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Expert Opin Investig Drugs. Author manuscript; available in PMC 2015 January 03.

Published in final edited form as:
Expert Opin Investig Drugs. 2014 February ; 23(2): 227–241. doi:10.1517/13543784.2014.856879.

Investigational peptide and peptidomimetic μ and δ opioid receptor agonists in the relief of pain

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Abstract

Introduction—Current methods for treating prolonged and neuropathic pain are inadequate and lead to toxicities that greatly diminish quality of life. Therefore, new approaches to the treatment of pain states are needed to address these problems.

Areas covered—The review primarily reviews approaches that have been taken in the peer-reviewed literature of multivalent ligands that interact with both μ and δ opioid receptors as agonists, and in some cases, also with pharmacophores for antagonist ligands that interact with other receptors as antagonists to block pain.

Expert opinion—Although there are a number of drugs currently on the market for the treatment of pain; none of them are 100% successful. In the authors' opinion, it is clear that new directions and modalities are needed to better address the treatment of prolonged and neuropathic pain; one drug or class clearly is not the answer for all pain therapy. Undoubtedly, there are many different phenotypes of prolonged and neuropathic pain and this should be one avenue to further develop appropriate therapies.

Keywords

agonist; multifunctionality; multivalency; opioid; pain; receptors

1. Introduction

There is no human being in the entire world who has not faced some kind of pain at some point of time in her/his life. It is essential for our survival. According to the International Association for the Study of Pain (IASP), pain is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage' [1]. Pain can be classified in numerous ways and accordingly, different types of pain are discussed in the literature. Pain has significant physical, economic and social impact. Approximately 1.5 billion people around the globe suffer from chronic pain [2]. The costs associated with pain treatment are much higher than that involved for the

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Declaration of interest
The research in the V Hruby group is supported in whole or in part from grants from the US Public Health Service, NIH and NIDA. Some of the compounds have patent coverage.

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treatment of heart disease or cancer [3]. Generally, acute pain associated with accidental injury or surgery is cured. But nearly 50% of patients who have gone through surgery face chronic pain [4]. Under-treatment of postsurgical acute pain has been found as a major reason for moderate to severe or even extreme pain in two thirds of these patients [5]. Support in favor of these statements comes from the observations made during the study on effective treatment of patients with acute pain [6,7].

In spite of having many serious side effects including respiratory depression, sedation, constipation, physical dependence and development of tolerance [8,9], opioid agonists have long been the mainstay analgesics for the treatment of various pain states because of their potency, efficacy and availability. Three classical opioid receptors, namely μ-, δ- and κ-opioid receptors (MOR, DOR and KOR, respectively), have been identified in the central nervous system (CNS) by pharmacological studies [10,11]. The common opioid drugs including morphine, codeine, oxycodone, methadone, heroin, morphine-6β-glucuronide (M6G), fentanyl, etc., which are used clinically for analgesic effects, mainly target the MOR. Most studies have confirmed that the μ-opioid receptor is primarily responsible for the antinociceptive activity. However, a number of studies have suggested that ligands with dual μ-and δ-agonist activities display better biological profiles compared to the ones acting selectively on MOR [12,13]. There is also evidence that the presence of DOR agonists can improve the analgesic efficacy of MOR agonists [14,15]. KORs, broadly found in the spinal cord, the dorsal ganglia, the periphery and the supraspinal regions, are associated with pain modulation, but are not discussed in this review due to page limitations. In addition, we do not discuss glycopeptides that target opioid receptors though they show great promise, nor other peptide conjugates that also may be useful. In addition, there is a huge amount of literature about non-peptide ligands that interact with opiate receptors that is not discussed here.

1.1 Probable paths to counteract the present problems

To overcome the difficulties in pain treatment described above, it has been proposed that new approaches to drug design are needed to deal with recent observations that in the development of prolonged and neuropathic pain states, there are critically important changes in the expressed genome in ascending and descending pain pathways, and in the CNS that result from upregulation of neurotransmitter receptors and their ligands that are stimulatory and thus can cause pain. These anti-opioid ligands and receptors need to be considered in drug design. Therefore, there is a need to develop approaches to design ligands that are multivalent and, therefore, can act at two, three or more receptors all with a single molecule. In this review, the authors will discuss some of these new approaches.

Here, it should be mentioned that the in-depth molecular-level understanding of the interactions between opioid ligands and their receptors is also very important for successful design of new drugs. Recent reports on how opioid ligands bind to their receptors by high-resolution crystal structures of three opioid receptor subtypes, that is the MOR [16], DOR [17] and KOR [18] have opened an additional opportunity to discover novel ligands targeting these G-Protein Coupled Receptors (GPCRs) that might ultimately be developed into more useful therapeutics [19,20]. However, it is important to note that the X-ray

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structures (conformations) of opioid receptors were occupied by antagonists and the conformation of agonist occupied receptors will clearly be different from an antagonist occupied receptor. Agonists and antagonists clearly have different structure-activity relationships (SARs) for opioid receptors.

In this review, our focus will be on agonists, which target the μ and δ opioid receptors. Some selective literature reports have been taken to discuss the drug design principles and SAR study. Emerging drug discovery approaches of multivalency/multifunctionality targeting both opioid and non-opioid targets all in a single molecule for the treatment of prolonged and neuropathic pain, also will be discussed. In addition, this review covers some selective potential drug candidates, which are in a preclinical investigational stage. Though some general aspects of non-peptide ligands for opioid receptors will be discussed, in this review the authors will focus primarily on peptide and peptidomimetic ligands for opioid receptors since the natural endogenous ligands for opioid receptors are peptides.

1.2 Literature search methodology

The literature search was carried out using SciFinder and PubMed. The phrases like ‘opioid agonist’, ‘opioid agonists for the treatment of pain’ and ‘peptide and peptidomimetic opioid ligands’ have been used during the search. Author search was conducted with well-known scientists’ names in this field. To make the review concise, we have taken our topic related publications starting from 2006 to the end of 2012, unless it was very much required for our discussion. Though there were scopes to include investigational drug candidates in Phase I and Phase II clinical trials, we left them out of this review because of the fact that we have limited access to the scientific data for those compounds.

New avenues of drug design are being developed which can provide novel ligands that are efficacious for the treatment of prolonged and neuropathic pain without the serious side effects of toxicities of current analgesics and which do not develop tolerance with prolonged use.

We strongly believe that the topic chosen and areas covered in this review are timely and very important in stimulating the scientific community to think of new approaches for management of the world’s most common health problem, that is unwanted pain in human daily life.

2. Background

Opioid agonists are the main clinically used drugs for the treatment of pain. Although they can act on all three subtypes of opioid receptors, most of the current drugs’ analgesic effects are mainly due to the activation of MOR present in the CNS. One of the key reasons of having limited a number of centrally acting drugs is due to the presence of the blood–brain barrier (BBB), which put forward some restriction for foreign molecules to enter into the brain. The BBB permits hydrophobic and selected molecules to pass through it. But hydrophobic agents are difficult to transport through blood which requires more hydrophilic nature of the drug candidates. These two opposite requirements by the blood and the BBB have made it a challenging job for scientists to discover and develop new drugs, which can

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be delivered into the CNS. Another very important issue associated with development of centrally acting opioid drugs is their metabolic stability. This is because of the fact that therapeutic agents should have half-lives in the acceptable range so that they can interact with their biological targets for a sufficient duration of time to produce the desired response.

3. Investigational opioid receptor agonists

To overcome the limitations of the existing opioid drugs, many approaches have been taken over the last few decades. Studies reported in the literature suggest the issues of metabolic stability and blood–brain barrier permeability should be taken into consideration at the very beginning stage of drug design. If a drug candidate is not stable enough to the enzymatic action in the physiological systems, it cannot reach the CNS. Again if it reaches the CNS, it should cross the BBB to activate the receptors in the brain. The scientific community has been trying to solve these two key issues. In the next sections, we will discuss the pharmacology and *in vivo* studies with some investigational opioid agonists.

