

## Hot Topics in Opioid Pharmacology: Mixed and Biased Opioids.

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**Details of authors' contributions:** All authors contributed to the construction of all drafts and all authors approved the final version of the article.

**Declaration of interests:** DGL is Chair of Board – British Journal of Anaesthesia and in the past has had funding from and held a consultancy with Grunenthal, the company that is developing cebranopadol. DGL is also a non-executive director of Cellomatics; an SME-CRO.

This review is based on a presentation at the 72<sup>nd</sup> NYSSA-PGA meeting in New York on 9<sup>th</sup> December 2018.

**Funding:** Research on opioids in the laboratory of DGL is funded by Biotechnology and Biological Sciences Research Council (BBSRC), British Journal of Anaesthesia and British Heart Foundation. AAHA is funded by a scholarship from HCED, Iraq.

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## **Abstract**

Analgesic design and evaluation has been driven by the desire to create high affinity, high selectivity MOP ( $\mu$ ;  $\mu$ ) agonists. Such ligands are the mainstay of current clinical practice and include morphine and fentanyl. Advances in this sphere have come from designing pharmacokinetic advantage; rapid metabolism for remifentanyl. These produce analgesia but also the adverse effect profile that currently defines this drug class; namely ventilatory depression, tolerance and abuse liability. The MOP receptor is part of a family and there are significant functional interactions between other members of the family (DOP;  $\delta$ ;  $\delta$ , KOP;  $\kappa$ ;  $\kappa$  and NOP; nociceptin/orphanin FQ). Experimentally MOP agonism and DOP antagonism produced antinociception (animals) with no tolerance and low doses of MOP and NOP ligands synergize to antinociceptive advantage. In this latter context lack of effect of NOP agonists on ventilation is an additional advantage. Recent development has been to move towards low selectivity multifunctional ‘mixed ligands’ (e.g., Cebranopadol) or ligand mixtures (e.g., Targinact). Moreover, the observation that  $\beta$ -arrestin coupling underlies the side effect profile for MOP ligands (from knockout animal studies) led to the discovery of biased (to G-protein and away from  $\beta$ -arrestin) MOP ligands (e.g., oliceridine). There is sufficient excitement in the opioid field to suggest that opioid analgesics without significant side effects may be on the horizon and the ‘opioid holy grail’ might be in reach.

**Running head:** Mixed and Biased Opioids

**Key words:** Opioids, Opioid receptors, Novel analgesics, Biased agonists.

## Introduction

G protein coupled receptors (GPCR) represent a target for more than one third of Food and Drug Administration (FDA) approved blockbuster drugs such as analgesics, antihistamines, neuroleptics and many cardiovascular drugs. One important GPCR class is the opioid receptor family. Opioid receptors are classified as  $\mu$  (mu;MOP),  $\delta$  (delta;DOP) and  $\kappa$  (kappa;KOP), these are the classical naloxone sensitive members of the family <sup>1</sup>. In addition there is a further non-classical member of the family the nociceptin/orphanin FQ (N/OFQ) receptor, NOP. This latter receptor is not sensitive to naloxone <sup>2</sup>. Opioids receptors couple to  $G_i/G_o$  G-proteins to enhance an efflux of  $K^+$  ions (producing hyperpolarisation in neurones), closing voltage gated  $Ca^{2+}$  channels (to reduce transmittter release in neurones) and inhibiting adenylate cyclase to reduce cAMP formation (affecting membrane repolarisation); signalling is switched off using the  $\beta$ -arrestin pathway, **Figure 1**. Via these co-ordinated cellular events <sup>1, 2, 3</sup> all members of the family are capable of producing analgesia (antinociception in non-humans) to varying degrees with differences in sites of action <sup>3, 4</sup>, **Table 1**. In addition opioid receptors couple to mitogen activated protein kinases (MAPK); including ERK1/2, p38 and JNK via G-protein and independent  $\beta$ -arrestin pathways, **Figure 1**.

Despite all members of the family being capable of producing analgesia the mainstay in the clinic is usually agonists for the MOP receptor (we will cover some emerging examples later in the review). These include drugs such as morphine, fentanyl and oxycodone. Attendant with the desired analgesic response opioids have a poor side effect profile; ventilatory depression, nausea and vomiting, constipation, tolerance and dependence to name the most important. Tolerance sets up a vicious cycle of dose escalation (especially in palliative care) and dependence is a worldwide problem linked to premature death and criminality.

Opioid receptors are members of a family and like all families there are important interactions that have the potential for clinical exploitation. It is well know that opioid receptors interact with each other in both a physical sense (dimers) and at a systems level <sup>5</sup>. As such the search for analgesics with reduced side effect profile has shifted away from selective MOP agonists to poorly seleticve ‘dirty’ opioids acting at multiple sites.

In this reivew we will address the question, *should analgesic research be focussed on high affinity MOP selective agonists or on the design and characterisation of non-selective mixed molecules?* We will build on previous reviews from Dietis and Calo et al <sup>5, 6</sup> to cover selected

preclinical development, clinical use of molecule mixtures and mixed opioid non-opioid ligands and explore the potential for biased agonism to drive analgesia with reduced side effects. In addition, we present some cautionary tales that arise when animal (usually rodents) studies are used to predict human efficacy. Before moving into the pharmacological arguments in this review a reminder of basic profile descriptors is provided in **Figure 2**. In its simplest form a ligand can interact with a receptor to produce an effect (agonist) or block an effect (antagonist). An agonist that produces the maximum possible effect (loosely defined as efficacy) in a particular tissue is a full agonist; anything with lower efficacy is a partial agonist. Antagonists have no efficacy in this context and some molecules reduce basal activity (or tone), these are inverse agonists (effect inverse of a classical agonist). Potency is the concentration or dose range over which a ligand produces its effect. In the examples shown in **Figure 2** this represents the x-axis.

