–1435


Multifunctional Opioid Ligands
Lenard NR, Daniels DJ, Portoghese PS, Roerig SC (2007) Absence of conditioned place preference or reinstatement with bivalent ligands containing mu-opioid receptor agonist and delta-opioid receptor antagonist pharmacophores. Eur J Pharmacol 566:75–82
substituted at position 3 with alkylated phenylalanine derivatives yield potent mixed mu-agonist/delta-antagonist and dual mu-agonist/delta-agonist opioid ligands. J Med Chem 50(12):2753–2766


Mosberg HI, Yeomans L, Anand JP, Porter V, Sobczyk-Kojiro K, Traynor JR, Jutkiewicz EM (2014) Development of a bioavailable μ opioid receptor (MOPr) agonist, δ opioid receptor...
(DOPr) antagonist peptide that evokes antinociception without development of acute tolerance. J Med Chem 57(7):3148–3153