3.1 μ-selective opioid agonists

Replacement of a disulfide bridge of bioactive peptides by an ethylene (–CH=CH–) or a bismethylene (–CH_2–CH_2–) moiety can provide a different biological profile. In an attempt to increase the metabolic stability of two dermorphin-derived cyclic tatrapeptide analogs H-Tyr-*c*[D-Cys-Phe-Cys]-NH_2 (Ligand **4**, Table 1) and H-Tyr-*c*[D-Cys-Phe-D-Cys]-NH_2 (Ligand **7**, Table 1) [21], which are agonists at MOR and DOR, Berozowska *et al.* [22] replaced the disulfide bridge by a bis-methylene moiety. All olefinic bond containing peptides **1**, **2**, and **5** (Table 1) showed considerably reduced μ and δ agonist potencies in the guinea pig ileum (GPI) and mouse vas deferens (MVD) assays, respectively. But, the –CH_2–CH_2–bridged peptide **3** with L-configuration in the fourth position showed comparable potency with its cysteine-containing parent compound **4** in both assays. The bis-methylene analog **6** with D-configuration in second and fourth positions became 10 – 27 less potent compared to its parent disulfide peptide **7**. Berozowska *et al.* [23] also synthesized dicarba analogs of two cyclic opioid penta-peptides H-Tyr-*c*[D-Cys-Gly-Phe-D(or L)-Cys]-NH_2 [24,25] evaluated for their pharmacological activities. The *trans* isomer **9** of H-Tyr-*c*[D-Allylgly-Gly-Phe-L-Allylgly]-NH_2 showed a μ and δ agonist ($IC_{50}{}^{\mu}$ = 0.898 nM, $IC_{50}{}^{\delta}$ = 0.275 nM) activities with high potency ($K_i{}^{\mu}$ = 0.616 nM, $K_i{}^{\delta}$ = 1.25 nM) at both receptors.

Weltrowska *et al.* [26] reported that analogs **16**, **19** and **22** of the opioid peptides H-Tyr-*c*[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]-NH_2 (non-selective ligand **17**) [27], H-Tyr-D-Arg-Phe-Lys-NH_2 (μ-selective ligand **20**) [28] and dynorphin A(1-11)-NH_2 (κ-selective ligand **23**) containing 4′-[N-((4′-phenyl)-phenethyl)carboxamido]phenylalanine (Bcp) in place of Tyr^1 maintained high μ opioid receptor binding affinity, but displayed very substantial differences in the opioid receptor profiles while compared to the corresponding Tyr^1-containing parent peptides (Table 2). The cyclic peptide H-Bcp-*c*[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]-NH_2 (**16**) appeared as a highly potent, μ-selective opioid agonist, whereas the Bcp^1-analogue of dynorphin A(1-11)-NH_2 showed partial agonism at the μ-receptor. These results suggest that the presence of bulky biphenylethyl moiety in these compounds might be involved in a hydrophobic interaction with a receptor sub site and thereby might play a role in the ligand's

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ability to induce a specific receptor conformation or to bind to a separate receptor conformation in a state of conformational receptor heterogeneity.

Ballet *et al.* [29] compared the opioid activity of two dermorphin analogs having an almost identical structure but different structural flexibility. Conformational restriction of the aromatic amino acid residues has been a successful strategy to increase the potency, selectivity and metabolic stability of peptides [30]. Compounds **25** and **26** were derived from the parent Compound **27** to increase the lipophilicity and conformational restriction (Table 2). The main difference between **25** and **26** is in their structural rigidity. In Ligand **25**, the aromatic side chains became the parts of lactam structures, which caused the conformational restriction of the ligand. In Ligand **26** they remained as such and as a result the side chains were flexible. Ligand **25** displayed comparable binding affinities for MOR and DOR (K_i^{μ} = 20.8 nM, K_i^{δ} = 17.8 nM), but eight times more agonist activity at μ compared to that at δ. The analog **25** showed the highest potency for *in vitro* GPI (IC_{50}^{μ} = 20.8 nM) assay.

However, both compounds (**25** and **26**) showed similar antinociception profile (AUC = 7820 and 8732, respectively, where AUC is the area under the curve) in the mouse tail flick test after intravenous administration of a dose of 2 mg/kg for each compound (Figure 1). This observation suggests that lipophilicity, rather than side chain flexibility, is the key decisive factor for BBB penetration.

In an effort to discover new drug candidates with better anti-nociception effect, Fichna *et al.* [31] synthesized [D-1-Nal^3] analogs of morphiceptin and endomorphin-2, and evaluated them for their *in vitro* (Table 2) and *in vivo* activities. Ligand **30** ([Dmt^1, D-1-Nal^3]morphiceptin) showed very high affinity and selectivity for MOR in the receptor binding assays (K_i^{μ} = 0.000549 nM, K_i^{δ} = 132 nM). It displayed high agonist activities at both the μ- and δ-receptors (IC_{50}^{μ} = 0.45 nM, IC_{50}^{δ} = 0.45 nM).

A profound supraspinal analgesia (around 100-fold more potent than the endogenous MOR ligand, endomorphin-2) has been reported for [Dmt^1, D-1-Nal^3]morphiceptin (**30**) by conducting the mouse hot plate test after administration of the ligand intracerebroventricularly (i.c.v.) (Figure 2). Study of time-course revealed that the antinociceptive activity of analog **30** could persist up to 2 h (Figure 3). This study suggested [Dmt^1, D-1-Nal^3]morphiceptin as a promising lead compound for the design of new analgesic drug candidates.

The opioid ligand H-Tyr-*c*[D-Cys-Phe-Phe-Cys]-NH_2 (**48**, Table 3) cyclized through a methylene dithiother has been shown as a potent and selective MOR agonist [32]. Berezowska and coauthors prepared dicarba analogs (ligands **35** – **47**, Table 3) **33** of **48** containing Tyr, 2′,6′-dimethyltyrosine (Dmt), 3-[2,6-dimethyl-4-hydroxyphenyl)propanoic acid (Dhp) or (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl) propanoic acid [(2*S*)-Mdp] in the position one, employing Fmoc/^{t}Bu solid-phase approach and substituting D-allylglycine and (2*S*)-2-amino-5-hexenoic acid in position 2 and 5, respectively, followed by ring-closing metathesis. On catalytic hydrogenation of *cis* and *trans* mixtures of isomeric olefinic peptides yielded the saturated –CH_2–CH_2–bridged peptides. All six Tyr^1- and

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Dmt[1]-dicarba analogs (**36** – **41**, Table 3) showed high μ- and δ-opioid agonist potency and produced only small or no preference for MOR over DOR. The six Dhp[1]- and (2*S*)-Mdp[1]-dicarba analogs (**42** – **47**, Table 3) showed antagonist activities at MOR but, surprisingly, displayed a range of different efficacies (agonism, partial agonism or antagonism) at the DOR. The results of this study suggested that the μ versus δ receptor selectivity and the efficacy at the DOR of these cyclic ligands depend on distinct conformational features of the 15-membered peptide ring framework, which might affect the orientation of the exocyclic residue and of the Phe[3] and Phe[4] side chains.

In an attempt to increase the bioavailability of the non-selective, cyclic enkephalin analogs H-Dmt-*c*[D-Cys-Gly-Phe-D(or L)-Cys]-NH_2 (Dmt = 2′,6′-dimethyltyrosine), Weltrowska *et al.* [34] prepared analogs containing *N*-methyl group at the Phe[4] and/or Cys[5] residue. In comparison with the non-methylated parent peptides (**55**, **56**), all mono- (**49** – **52**) and di-*N*-methylated (**53** and **54**) analogs in general showed high binding affinities at all three opioid receptors and high opioid agonist activities in functional opioid assays (Table 4). The observed results suggested that the local conformational restriction in these compounds by mono- and di-*N*-methylation did not greatly affect the *in vitro* opioid activity results. H-Dmt-*c*[D-Cys-Gly-*N*MePhe-L-*N*MeCys]-NH_2 (**54**), a conformationally restricted and low-energy conformer identified from the series, showed good spatial overlap of the crucial pharmacophoric subsites with those in the proposed MOR-bound conformation of the μ-selective opioid peptide JOM-**6** [H-Tyr-*c*(S-Et-S)[D-Cys-Phe-D-Pen]-NH_2] (Pen = penicillamine) [35]. This is in agreement with the moderate μ-selectivity determined for this compound. Substitution of Dmt[1] from **54** by (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(2*S*)-Mdp] resulted in Ligand **57** (Table 4), which turned out to be an opioid antagonist with high opioid receptor binding affinities and was expected to show enhanced bioavailability because of its further increased lipophilicity and reduced hydrogen bonding capacity.