#### **A. Mixed molecules and molecule mixtures.**

##### *i. Morphine and Oxycodone: MoxDuo*

MoxDuo or Q8003 is a mixture of morphine and oxycodone (3:2), the rationale for which is based on those presumptions from animal studies of co-administration opioid synergy and its potential to reduce the doses of the component drugs. Of note both morphine and oxycodone are MOP agonists but some interaction with  $\kappa_2$  (for which there is little if any structural evidence<sup>1</sup>) is considered a bonus. In addition to analgesic synergy a reduction in side effect profile is also predicted. The majority of the work has been performed in bunionectomy patients<sup>8-10</sup>. In a phase III double blind randomised trial Q8003 (12mg morphine : 8mg oxycodone) was compared with the individual components. 522 patients were randomised and 491 completed; 19 discontinued due to adverse events but this was comparable across the groups. There was a statistically significant improvement in efficacy for Q8003 compared to morphine or oxycodone (assessed as SPID; Sum of Pain Intensity Differences) but there was no evidence for synergy [Cambridge English dictionary: '*the combined power of a group of things when they are working together that is greater than the total power achieved by each working separately*']. Moreover, there was no improvement in adverse events. As noted in a brief note by Wolfe in 2014<sup>11</sup> that contextualised a failed FDA application the product failed to show synergy or safety advantage.

### *ii. Targinact*

Targinact is a mixture of oxycodone and naloxone that is administered orally. The mixture takes advantage of extensive metabolism of naloxone effectively limiting its action to the periphery. Naloxone undergoes first pass metabolism via the oral route such that it does not enter the CNS (in appreciable amounts). The actions of naloxone are therefore peripherally restricted by metabolism and act to prevent the actions of co-administered oxycodone on the GI tract. Oxycodone would enter the CNS to produce analgesia typical of a MOP agonist. The overall effects of targinact would be predicted to be analgesia (central effect) without constipation (peripheral GI effect)<sup>12</sup>. There are three index studies of note in moderate to severe chronic non-malignant and malignant pain where there was a significant improvement of bowel index (a measure of bowel function) compared to oxycodone<sup>13-15</sup>. Targinact appears to be a useful option in moderate to severe chronic pain that *can* be managed by opioids.

### *iii. Tramadol and Tapentadol*

Tramadol and tapentadol are centrally acting analgesics and represent examples of bifunctional ligands; they are effectively non-selective. Both drugs have a dual mechanism of action, the inhibition of catecholamine reuptake and agonism at the MOP receptor. Tramadol and tapentadol differ from the majority of examples in this review in that they target opioid and non-opioid sites. Both are MOP agonists and both potentiate descending inhibitory control. Pain is processed via afferent input into the spinal cord, spinal gating, ascension to the brain and a descending inhibition circuit<sup>16</sup>. Analgesia is produced by reducing afferent inflow, spinal gating and ascension to and processing in the brain. In addition, potentiating descending inhibition is analgesic. This latter pathway uses catecholamines so blocking their uptake will be analgesic and this is the presumed mechanism of analgesia produced by antidepressants<sup>17</sup>. In the case of tramadol and tapentadol analgesia is produced by a bifunctional (non-selective) action at MOP and catecholamine reuptake but there are important differences<sup>18-22</sup>. Tramadol is a chiral molecule, with the drug preparation being a racemic compound consisting of both left and right handed enantiomers (only one of which is active). Overall tramadol has a low affinity at MOP with the first M1 metabolite of the drug having improved affinity (microM to nanoM affinity) for the MOP receptor, unfortunately some patients are unable to metabolise tramadol. Tramadol inhibits the uptake of both norepinephrine and serotonin and therefore there is a possibility of central serotonin syndrome<sup>23, 24</sup>.

Tapentadol is presented as a single enantiomer; a resolved compound in which the original racemic mixture is purified. Tapentadol has higher affinity for MOP and does not require metabolism. Moreover, there is a degree of selectivity for norepinephrine uptake. Both tramadol and tapentadol are effective in nociceptive, inflammatory and neuropathic pain although efficacy of tramadol in neuropathic pain has been questioned <sup>25</sup>. Tapentadol IR improved pain intensity similar to oxycodone and was therefore classified as ‘non-inferior’ to oxycodone <sup>26</sup>. Responder rates were better with tapentadol compared to oxycodone and there was a side effect profile typical of MOP agonist. However, there was reduced nausea and vomiting compared to oxycodone <sup>26</sup>.

#### *iv. Mixed MOP/DOP*

One particular experimental example worthy of further mention is the interaction between MOP and DOP. A significant demonstration to reveal how a mixed opioid approach could be beneficial in terms of analgesia with a reduced tolerance profile was a study by Zhu et al using DOP knock-out (KO) mice, a genetic approach in which the gene encoding for the DOP receptor is removed. In wildtype mice, naturally expressing DOP, analgesic responses to morphine were lost following 5 days fixed drug administration, however, in DOP KO animals the same daily administration of morphine for 8 days failed to reveal tolerance. The study underlies the importance of DOP receptor expression in the development of morphine tolerance <sup>27</sup>. Pharmacological approaches, using MOP agonists and DOP antagonists also produce antinociception in animal models with a reduction in the development of tolerance. When mice are implanted with a morphine pellet, tolerance develops such that more morphine is required to produce the same degree of antinociception, this can be reversed by administration of the selective DOP antagonist, naltrindole <sup>28</sup>. Moreover, in a similar protocol but replacing DOP antagonism with antisense DOP knockdown Kest et al <sup>29</sup> demonstrated that, where there is less DOP (a form of genetic DOP ‘antagonism’), tolerance to morphine was again reduced. In a more elegant experiment Nitsche et al <sup>30</sup> used DOP and ppENK (pre-pro-Enkephalin) knockout animals; ppENK is the precursor for the natural peptide ligand for DOP. In wild type animals tolerance to morphine rapidly developed; this was absent in animals that lacked either ppENK (the ability to activate DOP) or the DOP receptor itself.

Using a series of compounds that act as MOP agonists and DOP antagonists, in a series of *in vitro* and *in vivo* experiments, Anand et al started to elucidate the pharmacological characteristics required for optimal production of antinociception without the development of

tolerance and dependence <sup>31</sup>. It was recognised that a simple combination of MOP agonist activity with DOP antagonist activity is not sufficient to prevent the development of tolerance. High binding affinity at DOP was deemed to protect against tolerance, however compound efficacy *in vitro* or *in vivo* did not relate to a lack of tolerance development <sup>31</sup>.

Collectively these data underscore a powerful receptor interaction with important functional consequences; sadly there are no mixed MOP-agonist:DOP-antagonist drugs or drug combinations currently in the clinic.

#### *v. Buprenorphine*

Buprenorphine, a semi-synthetic opioid which can be derived from the naturally occurring alkaloid of the opium poppy thebaine, is a mixed opioid already in clinical use. Buprenorphine is approved in the treatment of opioid dependency, as an analgesic and has a mixed opioid pharmacology consisting partial agonist activity at MOP and NOP and low partial agonist activity at KOP and DOP reported by some groups <sup>32, 33</sup> while others have determined DOP antagonism and KOP inverse agonism <sup>34</sup>. This is a multifunctional molecule.

Much of the early detailed pharmacological data characterising buprenorphine has come from studies in rodents, defining what has now become a trademark characteristic of the drug, a bell shaped dose response curve <sup>35-37</sup>. This was observed with specific antinociceptive paradigms; in the mouse tail-flick pain model the intensity of nociceptive stimuli determined whether or not a bell shaped curve to the drug manifest. When low stimulus intensity is used a ceiling effect for the antinociceptive action of buprenorphine is seen, however when a higher intensity is used a bell shape curve is apparent, with greater antinociception being observed at 1mg/kg compared with 0.3mg/kg and lower antinociception at 3mg/kg compared with 1mg/kg buprenorphine <sup>35, 36</sup>. In an extensive exploration in rodent models of acute and chronic pain, the drug had full analgesic efficacy for acute thermal and visceral pain as well as for chronic inflammatory and neuropathic pain. Interestingly for pain models using a chemical stimulus a bell shape is not seen, highlighting the nature of the painful stimulus, rather than intensity, as influential in the shape of the dose response curve <sup>37</sup>.

The ceiling effect to buprenorphine antinociceptive action is easily explained by its partial agonist activity. However the bell-shaped response curve is more complex and proposed to be the result of mixed opioid activity, resulting in activation of the NOP receptor which leads to

the suppression of MOP-mediated antinociception via the anti-opioid action of NOP receptor activation (see below), certainly in acute models of pain (**Figure 3**). If this proposal is correct then NOP receptor antagonists should potentiate the antinociception of buprenorphine. This was shown in the mouse tail-flick assay using the NOP selective antagonist J-113397 <sup>36, 38</sup>. Indeed, antagonism of the NOP receptor, or its absence in knockout mice, leads to buprenorphine having a steeper dose response curve and a full agonist profile.

It is important to recognise in analgesic development that rodents display noticeable behavioural and pharmacological differences to non-human primates, with the latter being more 'human' in their pain physiology and pharmacology. In primates, systemic buprenorphine dose-dependently produces antinociception, ventilatory depression and itch/scratching up to 0.1mg/kg <sup>33</sup>. Increasing the dose to 1mg/kg sees buprenorphine retaining its antinociceptive action without increasing the ventilatory depression, and it is this ceiling action regarding the ventilatory depression that gives the drug such a favourable therapeutic window <sup>33</sup>. This suggests that much of the complex pharmacological dose-response curve data for buprenorphine are a rodent phenomenon.

In non-human primates MOP and NOP antagonists have differing actions on buprenorphine induced responses, such that pre-treatment with a MOP antagonist leads to a large rightward shift in the buprenorphine dose-response curve for a range of physiological responses including antinociception, ventilatory depression, itch/scratching. The degree of shift is similar to that seen with the antagonism of selective MOP agonists, suggesting buprenorphine-induced antinociception, ventilatory depression, and itch/scratching in primates is from agonist activity at MOP receptors alone <sup>33</sup>. This is not unexpected given that the affinity of buprenorphine for NOP is significantly lower than at the classical opioids receptors, 77nM at NOP compared to 1.5nM at MOP in human opioid receptors expressed in Chinese Hamster Ovary cells <sup>38</sup>.

Accounting for any possible NOP receptor element in buprenorphine action, the NOP antagonist J-113397, at a dose which is capable of antagonising the actions of the NOP selective agonist Ro 64-6198 in primates, produced no shift in the dose response curves of buprenorphine. Therefore, and fundamentally different to rodent models, NOP receptors are not involved in the physiological responses to buprenorphine in primates <sup>33</sup>. Furthermore, in an interesting series of experiments a number of NOP agonists were co-administered with buprenorphine and the combined dose saw a synergistic antinociceptive response in primates.