Novoa *et al.* [36] studied the effect of variation of the net charge, lipophilicity and side chain flexibility in Dmt[1]-DALDA (**58**, Table 4) on opioid activity and biodistribution. [37]. The authors examined the impact of charges on the side chain of the second and fourth amino acid residues in the peptidic MOR lead agonist Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt[1]]-DALDA, **58**). The final amide bond was *N*-methylated to increase the overall lipophilicity of [Dmt[1]]-DALDA (**58** – **65**, Table 4). Phenylalanine (Phe) at position three was substituted by a constrained aminobenzazepine analog to investigate the effect side chain flexibility (**66** – **70**). The authors conducted *in vitro* receptor binding and activity studies on the peptidic ligands. They also studied the ligands' *in vivo* transport (brain in- and efflux, and tissue biodistribution) and antinociceptive properties after peripheral administration (ip and sc) in mice. Appreciable shifts of receptor binding, activity and transport properties were reported because of the structural modifications. Important observation was made when antinociceptive properties of these ligands were examined. While [Dmt[1]]-DALDA (**58**) and its *N*-methyl analog, Dmt-D-Arg-Phe-*N*MeLys-NH_2 (**62**) displayed a long-lasting antinociceptive effect (> 7 h), the peptides with D-Cit[2] (**64**) produced effective antinociception faster (maximal effect at 1 h post injection) but also lost their analgesic

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activity quickly when compared to [Dmt^1]-DALDA (**58**) and [Dmt^1, *N*MeLys^4]-DALDA (**62**).

Guillemyn *et al.* has reported [38] that tetrapeptide analogs of the type H-Dmt^1-Xxx^2-Yyy^3-Gly^4-NH_2 are able to cross the BBB after intravenous and subcutaneous administration and successfully activate the MOR and DOR more efficiently and over longer periods of time compared to morphine. The authors have shown a comparison in potency of antinociception (using the hot water tail flick test as the animal model) between a side chain conformationally constrained analogue containing the benzazepine ring (BVD03: H-Dmt-NMe-D-Ala-Aba-Gly-NH_2, where Xxx^2 = NMe-D-Ala and Yyy^3 = Aba), and a 'ring opened' analog (BVD02: H-Dmt-NMe-D-Ala-Aba-Gly-NH_2, where Xxx^2 = NMe-D-Ala and Yyy^3 = Phe). The results demonstrate that combination of increased lipophilicity by amide bond *N*-methylation and the conformational constraint introduced at the Phe^3 side chain displays prolonged antinociception. Enhanced potency and maintained antinociception have been observed on substitution of *N*-Me-D-Ala^2 by D-Arg^2 in the tetrapeptide sequence as it is shown for AN81 (H-Dmt-D-Arg-Aba-Gly-NH_2, where Xxx^2 = D-Arg and Yyy^3 = Aba) versus BVD03. However, a considerable decrease in antinociception has been reported after a regular injection of the studied opioid ligands over a time period of 5 days. In addition, the authors studied a compact opioid agonist and NK1 antagonist hybrid SBCHM01 (H-Dmt-D-Arg-Aba-Gly-NMe-3′,5′Bn$(CF_3)_2$). But it could not overcome the development of opioid induced tolerance, which is in contrast to the results manifested by a similar kind of hybrid TY005 (H-Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Trp-O-3′,5′Bn$(CF_3)_2$) [39]. Here, we suggest that a certain distance between opioid and NK1 pharmacophores is required to display their synergistic effects.

3.2 δ/μ opioid agonists

The analgesia mediated by activation of MOR is accompanied by typical side effects of opiates. But the stimulation of DOR exhibits antinociceptive effects with reduced respiratory depression, low constipation and minimal potential for physical dependence, suggesting this opioid receptor subtype is a potential therapeutic target for the development of analgesics with better biological profiles [40–42]. As there is evidence for synergistic effects exerted through the simultaneous δ and μ receptors activation, some of the potent investigational ligands will be discussed here.

Zieleniak *et al.* [43] synthesized cyclic heptapeptides (**71** – **78**, Table 5) related to the full sequence of deltorphin. *In vitro* functional assay of these peptides at MOR and DOR showed high δ opioid agonist potency and high selectivity for δ receptors. Ligands **73** (IC_{50}^{δ} = 0.64 nM), **74** (IC_{50}^{δ} = 0.483 nM), **75** (IC_{50}^{δ} = 2.73 nM) and **76** (IC_{50}^{δ} = 0.814 nM) produced enhanced agonist activity in MVD assay compared to [Leu^5]enkephalin (**35**, IC_{50}^{δ} = 11.4 nM, Table 2). The authors studied the conformations of these peptides using 2D-NMR in H_2O/D_2O and molecular dynamics, which revealed that the backbone rings had well defined conformations, while the side chains of Tyr^1 and Phe^3, and the exocyclic part of the *C*-terminal had significant conformational flexibility. These results, when compared with the previously reported data for 1 – 4 dermorphin analogs [44,45], suggested that presence of the address segment at the C-terminal played a role for δ-receptor selectivity and binding

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affinity of message region. The results observed during this study provided insights regarding the putative bioactive conformations and bioactivity.

Ciszewska *et al*. [47] prepared *N*-(ureidoethyl)amides of cyclic enkephalin analogs and studied for opioid receptor activities by GPI and MVD assays (**79** – **86**, Table 5). Depending on the size of the ring, the ligands produced diverse opioid activities. In comparison with [Leu^5]enkephalin (**35**), all ligands (**79** – **86**) showed higher potency in the GPI assay (between 125 and 12 times), and **79** appeared to be almost equipotent in the GPI and MVD assay (Table 5). Ligands **79** ($IC_{50}{}^{\mu}$ = 1.97 nM, $IC_{50}{}^{\delta}$ = 1.81 nM) and **84** ($IC_{50}{}^{\mu}$ = 3.36 nM, $IC_{50}{}^{\delta}$ = 11.6 nM) containing 18-membered ring were found to the most potent. Though enkephalins are δ-selective peptides, these cyclic derivatives showed very similar potencies at MOR and DOR.

In an attempt to increase the bioavailability of peptide-derived ligands at DOR and MOR in the CNS, Lee at al. [48]. introduced lipophilic aromatic amino acid Dmt in place of Tyr at position one in enkephalin-like tetrapeptide analogs with an *N*-phenyl-*N*-piperidin-4-ylpropionamide moiety at the C-terminal. SAR studies led to the discovery of non-selective ligands with high potency at μ- and δ-opioid receptors. Ligand **87** (Figure 4) containing the D-Nle^2 and 4-fluoro-substituted aromatic ring in Phe^4 showed high opioid activities at both the receptors ($K_i{}^{\delta}$ = 0.4 nM, $K_i{}^{\mu}$ = 0.02 nM; $IC_{50}{}^{\delta}$ = 0.37 nM, $IC_{50}{}^{\mu}$ = 0.26 nM). Ligand **88** (Figure 4) having the D-Ala^2 and 4-chloro-substituted aromatic ring in Phe^4 also produced potency at both the receptors ($K_i{}^{\delta}$ = 0.14 nM, $K_i{}^{\mu}$ = 0.14 nM; $IC_{50}{}^{\delta}$ = 0.70 nM, $IC_{50}{}^{\mu}$ = 2.6 nM). In addition, Ligand **88** with impressive *in vitro* biological profiles displayed potent *in vivo* antihyperalgesic and antiallodynic effects in the tail-flick assay.