Importantly, and unlike in rodents, NOP activation does not have an anti-opioid effect diminishing MOP induced antinociception but supports a potentiated antinociceptive action from the synergistic actions of combined activation of NOP and MOP agonists .

Activation of the NOP receptor not only modulates the analgesic activity of opiates but also the rewarding properties of a number of drugs of abuse <sup>39-41</sup>. In relation to the mixed opioid receptor activity of buprenorphine, when administered to Sardinian alcohol preferring rats at low doses there was increased alcohol consumption while at higher doses alcohol consumption was attenuated <sup>42</sup>. In the presence of the NOP antagonist UFP-101, the high dose buprenorphine reduction in alcohol consumption was absent. In these animal paradigms of reward buprenorphine is seen to act through the MOP receptor at low doses, but at higher doses there is an activation of the NOP receptor with its anti-opioid effects leading to attenuated alcohol consumption <sup>42</sup>. Combining NOP activation with MOP activation (see below) may provide an effective way to attenuate the opioid reward pathways, and may account for the reduced abuse liability of buprenorphine in man.

While buprenorphine acts with full analgesic efficacy, pharmacologically at the receptor it is a partial agonist at MOP, which could hold implications in chronic models of pain, where opioid receptor expression is likely to change. Appreciation of drug intrinsic activity (efficacy) is important; opioid receptor densities will vary from region to region, or during disease states, which could see a partial agonist acting as a full agonist for one response, say analgesia, but having a partial agonist activity for another, such as ventilatory depression. In rodent models of chronic pain, both inflammatory and neuropathic, where opioid receptor densities are likely to be unstable, buprenorphine proved a potent analgesic <sup>37</sup>. In the clinical setting buprenorphine provides analgesia paralleling that seen with full MOP agonists, and without a ceiling effect, suggesting either a high partial intrinsic efficacy for buprenorphine or a large MOP receptor density in those neuronal populations/regions leading to analgesia. However, the ceiling effect for ventilatory depression induced by buprenorphine is suggestive that the latter is true and buprenorphine is a clear partial agonist.

#### vi. Mixed MOP/NOP ligands

There is growing interest in mixed MOP/NOP ligands. This is based on a number of animal studies <sup>43</sup>. Of particular interest is an elegant study from Ko and McNaughton in primates who

show that low doses of N/OFQ and morphine that are ineffective as analgesics (and hence no side effects) alone produce marked synergism <sup>44</sup>. In this context it is also worth noting that NOP activation is not associated with ventilatory depression <sup>2</sup>. There are published data on several mixed MOP/NOP ligands including, cebranopadol <sup>45</sup>, BU08028 <sup>46</sup>, SR16435 <sup>47</sup> and AT-121 <sup>48</sup>; cebranopadol and AT-121 are described in more detail. Cebranopadol is a mixed opioid – NOP agonist, developed by Grunenthal for which there is a substantial basic profile and on-going clinical evaluation. Basic profile is of a relatively high affinity MOP and NOP agonist with partial agonism at the NOP receptor. Recent data suggests that cebranopadol is a MOP and particularly NOP G-protein biased agonist<sup>49</sup> (see below). Activation produces antinociception (in rodents) with higher potency in models of neuropathic pain; a massive advantage over current opioids. There are limited ventilatory effects and tolerance develops very slowly; days with morphine and weeks with cebranopadol <sup>45</sup>. There are 9 studies on cebranopadol that have been completed in patients with diabetic polyneuropathy, bunionectomy, chronic low back pain, osteoarthritis of the knee, and cancer <sup>6</sup> [clinicaltrial.org]. The data outline early translation from the lab to the clinic and an innovative analgesic profile with significant efficacy in neuropathic pain <sup>6,50</sup>. We have reviewed this compound recently <sup>6</sup>.

Very recently Astrea Therapeutics introduced a further mixed opioid - NOP agonist; AT-121. This ligand also displayed relatively high affinity for both MOP and NOP and a partial agonist profile at both receptors. In non-human primates this molecule produced antinociception and suppressed the reinforcing effects of oxycodone. There was no ventilatory depression, opioid induced hyperalgesia, or dependence. There are no clinical data available <sup>48</sup>.

## **B. Biased agonists**

Signalling via GPCRs is initiated by a conformational change in the receptor leading to G-protein activation. Agonist efficacy, driven by this interaction can be classified as full agonist, partial agonist, antagonist and inverse agonist <sup>51</sup>, these descriptors cover the ligands discussed in section A of this review; illustrated in **Figure 2**. A further descriptor for functional activity can be driven by ligand bias. In its simple terms biased agonism (or functional selectivity) is the ability of a particular ligand to drive one signalling pathway over another; to show bias. In order to quantify bias of the ligand the other (often experimental) sources of bias in the system such as receptor (different expression in different experimental systems) and observational bias should be removed or controlled for <sup>52</sup>. There are several methods for bias quantification, these are driven by selection of the reference unbiased comparator and the pathways under study;

these considerations are outside the scope of this review<sup>53</sup>. Much of the literature on bias comes from the cardiovascular system so we include a brief description.