4. Emerging drug design strategies: alliance of opioid agonist/NK1 or CCK antagonist activities

Most of the clinically used opioid drugs target MOR [49] to exert their antinociceptive effects. Many side effects are also known to be due to interaction of drugs with MOR. Development of addiction liability is one of the major problems in targeting MOR [50,51]. The side effects associated with the clinical use of opioid drugs as analgesics significantly decrease the patients' quality of life. The mechanistic pathways responsible for these side effects are largely unknown. It has been observed that prolonged pain states lead to neuroplastic changes in both ascending and descending pathways in the spinal column which cause an increased release of neurotransmitters (e.g., substance P, Cholecystokinin, CCK) that enhance pain and overexpression of the corresponding receptors (NK1 and CCK) for those newly released pain-promoting ligands (see [52–54] for Substance P and NK1 receptor and [55–59] for CCK and CCK receptor). Current drugs used for the treatment of sustained pain generally can only modulate pain and cannot counteract against these induced neuroplastic modifications. Thus, it is not unexpected that current analgesics do not work effectively in these pathological conditions.

Coadministration of a δ/μ opioid agonist and a neurokinin 1 (NK1) antagonist has been reported to produce the facilitatory role of the substance P-NK1 system in opioid signal transmission [60,61]. This combination explains many important biological effects such as

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high potency in acute pain models and inhibition of opioid-induced tolerance in chronic tests using rats. A study reported that mice lacking NK1 receptor did not produce the rewarding properties of morphine [62]. Thus, the alliance of an agonist activity at the opioid receptors and an antagonist at NK1 receptors may have enhanced effects in the treatment of prolonged pain states that involve increased substance P activity. Poor patient compliance, complications in drug metabolism, distribution and possible drug–drug interactions limit the use of drug mixtures as therapeutics. Over the last several years, the Hruby Research Group and a few other groups have taken a new approach to combine these two activities in one ligand would have simple metabolic and pharmacokinetic properties. The anticipated activities of this kind of ligand would include potent analgesic activities in both acute pain and in neuropathic pain states without the development of tolerance [63]. The concept of drug design by this strategy is based on design of ligands with the adjacent and overlapping pharmacophores, in which the μ-/δ-opioid agonist pharmacophore is incorporated at the *N*-terminus, and the NK1 antagonist pharmacophore is introduced at the C-terminus of a single peptide derived ligand. The opioid pharmacophore of these hybrid molecules are designed based on the sequence of well-known opioid pharmacophore, for example. biphalin and DADLE [64,65], while the structural modifications were made on the basis of a 3′,5′-(bistrifluoromethyl)-benzyl ester of *N*-acylated tryptophan, a known NK1 antagonist pharmacophore at the C-terminus [66–68]. The two pharmacophores are combined either directly or through an additional address segment. The linker may also work as an address region for both pharmacophores [65,69–72]. The designed multivalent molecules may have additional advantages over a cocktail of individual drugs for easy administration such as simple ADME properties and no drug–drug interactions. A higher local concentration is also expected than that in the coadministration of two drugs because the expressions of the NK1 and opioid receptors show a significant degree of anatomical overlap in the CNS, leading to synergies in potency and efficacy [73–75]. In fact, the results published through a highly collaborative research from the groups of Hruby, Porreca, Vanderah and Lai showed that the lead multifunctional compounds, TY005 (H-Tyr^1-D-Ala^2-Gly^3-Phe^4-Met^5-Pro^6-Leu^7-Trp^8-O-[3′,5′-Bzl(CF$_3$)$_2$]: K_i^{μ} = 36 nM, K_i^{δ} = 2.8 nM, K_i^{rNK1} = 0.29 nM; IC_{50}^{μ} = 360 nM, IC_{50}^{δ} = 22 nM; K_e^{NK1} = 25 nM; EC_{50}^{μ} = 32 nM, EC_{50}^{δ} = 2.9 nM; E_{max}^{μ} = 42%, E_{max}^{δ} = 45%) [65], and TY027 (H-Tyr^1-D-Ala^2-Gly^3-Phe^4-Met^5-Pro^6-Leu^7-Trp^8-[NH-3′,5′-Bzl(CF$_3$)$_2$]: K_i^{μ} = 16.0 nM, K_i^{δ} = 0.66 nM, K_i^{rNK1} = 7.3 nM; IC_{50}^{μ} = 490 nM, IC_{50}^{δ} = 15 nM; K_e^{NK1} = 10 nM; EC_{50}^{μ} = 7.0 nM, EC_{50}^{δ} = 8.6 nM; E_{max}^{μ} = 55%, E_{max}^{δ} = 58%) [69] having μ-/δ-opioid agonist/NK1 antagonist activities can reverse neuropathic pain in a rodent model with BBB permeability, no sign of opioid-induce tolerance, and no development of reward liability, validating the hypothesis that a single compound possessing opioid agonist/NK1 antagonist activities is effective against neuropathic pain [39,63,76–78]. The multifunctional ligand TY027 has recently been reported [79] to show a preclinical profile of excellent antinociceptive efficacy, low abuse liability and no opioid-related emesis or constipation. In rodent models of acute and neuropathic pain, TY027 exhibited potent analgesic efficacy after central or systemic administration with a plasma half-life of over 4 h and CNS penetration. The data presented in that report showed that a multifunctional ligand can combat the pathological conditions created by chronic pain and sustained opioid use with antinociceptive efficacy in rodent models with significantly fewer side effects than morphine. In these *in vivo* studies it was established that peptides such as **TY027** are

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bioavailable after peripheral administration, cross the BBB well, have minimal or none of the toxic side effects of morphine, and are potent against neuropathic pain states in animal models (e.g., see **77** – **79**). *In vivo* studies are in progress with another potent opioid agonist/NK1 antagonist ligand **TY032** (H-Dmt^1-D-Ala^2-Gly^3-Phe^4-Met^5-Pro^6-Leu^7-Trp^8-[NH-3′,5′-Bzl(CF$_3$)$_2$]: K_i^{μ} = 2.0 nM, K_i^{δ} = 0.18 nM, K_i^{rNK1} = 2.3 nM; IC_{50}^{μ} = 19 nM, IC_{50}^{δ} = 1.8 nM; K_e^{NK1} = 7.5 nM; EC_{50}^{μ} = 29 nM, EC_{50}^{δ} = 1.0 nM; E_{max}^{μ} = 72%, E_{max}^{δ} = 94%) [80]. Thus, such rationally designed, multifunctional ligands have promising activities for treating acute and chronic pain.

Upregulation of CCK has been reported following the chronic administration of morphine to animals indicating the possibility of this endogenous peptide having pronociceptive effects [59]. Evidence is also there for neuropeptide CCK to act as an antagonist of opiate analgesia [81]. These complex interactions between opioids and endogenous CCK receptor systems have led to the suggestion that chronic and neuropathic pain can be treated by combining agonist activity at opioid receptors and antagonist activity at CCK receptor. The strategy is similar to opioid agonist/NK1 antagonist strategy. A number of reports are available in the literature focusing on the design, synthesis and SAR study of this class of ligands [82–86]. Hanlon *et al.* [87] studied the *in vivo* efficacy of novel *μ*-/*δ*-opioid agonist/CCK antagonist ligands **RSA504** (H-Tyr^1-D-Nle^2-Gly^3-Trp^4-Nle^5-Asp^6-Phe^7-NH$_2$: $K_{i\ \mu}$ = 26 nM, $K_{i\ \delta}$ = 2.6 nM, $K_i^{CCK\text{-}1}$ = 9.6 nM, $K_i^{CCK\text{-}2}$ = 15 nM; IC_{50}^{μ} = 210 nM, IC_{50}^{δ} = 23 nM; EC_{50}^{μ} = 0.46 nM, EC_{50}^{δ} = 4.5 nM; E_{max}^{μ} = 88%, E_{max}^{δ} = 81%), and **RSA601** (H-Tyr^1-D-Phe^2-Gly^3-D-Trp^4-*N*MeNle^5-Asp^6-Phe^7-NH$_2$: K_i^{μ} = 5.9 nM, K_i^{δ} = 0.42 nM, $K_i^{CCK\text{-}2}$ = 1.1 nM; IC_{50}^{μ} = 71 nM, IC_{50}^{δ} = 24 nM; EC_{50}^{μ} = 530 nM, EC_{50}^{δ} = 29 nM; E_{max}^{μ} = 50%, E_{max}^{δ} = 14%). **RSA504** and **RSA601** produced antihypersensitivity to mechanical and thermal stimuli *in vivo* using the spinal nerve ligation model of neuropathic pain. Intrathecal administration of **RSA504** and **RSA601** did not display antinociceptive tolerance over 7 days of administration and did not produce motor impairment or sedation using a rotarod. These results suggested multi-targeted molecules can address the pathology of neuropathic pain. Thus ligands with *δ*-/*μ*-opioid agonist activities and CCK antagonist activity within one molecule can offer a novel approach with efficacy for neuropathic pain while lacking the side effects typically caused by conventional treatment with opioid drugs.