#### *i. Biased agonists and the cardiovascular system*

Expression of GPCRs in the cardiovascular system is high and a wide range of cardiovascular diseases are linked to GPCRs and their signalling pathways<sup>54</sup>. Well characterised examples include Angiotensin II (AngII) Type 1 Receptor (AT1R) and  $\beta$ -adrenergic receptors, with roles in hypertension<sup>55, 56</sup>. In addition to classical GPCR-G protein signalling, functional responses can be mediated by other transducers such as  $\beta$ -arrestins and GRKs (Smith et al., 2018).  $\beta$  arrestins were initially shown to be responsible for the inhibition of  $\beta$ -adrenoceptor function. The name arrestin was coined to indicate a role in turning off receptor responsiveness<sup>57</sup>. There appears to be some advantage to driving signalling via the  $\beta$ -arrestin pathway, **Table 2**, this is the opposite in analgesic research where  $\beta$ -arrestin may support the adverse event profile. G protein biased ligand for angiotensin II causes vasoconstriction and fluid retention while  $\beta$ -arrestin biased ligand (TRV120027) reduces arterial pressure and increases cardiac contractility. Carvedilol which is a  $\beta$ 1-AR  $\beta$ -arrestin-biased ligand has a cardioprotective effect because it is believed that sustained  $\beta$ 1-AR activation is cardiotoxic in terms of increasing apoptosis, heart rate, and blood pressure via  $G_s$  signalling. Also carvedilol activates epidermal growth factor receptor (EGFR) through  $\beta$ -arrestin pathway. EGFR has a significant role in nitric oxide production and eventually induces relaxation of various vascular beds<sup>58-60</sup>.

#### *ii. Biased agonists and opioid receptors*

As noted above in **Table 1** opioid receptors couple to multiple signalling pathways and therefore have ‘pluridimensional efficacy’<sup>61</sup> and represent a target for the design of biased ligands<sup>62</sup>, **Table 2**. The proposal of targeting specific signalling transduction pathways in opioid receptors arose from an important study in 1999 by Bohn and colleagues. They blocked the function of  $\beta$ -arrestin 2 protein in mice by gene deletion (Knock out; KO). The results showed improvement in the analgesic effect of morphine and less tolerance in comparison with wild type animals; a result of effectively blocking of  $\beta$ -arrestin desensitization process<sup>63</sup>. Naloxone administration to both groups diminished the analgesic effect of morphine while naltrindole and nor-binaltorphimine administration acting at DOP and KOP respectively were ineffective. These  $\beta$ -arrestin 2 KO animals also showed reduced morphine induced constipation (animal models of GI transit) and ventilatory depression<sup>64</sup>. Bias in favour of G-protein

signalling at the expense of  $\beta$ -arrestin signalling has the potential to produce analgesia with reduced side effects, **Figure 4**.

#### *Oliceridine or TRV130*

This is the first G-protein biased ligand which has been developed by Trevena pharmaceutical company. In 2013, DeWire and colleagues found that TRV130 was superior to morphine in terms of analgesia and side effects. In vitro studies were carried out in human embryonic kidney cell (HEK) lines where TRV130 showed remarkable G-protein stimulation, a reduction of  $\beta$ -arrestin recruitment and reduced internalization of MOP receptors. Our data revealed that TRV130 is weak (partial) agonist at G-protein activation and is inactive at  $\beta$ -arrestin (Unpublished data and also a similar profile for PZM21). The results of in vivo studies showed significant improvement in antinociceptive effects in TRV130 treated rats and mice. Also they reported a decrease in ventilatory depression and constipation. These data support the idea that the recruitment of  $\beta$ -arrestin is linked to the incidence of adverse effect of opioids and also facilitate identification of selective biased ligands<sup>65, 66</sup>.

Clinical studies with TRV130 have been running since 2014 and are ongoing. Most of the clinical studies revealed that intravenous TRV130 is well tolerated and has the same or better analgesic effect compared to morphine with a prominent decrease in side effects. TRV130 has passed through Phase I, II and III trials<sup>67-70</sup>. Recently, Altarifi and colleagues show that TRV130 has sustained antinociceptive effects in repeated use and is resistant to antinociceptive tolerance, similar to previous data, while gastrointestinal function was inhibited. In addition, they found that repeated TRV130 use enhanced abuse liability similar to morphine, measured by an intracranial self-stimulation procedure (ICSS)<sup>71</sup>.

In October 2018 the FDA considered oliceridine and were not convinced by the safety data voting 8:7 against approval. Concerns were expressed by the Anesthetic and Analgesic Drug Products Advisory Committee (AADPAC) regarding how the suggested dosing strategy may not be adequate in treating moderate to severe pain and failing to demonstrate improvement over an active comparator. In relation to this, further uncertainties surrounded the possible use of higher doses, to establish adequate pain treatment and how this may lead to adverse events, QT prolongation and ventilatory depression were a suggested issue in the report. The FDA asked for more safety data.

### *PZM21*

This is another biased MOP agonist, produced by computational docking of 3000 compounds. *In vivo* and *in vitro* studies revealed that this compound has high selectivity for MOP receptors with a weak KOP antagonist action. Also, G-protein activation is similar to TRV130 along with weak  $\beta$ -arrestin recruitment, even with co expressed GRK <sup>72, 73</sup>. Hill and colleagues showed that PZM21 has low efficacy in G-protein activation and also, it depresses ventilation and induces tolerance similar to morphine in animal studies. In contrast to the previous results, PZM21 is similar to the traditional ligands in the incidence of the side effects <sup>74</sup>.

With respect to TRV130 and PZM21 it is worth exploring the pharmacology a little more carefully. If both molecules are partial agonists and if the arrestin pathway is poorly amplified then an absolute lack of efficacy could be simply explained as partial agonist behaviour rather than resorting to the ascription of a bias label. This is currently speculation and will require rigorous experimental validation.