5. Concluding discussion

The natural ligands which modulate pain in the human are peptides (e.g., *β*-endorphin, enkephalins, etc.) and yet all treatments primarily use alkaloids and other heterocyclic compounds. None of these are peptides or peptide derivatives. Strangely, essentially all treatments for pain, the most ubiquitous disease, have high toxicities and for prolonged and neuropathic pain, often poor efficacies and all lead to the development of tolerance with long-term use, yet the medical profession continues to use them. Moreover, their patients often abuse current opioid drugs. As we have discussed here, there are new alternatives that in animal models of pain overcome many of the problems of current analgesics. Yet astonishingly none of these peptides have been subjected to Phase III clinical trials. Why? Are the power brokers of our pharmaceutical, medical and investment communities afraid they may work? It is mysterious and the reality is there for all to see. Hopefully, our brief and incomplete review of the opioid ligands of the past and the current ones under

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investigation will stimulate interest in the medical and pharmaceutical industries to further develop these ligands, and give HOPE to some of our billion fellow human beings that suffer daily from pain.

6. Expert opinion

Although there are a number of drugs for the management of pain, none of them are 100% successful in controlling it, especially when the treatment of prolonged and neuropathic pain is concerned. Several therapeutic agents including opioid analgesics, anticonvulsants, antidepressants, topical anesthetics (lidocaine patch), etc., with different biological targets and activities are currently in clinical use. These drugs can offer moderate pain relief. During the use of these drugs, there are considerable concerns of serious and unwanted side effects.

The most common feature among all available drugs and the drugs in advanced stages of clinical trials is that almost all of them target μ-opioid or other receptors or ion-channels on neurons. It is well known that most of the biological targets have subtypes, and each subtype is responsible for different kinds of biological activities. It is emerging in the literature that these biological targets have a number of conformations as well as binding sites that elicit different responses. If binding to a particular site establishes equilibrium between more than one conformations of a receptor, this can lead to several biological responses, including unwanted side effects. It is known that small molecules are generally less selective for their biological targets, often resulting in unwanted activation or deactivation of other biological systems which produce undesirable side effects. Elimination of these limitations of current drugs is an important goal. Peptide and peptidomimetic ligands have been found to be more selective for a specific target. But, some of the major problems associated with these types of ligands can include less stability in biological conditions and a reduced chance of reaching their targets. Elimination of these problems will open an extraordinary avenue in the drug discovery horizon. An approach, which might be very useful, is to design ligands that can interact with multiple biological targets with the specific desired activity at each individual target. The authors of this review suggest that such drugs with novel biological profiles will lead to better medical benefits for the treatment of pain. Molecules with high selectivity for binding sites and unique conformations can provide a new and better path to achieve these goals. The development of such conformationally and functionally biased drug candidates is a real challenge, but it can be addressed. The application of this latest approach to drug design and discovery with multivalency/ multifunctionality has been discussed here. As there is evidence for peripherally and centrally acting opioid agonists to show analgesic effects, design of drug candidates having activities at both locations can be interesting to consider.

Clearly new directions and new modalities are needed for the treatment of prolonged and neuropathic pain. Clearly also ‘one shoe’ will not fit all. Undoubtedly, there are many different phenotypes of prolonged and neuropathic pain. Hopefully, this brief review will give some stimulation to others to discover new approaches to the development of ligands to treat our most ubiquitous and poorly treated diseases, prolonged and neuropathic pain.

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87. Hanlon KE, Herman DS, Agnes RS, et al. Novel peptide ligands with dual acting pharmacophores designed for the pathophysiology of neuropathic pain. Brain Res. 2011; 1395:1–11. [PubMed: 21550594]

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Article highlights

- Mixed μ/δ opioid receptor agonist ligands have no or reduced toxicities relative to μ opioid ligands such as morphine.
- Highly potent μ/δ opioid ligands can have patent antinociceptive activities for neuropathic pain.
- Highly potent multivalent ligands with μ/δ receptor opioid agonist activity and neurokinin-1 receptor antagonist activities have minimal toxicities of current opioid ligands in clinical use.

This box summarizes key points contained in the article.

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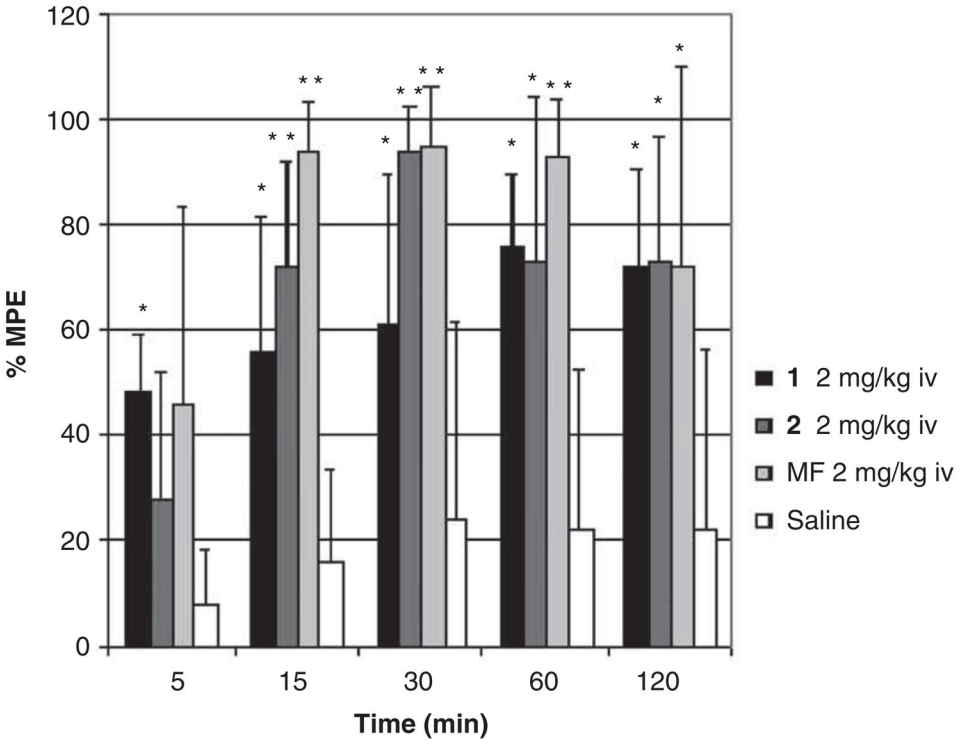

Figure 1.

Analgesic effect as % MPE of 25 (1) and 26 (2) in the mouse tail-flick assay at different times after intravenous administration.

**P < 0.001; *P < 0.05.

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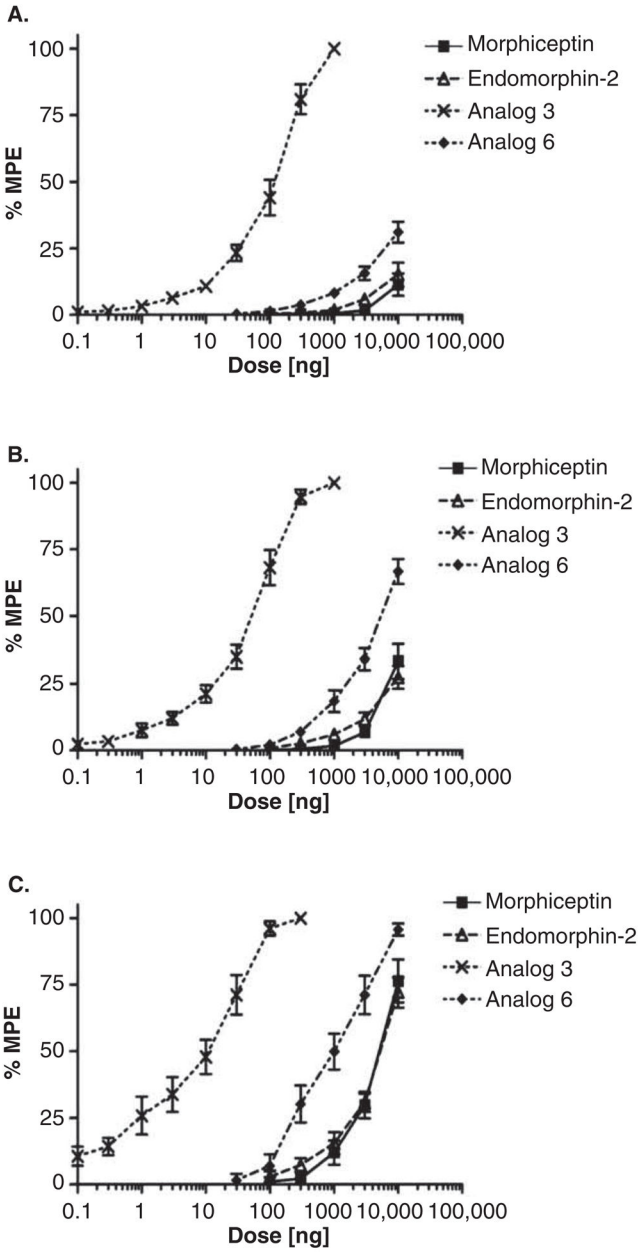

Figure 2.

Dose–response curves determined in the hot plate test for the inhibition of paw licking (**A**), rearing (**B**), and jumping (**C**) induced by i.c.v. injection of morphiceptin, EM-2, [Dmt^1, D-1-Nal^3]morphiceptin (Analog **30** (**3**)), and [Dmt^1, D-1-Nal^3]EM-2 (Analog **33** (**6**)). The data represent the mean – SEM of 10 mice per group.

Adapted from [31] with permission from Elsevier.

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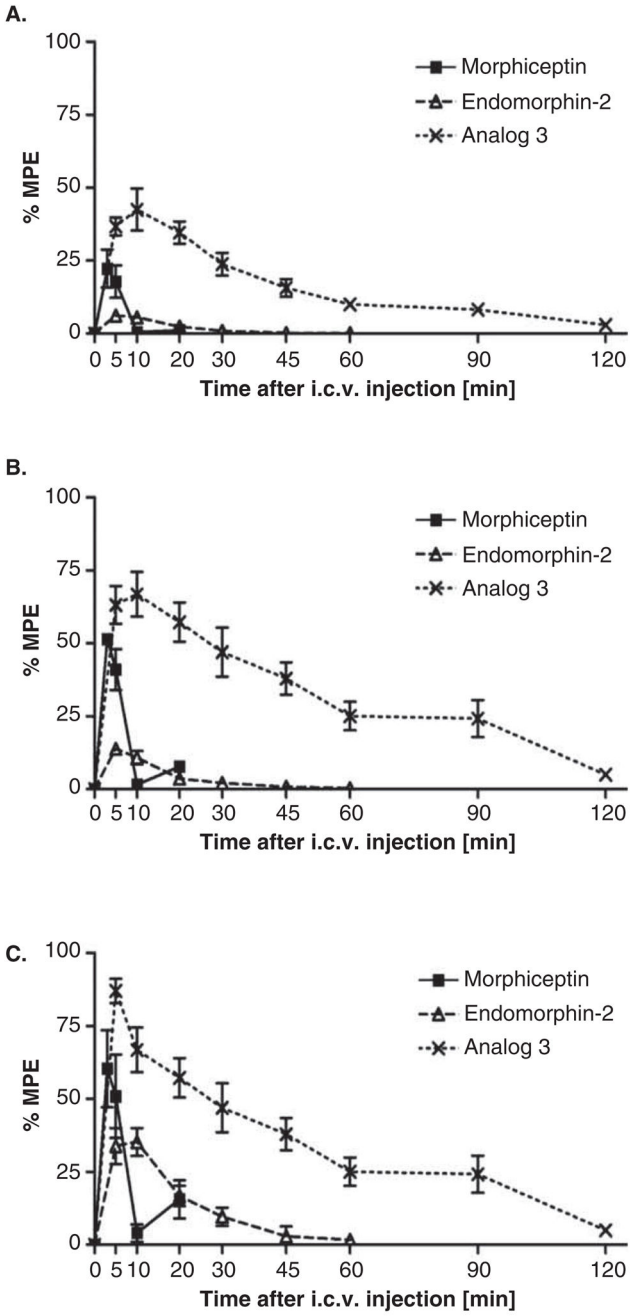
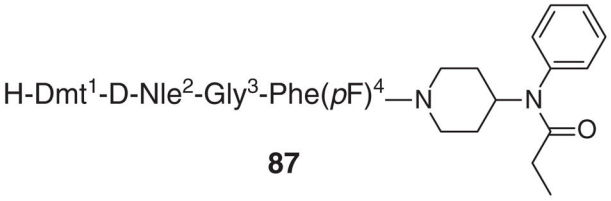


Figure 3.

Time-course of the changes in inhibition of paw licking (**A**), rearing (**B)**, and jumping (**C**) induced by i.c.v. injection of morphiceptin (10 μg), EM-2 (3 μg), and [Dmt[1], D-1-Nal[3]]morphiceptin (Analog **30** (**3**), 0.1 μg), determined in the hot plate assay. Adapted from [31] with permission from Elsevier.



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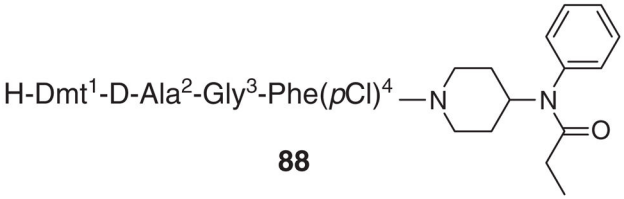

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Figure 4.
Structures of two potent opioid ligands.

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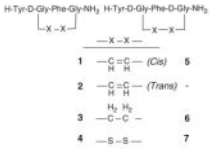
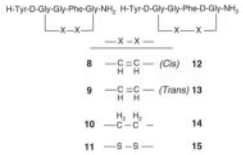
Table 1

Pharmacological data of Dicarba analogs of H-Tyr-*c*[D-Cys-Phe-L(or D)-Cys]-NH_2 and H-Tyr-*c*[D-Cys-Gly-Phe-L(or D)-Cys]-NH_2 at opioid receptors (OR).





Compound number	GPI	MVD	GPI/MVD	OR binding affinity			Binding ratio
	IC_{50} (nM)	IC_{50} (nM)	IC_{50} ratio	K_i^{μ} (nM)	K_i^{δ} (nM)	K_i^{κ} (nM)	$K_i^{\mu}/K_i^{\delta}/K_i^{\kappa}$
1	436	460	1/1.06	–	–	–	–
2	162	444	1/2.74	–	–	–	–
3	76.5	655	1/8.56	–	–	–	–
4	64.7	740	1/11.4	11.0	373	–	1/34/–
5	176	246	1/1.40	–	–	–	–
6	208	786	1/3.78	–	–	–	–
7	20.0	28.8	1/1.44	–	–	–	–
8	1.81	0.496	3.65/1	2.4	6.55	200	1/3/83
9	0.898	0.275	3.27:1	0.616	1.25	57.6	1/2/94
10	1.02	3.19	1/3.12	2.34	5.87	309	1/3/132
11	1.65	0.603	2.74/1	1.74	1.61	40.1	1/1/23
12	11.9	9.91	1.2/1	2.27	7.04	498	1/3/219
13	5.63	4.38	1.3/1	1.05	1.92	30.4	1/2/29
14	11.2	3.64	3.1/1	1.17	3.34	71.5	1/3/61
15	1.30	0.562	2.31/1	0.550	0.822	44.9	1/1/82

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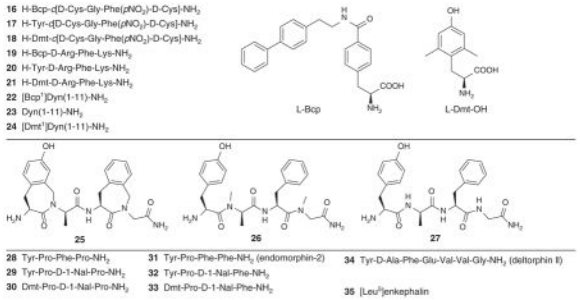
Table 2

Binding affinity and functional activities at ORs.