### *Other opioid biased agonists*

*SHR9532*: Li and colleagues have made structural changes to TRV130 to produce a highly efficacious new compound SHR9352. They found that this molecule has high affinity toward MOP, compared with KOP and DOP receptors. Furthermore, it shows high selectivity toward G protein activation and less  $\beta$ -arrestin recruitment in *in vitro* and animal studies <sup>75</sup>.

*Dmt-c[D-Lys-Phe-Asp]NH<sub>2</sub>*: In contrast, Gach-Janczaka and colleagues described the cyclic peptide (Dmt-c[D-Lys-Phe-Asp]NH<sub>2</sub>) as the first MOP and DOP  $\beta$ -arrestin biased ligand, it displays and enhanced antinociceptive profile in the hot-plate test but decreased gastrointestinal transit in animals <sup>76</sup>. The improvement in analgesic effect might be attributed to simultaneous activation of both MOP and DOP receptors while GIT side effect might be attributed to  $\beta$ -arrestin recruitment.

*Mitragynine pseudoindoxyl*: A mixed a MOP agonist / DOP antagonist which has a G-protein pathway signalling bias when assayed *in vitro* (see also the section above on mixed MOP agonism and DOP antagonism). In *in vivo* mouse models the compound produced a potent antinociceptive response and importantly no reward or aversion were observed along with diminished antinociceptive tolerance, ventilatory depression and GI transit inhibition. These findings suggest this combination of MOP agonism/DOP antagonism coupled with the non-

recruitment of beta-arrestin2 may allow the antinociceptive responses to be separated from many of the common MOP agonist side effects<sup>77</sup>.

*SR-17018*: Schmid et al tested a series of MOP compounds, consisting of a piperidine core structure, in a number systems and characterised the functional selectivity between G-protein signalling and  $\beta$ -arrestin2 recruitment. The *in vivo* antinociceptive efficacies of these compounds were then compared with the ventilatory suppressant effects to determine therapeutic window i.e. best pain relief with least ventilatory depression. They showed that those compounds with  $\beta$ -arrestin2 bias, over G-protein signalling, had a reduced therapeutic window relative to morphine i.e. induction of ventilatory depression at lower doses. Importantly those compounds which had an improved and broader therapeutic window relative to morphine showed a bias for G-protein signalling relative to the recruitment of  $\beta$ -arrestin2<sup>78</sup>,<sup>79</sup>. Of the series tested, SR-17018 showed the greatest G-protein signalling bias and a high ED<sub>50</sub> for ventilatory suppression<sup>32</sup>. These findings reveal a positive association between the *in vitro* determined bias factor, the degree of difference between G-protein signalling and  $\beta$ -arrestin2 recruitment, the therapeutic window, and the ability to separate antinociception and ventilatory depression<sup>78</sup>.

## Summary

Old pharmacological doctrine dictates that high affinity highly selective MOP agonists are the perfect analgesic and in a sense this is right as MOP agonism produces analgesia. However, MOP agonism also produces the side effect profile that currently classifies (and villifies) opioids; ventilatory depression, tolerance and abuse potential. In his 2006 review Corbett<sup>80</sup> described the quest for opioids without side effects as the holy grail. He indicated this is an exciting but vain quest. Maybe, 12 years on from the Corbett review we may be making progress but by utilising interactions between opioid receptors and designing non-selective and biased ligands.

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**Acknowledgment:** The authors are grateful to Prof G. Calo of University of Ferrara for a critical review of the manuscript.

**Table-1:** Opioid receptor classification and basic characteristics.

	<b>MOP</b>	<b>DOP</b>	<b>KOP</b>	<b>NOP</b>
<b>Other terminology</b>	mu:μ	delta:δ	kappa:κ	ORL-1
<b>Evidence for subtypes</b>	No	No	No	No
<b>G-protein coupling</b>	Yes-G <sub>i</sub>	Yes-G <sub>i</sub>	Yes-G <sub>i</sub>	Yes-G <sub>i</sub>
<b>Crystal structure</b>	Yes	Yes	Yes	Yes
<b>Signalling (all)</b>	<sup>1</sup> K <sup>+</sup> channel, Ca <sup>2+</sup> channel, MAPK and inhibition of cAMP			
<b>Arrestin recruitment</b>	Yes	Yes	Yes	Yes
<b>Natural ligand</b>	<sup>2</sup> E1/2	Enkephalin	Dynorphin	N/OFQ
<b>Analgesia</b>	Yes	Yes	Yes	Yes
<b>Clinical ligand</b>	Morphine	None	<sup>3</sup> None	<sup>4</sup> None
<b><sup>5</sup>Naloxone sensitivity</b>	Yes	Yes	Yes	No

<sup>1</sup>Efflux of K<sup>+</sup> and reduced influx of Ca<sup>2+</sup>; MAPK – mitogen activated protein kinase

<sup>2</sup>E1/2 is Endomorphin-1/2; no precursor has yet been identified

<sup>3</sup>Buprenorphine as activity at KOP

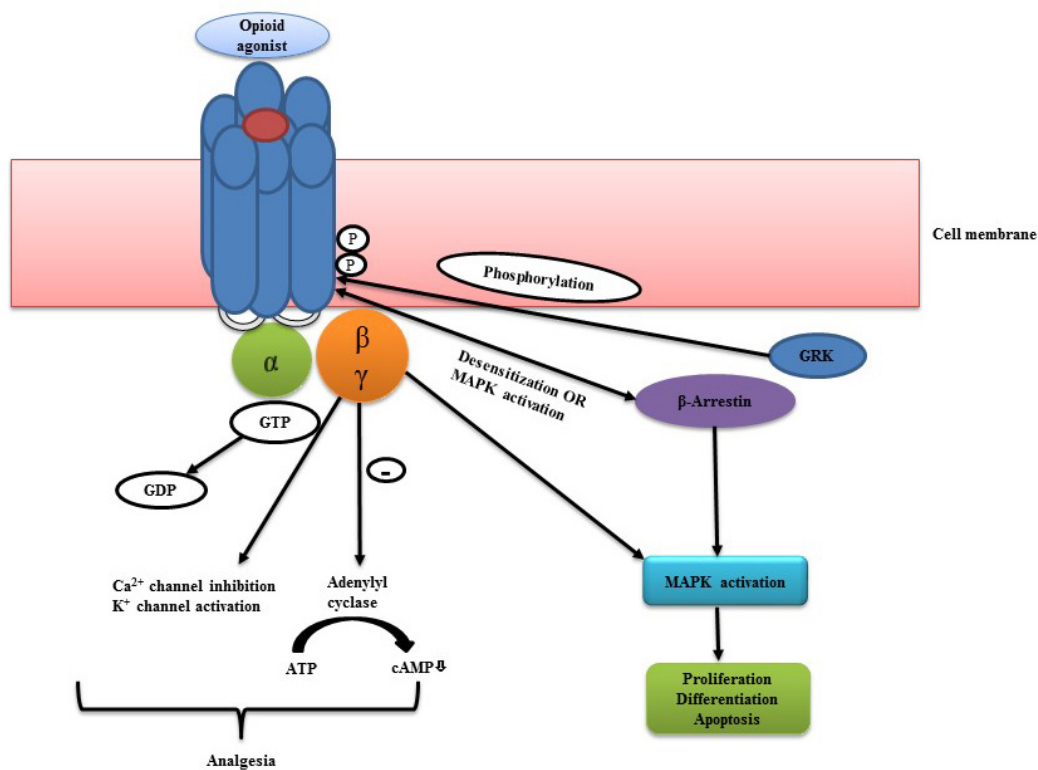
<sup>4</sup>Cebranopadol is close to the clinic

<sup>5</sup>MOP/DOP/KOP are defined as classical opioid receptors due to their naloxone sensitivity

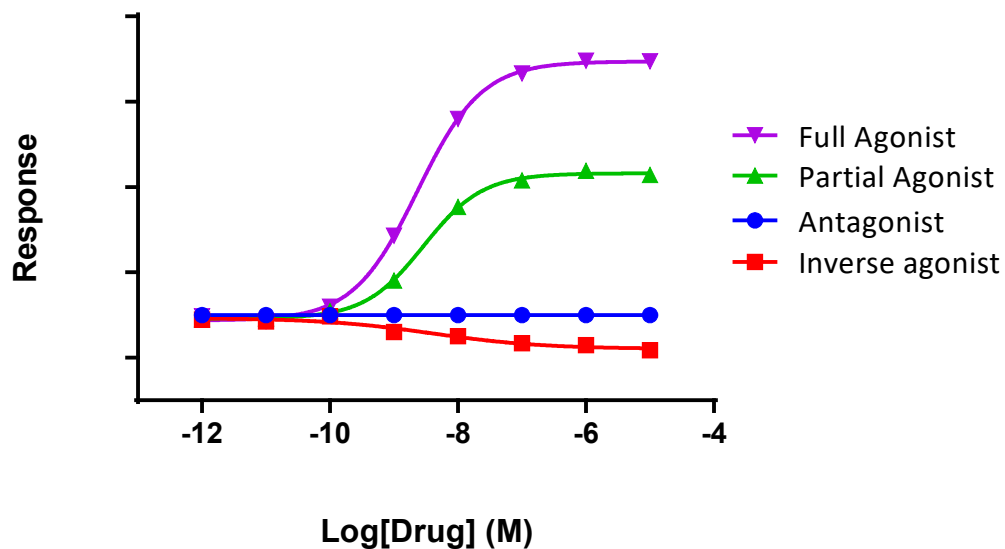
**Table-2:** Examples of biased ligands for cardiovascular and opioid receptors<sup>54, 81</sup>

<b>Ligand</b>	<b>Receptor</b>	<b>Signalling bias</b>
Oliceridine (TRV130)	$\mu$ -opioid	G protein
TRV734	$\mu$ -opioid	G protein
Triazole 1.1	$\kappa$ -opioid	G protein
RB-64	$\kappa$ -opioid	G protein
PZM21	$\mu$ -opioid	G protein
Dmt-c[D-Lys-Phe-Asp]NH <sub>2</sub>	$\mu$ and $\delta$ opioid	$\beta$ -arrestin biased
Carvedilol	$\beta_1$ -adrenergic receptor	$\beta$ -arrestin-biased
Alprenolol	$\beta_1$ -adrenergic receptor	$\beta$ -arrestin-biased
ApoM+HDL-S1P	Sphingosine 1-phosphate receptor-1 (S1P <sub>1</sub> )	$\beta$ -arrestin-biased