Compound number	GPI	MVD	GPI/MVD	OR binding affinity			Binding ratio
	IC_{50} (nM)	IC_{50} (nM)	IC_{50} ratio	K_i^{μ} (nM)	K_i^{δ} (nM)	K_i^{κ} (nM)	$K_i^{\mu}/K_i^{\delta}/K_i^{\kappa}$
16	0.226	0.319	1/1.4	0.293	9.99	6.93	1/34/24
17	0.717	0.211	3.4/1	0.443	0.389	1.53	1/0.9/3.5
18	0.542	0.182	3/1	0.247	0.704	3.77	1/3/15
19	0.984	4.60	1/4.7	0.948	102	1.39	1/108/1.5
20	254	781	1/3	1.69	19200	4230	1/11360/2503
21	1.41	23.1	1/16	0.143	2100	22.3	1/14685/156
22	0.591*	1.07	–	3.41	13.9	1.56	1/4/0.5
23	0.376	15.2	1/40	0.653	13.5	0.087	1/21/0.13
24	0.502	0.377	1.3/1	0.322	0.435	1.18	1/1.4/3.7
25	3.64	30.3	1/8	20.8	17.8	–	1.2/1/–
26	22.0	79.7	1/4	28.1	7.1	–	4/1/–
MF/Morphine	29.3	155	1/5	9.77	112.2	–	1/11/–
28	318	4800	1/15	19.8	> 1000	–	1/> 50/–
29	9.57	35.4	1/3.6	0.47	> 1000		1/> 2127/–
30	0.45	0.64	1/1.4	0.00055	132	–	1/24044/–
31	7.71	15.3	1/2	0.79	> 1000	–	1/> 1266/–
32	872	2040	1/2.3	22.3	> 1000	–	1/> 45/–
33	1.53	1.63	1/1.1	0.93	> 1000		1/> 1075/–

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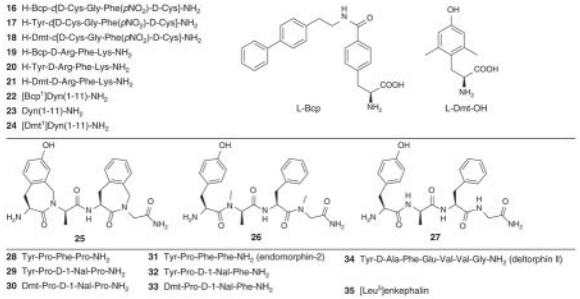

Compound number	GPI	MVD	GPI/MVD	OR binding affinity			Binding ratio
	IC_{50} (nM)	IC_{50} (nM)	IC_{50} ratio	K_i^{μ} (nM)	K_i^{δ} (nM)	K_i^{κ} (nM)	$K_i^{\mu}/K_i^{\delta}/K_i^{\kappa}$
34	–	–	–	–	0.56	–	–/–/–
35	246	11.4	21.6/1	–	–	–	–/–/–

*Partial agonist.

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Table 3

In vitro binding affinity and functional activity data of dicarba-cyclic ligands.

H-Yyy-D-Gly-Phe-Phe-Gly-NH_2 (X–X–X)

No.	Yyy	—X–X–X—
36	Tyr	—C(H)=C(H)–C(H_2)– (Cis)
37	Tyr	—C(H)=C(H)–C(H_2)– (Trans)
38	Tyr	—C(H_2)–C(H_2)–C(H_2)–
39	Dmt	—C(H)=C(H)–C(H_2)– (Cis)
40	Dmt	—C(H)=C(H)–C(H_2)– (Trans)
40	Dmt	—C(H_2)–C(H_2)–C(H_2)–
42	Dhp	—C(H)=C(H)–C(H_2)– (Cis)
43	Dhp	—C(H)=C(H)–C(H_2)– (Trans)
44	Dhp	—C(H_2)–C(H_2)–C(H_2)–
45	(2*S*)-Mdp	—C(H)=C(H)–C(H_2)– (Cis)
46	(2*S*)-Mdp	—C(H)=C(H)–C(H_2)– (Trans)
47	(2*S*)-Mdp	—C(H_2)–C(H_2)–C(H_2)–
48	Tyr	—S–C(H_2)–S—

Compound number	GPI		MVD		GPI/MVD	OR binding affinity			Binding ratio
	IC_{50}(nM)	K_e (nM)	IC_{50} (nM)	K_e (nM)	IC_{50} ratio	K_i^{μ} (nM)	K_i^{δ} (nM)	K_i^{κ} (nM)	$K_i^{\mu}/K_i^{\delta}/K_i^{\kappa}$
36	0.401	–	0.851	–	1/2	0.281	0.92	9.36	1/3/12
37	1.34	–	4.51	–	1/3	0.332	1.53	15.2	1/5/14
38	1.05	–	0.740	–	1.4/1	1.04	2.24	11.3	1/2/11
39	0.161	–	0.191	–	1/1.2	0.528	0.49	12.8	1/1/24
40	0.407	–	0.0444	–	9/1	0.438	0.29	4.97	1/1/11
41	0.509	–	0.0655	–	8/1	1.54	1.20	15.8	1/1/10
42	–	32.2	PA (e = 0.38)	–	–	23.5	86.3	266	1/4/11
43	–	44.3	239	–	–	16.7	27.0	> 500	1/2/> 30
44	–	41.7	7500	–	–	8.95	39.2	> 500	1/4/> 56
45	–	32.4	PA	–	–	7.44	7.67	185	1/1/25
46	–	157	PA (e = 0.33)	–	–	323	35.3	635	1/0.1/2
47	–	25.7	9.91	147	–	18.3	18.7	302	1/1/17
48	–	–	–	–	–	0.016	1.8	2.5	1/112/156

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Table 4

Binding and functional assay data of cyclic ligands at opioid receptors (OR).

49 H-Dmt-c[D-Cys-Gly-*N*MePhe-D-Cys]-NH_2
50 H-Dmt-c[D-Cys-Gly-*N*MePhe-L-Cys]-NH_2
51 H-Dmt-c[D-Cys-Gly-Phe-D-*N*MeCys]-NH_2
52 H-Dmt-c[D-Cys-Gly-Phe-L-*N*MeCys]-NH_2
53 H-Dmt-c[D-Cys-Gly-*N*MePhe-D-*N*MeCys]-NH_2
54 H-Dmt-c[D-Cys-Gly-*N*MePhe-L-*N*MeCys]-NH_2
55 H-Dmt-c[D-Cys-Gly-Phe-D-Cys]-NH_2
56 H-Dmt-c[D-Cys-Gly-Phe-L-Cys]-NH_2
57 (2*S*)-Mdp-c[D-Cys-Gly-*N*MePhe-L-*N*MeCys]-NH_2

58 H-Dmt-D-Arg-Phe-Lys-NH_2
59 H-Dmt-D-Arg-Phe-Nle-NH_2
60 H-Dmt-D-Cit-Phe-Lys-NH_2
61 H-Dmt-D-Cit-Phe-Nle-NH_2
62 H-Dmt-D-Arg-Phe-*N*MeLys-NH_2
63 H-Dmt-D-Arg-Phe-*N*MeNle-NH_2
64 H-Dmt-D-Cit-Phe-*N*MeLys-NH_2
65 H-Dmt-D-Cit-Phe-*N*MeNle-NH_2
66 H-Dmt-D-Arg-Aba-Lys-NH_2
67 H-Dmt-D-Arg-Aba-Nle-NH_2
68 H-Dmt-D-Cit-Aba-Lys-NH_2
69 H-Dmt-D-Cit-Aba-Nle-NH_{2C}
70 H-Dmt-D-Arg-Aba-Gly-NH_2

Compound number	GPI			MVD		GPI/MVD	OR binding affinity			Binding ratio
	IC_{50} (nM)	K_e^{μ} (nM)	K_e^{κ}(nM)	IC_{50} (nM)	K_e^{δ}(nM)	IC_{50} ratio	K_i^{μ}(nM)	K_i^{δ}(nM)	K_i^{κ}(nM)	$K_i^{\mu}/K_i^{\delta}/K_i^{\kappa}$
49	1.07	–	–	1.11	–	1/1	0.496	2.29	0.45	1/5/1
50	0.457	–	–	0.884	–	1/2	0.354	2.36	0.86	1/7/2
51	1.81	–	–	0.352	–	5/1	0.504	0.525	1.01	1/1/2
52	1.36	–	–	0.122	–	11/1	0.586	0.776	0.89	1/1/2
53	1.34	–	–	3.72	–	1/3	0.876	6.07	1.42	1/7/2
54	0.394	–	–	1.95	–	1/5	0.641	1.79	0.88	1/3/1
55	0.586	–	–	0.0530	–	11/1	0.412	0.202	0.68	1/0.5/1.6
56	0.812	–	–	0.115	–	7/1	0.282	0.306	0.68	1/1/2
57	–	71.0	151	–	277	–	14.4	35.9	29.5	1/2/2
58	1.41	–	–	23.1	–	1/16	0.143	2100	22.3	1/11400/156
59	2.2	–	–	6.2	–	1/2.8	0.23	15.2	16.2	1/66/70
60	0.31	–	–	1.4	–	1/4.5	0.57	50.0	20.9	1/88/37
61	0.89			4.7		1/5.3	0.79	3.98	98.5	1/5/125
62	8.3	–	–	7.0	–	1.2/1	0.26	309	56.1	1/1190/216
63	4.35	–	–	1.5	–	2.9/1	0.26	16.0	14.4	1/62/55
64	1.01	–	–	4.4	–	1/4.4	0.94	39.2	11.5	1/42/12
65	0.65	–	–	1.5	–	1/2.3	2.92	5.03	> 10,000	1/2/> 3420
66	76.0	–	–	64 (IC_{25})	–	–	0.61	38.60	ND	1/64/–
67	1.45	–	–	3.1	–	1/2.1	0.43	7.46	1.12	1/17/3
68	49.0	–	–	1000	–	1/20	2.62	56.9	ND	1/22/–
69	0.78 (IC_{35})	–	–	1.16	–	–	0.93	3..18	11.1	1/3/12

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49 H-Dmt-c[D-Cys-Gly-*N*MePhe-D-Cys]-NH_2
50 H-Dmt-c[D-Cys-Gly-*N*MePhe-L-Cys]-NH_2
51 H-Dmt-c[D-Cys-Gly-Phe-D-*N*MeCys]-NH_2
52 H-Dmt-c[D-Cys-Gly-Phe-L-*N*MeCys]-NH_2

53 H-Dmt-c[D-Cys-Gly-*N*MePhe-D-*N*MeCys]-NH_2
54 H-Dmt-c[D-Cys-Gly-*N*MePhe-L-*N*MeCys]-NH_2
55 H-Dmt-c[D-Cys-Gly-Phe-D-Cys]-NH_2
56 H-Dmt-c[D-Cys-Gly-Phe-L-Cys]-NH_2
57 (*2S*)-Mdp-c[D-Cys-Gly-*N*MePhe-L-*N*MeCys]-NH_2

58 H-Dmt-D-Arg-Phe-Lys-NH_2
59 H-Dmt-D-Arg-Phe-Nle-NH_2
60 H-Dmt-D-Cit-Phe-Lys-NH_2
61 H-Dmt-D-Cit-Phe-Nle-NH_2

62 H-Dmt-D-Arg-Phe-*N*MeLys-NH_2
63 H-Dmt-D-Arg-Phe-*N*MeNle-NH_2
64 H-Dmt-D-Cit-Phe-*N*MeLys-NH_2
65 H-Dmt-D-Cit-Phe-*N*MeNle-NH_2

66 H-Dmt-D-Arg-Aba-Lys-NH_2
67 H-Dmt-D-Arg-Aba-Nle-NH_2
68 H-Dmt-D-Cit-Aba-Lys-NH_2
69 H-Dmt-D-Cit-Aba-Nle-NH_{2c}
70 H-Dmt-D-Arg-Aba-Gly-NH_2

Compound number	GPI			MVD		GPI/MVD	OR binding affinity			Binding ratio
	IC_{50} (nM)	K_e^{μ} (nM)	K_e^{κ}(nM)	IC_{50} (nM)	K_e^{δ} (nM)	IC_{50} ratio	K_i^{μ}(nM)	K_i^{δ} (nM)	K_i^{κ} (nM)	$K_i^{\mu}/K_i^{\delta}/K_i^{\kappa}$
70	0.32	–	–	0.42	–	1/1.3	0.15	0.60	118	1/4/787

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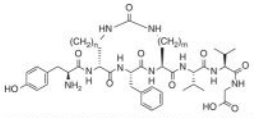
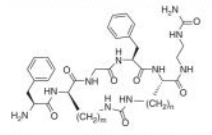
Table 5

GPI and MVD assay data for cyclic deltorphin (1 – 7) analogs and *N*-(ureidoethyl)amides of cyclic enkephalin analogs.



71 {[H-Tyr-D-Lys(&[1])-Phe-Lys(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
72 {[H-Tyr-D-Lys(&[1])-Phe-Orn(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
73 {[H-Tyr-D-Lys(&[1])-Phe-Dab(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
74 {[H-Tyr-D-Lys(&[1])-Phe-Dap(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
75 {[H-Tyr-D-Orn(&[1])-Phe-Lys(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
76 {[H-Tyr-D-Orn(&[1])-Phe-Orn(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
77 {[H-Tyr-D-Orn(&[1])-Phe-Dab(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];
78 {[H-Tyr-D-Orn(&[1])-Phe-Dap(&[2])-Val-Val-Gly-NH$_2$][&[1]CO&[2]];



79 {[H-Tyr-D-Lys(&[1])-Gly-Phe-Dap(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
80 {[H-Tyr-D-Lys(&[1])-Gly-Phe-Dab(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
81 {[H-Tyr-D-Lys(&[1])-Gly-Phe-Orn(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
82 {[H-Tyr-D-Lys(&[1])-Gly-Phe-Lys(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
83 {[H-Tyr-D-Orn(&[1])-Gly-Phe-Dap(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
84 {[H-Tyr-D-Orn(&[1])-Gly-Phe-Dab(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
85 {[H-Tyr-D-Orn(&[1])-Gly-Phe-Orn(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];
86 {[H-Tyr-D-Orn(&[1])-Gly-Phe-Lys(&[2])-NHCH$_2$CH$_2$NHCONH$_2$][&[1]CO&[2]];

Nomenclature, ref. # 46

Compound number	GPI	MVD	GPI/MVD	Compound number	Ring size	GPI	Relative potency	MVD	Relative potency	GPI/MVD
	IC_{50} (nM)	IC_{50} (nM)	IC_{50} ratio			IC_{50} (nM)		IC_{50} (nM)		IC_{50} ratio
71	888	11.8	75/1	**79**	18	1.97	125	1.81	6.30	1.1/1
72	> 10,000	67.0	> 149/1	**80**	19	5.55	44.3	6.0	1.90	1/1.1
73	65.4	0.640	102/1	**81**	20	18.1	13.6	22.7	0.502	1/1.3
74	25.4	0.483	53/1	**82**	21	20.2	12.2	26.6	0.429	1/1.3
75	1020	2.73	374/1	**83**	17	11.3	21.8	14.1	0.809	1/1.2
76	159	0.814	195/1	**84**	18	3.36	73.2	11.6	0.983	1/3.5
77	460	106	4.3/1	**85**	19	6.87	35.8	16.5	0.691	1/2.4
78	> 10000	27.1	> 369/1	**86**	20	16.1	15.3	28.2	0.404	1/1.8
–	–	–	–	**35**	–	246	1	11.4	1	21.6/1