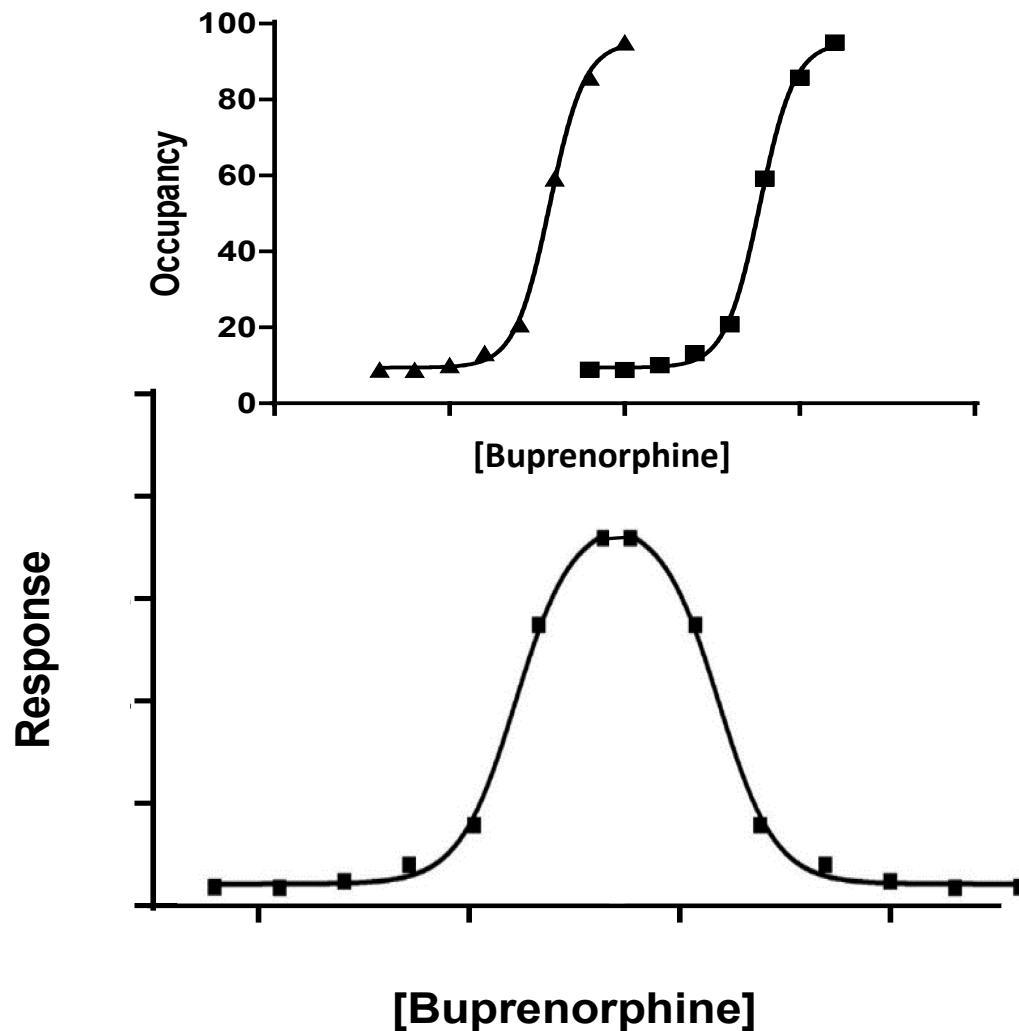
**Figure-1.** Intracellular pathways that follow agonist binding to opioid receptors. Following agonist binding to an opioid receptor conformation changes in the receptor take place and the activation (via GDP – GTP exchange at the G-protein) of different signalling pathways. *Predominantly left side of the figure:* G-protein signalling pathways, by which opioid receptors cause the dissociation of G-protein into  $\alpha$  and  $\beta/\gamma$  subunits leading to the inactivation of calcium channels, activation of potassium channels (reducing the excitability of the cell membrane), inhibition of adenylate cyclase and stimulation of mitogen associated protein kinases (MAPKs) cascades. Bias towards G-protein signalling has been linked to anti-nociceptive profile. *Predominantly right side of the figure:*  $\beta$ -arrestin signalling pathways, G-protein receptor kinases phosphorylate active G-protein coupled receptors enabling the recruitment of  $\beta$ -arrestins leading to the internalisation/desensitisation of opioid receptors **and** the activation of mitogen associated protein kinases (MAPKs) cascades. Bias towards  $\beta$ -arrestin has been linked to side effect profile.



**Figure-2:** Graphical representation of typical pharmacological 'behaviours'. A full agonist produces the maximal possible response (loosely describing efficacy) on the y-axis in arbitrary units. The green curve shows an agonist with lower maximum (efficacy) and this is a partial agonist. A neutral antagonist produces no response alone (but reverses the effects of an agonist). An inverse agonist reduces basal activity (often referred to as tone) and models an inverse sigmoid. Drug potency is depicted on the x-axis ( $\text{Log}[\text{Drug}]$ ) and a typical measure would be the concentration or dose to produce 50% of the maximal response ( $\text{EC}_{50}$  or  $\text{ED}_{50}$ ).



**Figure-3:** Graphical representation of the relationship between buprenorphine mixed opioid receptor occupancy, at MOP (upper: triangle) and NOP (upper: square), and the bell-shaped analgesic response curve in rodents. Occupancy of MOP at low concentrations of buprenorphine results in analgesia, as the concentration of buprenorphine is increased, occupancy at NOP and via the anti-opioid action results in the falling analgesic response.



**Figure-4:** Stylised diagram depicting biased agonism at the MOP receptor. Three possibilities are shown coupling to G-protein and/or arrestin. On the left is an unbiased agonist that interacts equally with both pathways and is predicted to produce analgesia and side effects. In the centre bias to the G-protein is shown and this will be predicted to show analgesia and reduced side effects. To the right the opposite bias is depicted; arrestin bias would be predicted to produce more side effects and display reduced analgesic profile.

