TECHNICAL NARRATIVE

Addiction and death from opioid abuse is "a major public health problem that is getting worse rapidly"

Opioids are the most widely prescribed drugs for treatment of moderate to severe pain. They are also the most powerful analgesics for treatment of acute and chronic pain. Whether after surgery, or on a battlefield, or simply after a root canal, opiates are prescribed in rapidly increasing numbers. In 1990 there were 78 million prescriptions written for opiate medications in the US; by 2010 this value nearly tripled to 210 million prescription [1]. However, their use is plagued by serious side effects, including abuse and addiction, constipation, respiratory depression, and death from overdose. In the US, more than 2 million people are addicted to opioids – and this number is increasing steadily [2]. In 2011, ~500,000 people were sent to the emergency room for treatment of opioid-related problems. Abuse of prescription drugs is highest among young adults aged 18 to 25, with 5.9% reporting nonmedical use in a month [2].

From a CDC report in 2011: US death rates have declined over the past ten years for all major causes of death *except for death from prescription opioid abuse*. The CDC has declared this to be "a major public health problem that is getting worse, and getting worse rapidly" [3]. A follow-up report in 2012 stated that the epidemic of overdoses of opioid pain relievers (OPRs) has continued to worsen. OPR deaths represent nearly 75% of all prescription drug overdose deaths. Drug overdose deaths have now exceeded deaths from motor vehicles as the #1 cause of accidental death in the US [4].

While alternatives to opioids exist, these are largely inadequate for treating the kinds of pain treated by opioids. As a considerable amount of pain is due to inflammation, both steroids and non-steroidal anti-inflammatory drugs (NSAIDS) attenuate the pain at its source by reducing inflammation. These types of drugs are successful for mild pain, and some powerful anti-inflammatory compounds are effective for moderate pain. They are generally safer than opiates because they lack the rewarding aspects that cause dependence and addiction. However, they are generally ineffective for severe pain, such as is incurred in combat and military training. Moreover, NSAIDS have their own side effects, leading to increased bleeding and stomach problems: the last thing a patient would want after a battlefield injury would be something that prevents clotting. This leaves opiates as the only currently acceptable option for treatment of injuries on site and they are still the most highly used treatment once removed from the battlefield, often as part of a multi-modal pain treatment plan.

Opioids remain extremely problematic because of the poor outcomes associated with their side effects. But it should be recognized that all of the leading opioid analgesics on the market today are **mu opioids** – i.e. opioids acting at the **mu opioid receptor**. The mu receptor produces euphoria, and it is that euphoric "high" that leads to abuse and addiction. Furthermore, withdrawal from mu opioids induces very severe physical and psychological symptoms that often lead to relapse. Without that euphoric high and severe withdrawal, the drugs would not be abused and would not be addicting. Mu opioids also create other serious side effects including constipation and respiratory depression. Mu-opioid side effects and the benefits of non-mu opioid analgesia are discussed in detail below.

Mu opioid receptor agonists are the current standard for opioid pain relief

Opiates treat pain by blocking the pain signal from reaching the pain centers of the brain, either at the level of the spinal cord or in the brain directly. There are four receptors in the opioid receptor family: mu (μ), delta (δ), kappa (κ), and NOP, where systemic administration of small molecule agonists of the first three receptors mediate an analgesic response [5]. Historically, most opiate analgesics have been mu agonists, due to their potent analgesic activity. These include the natural product morphine, short acting potent analgesics such as fentanyl, and long-lasting compounds such as methadone. More recently the orally active powerful mu agonists oxycodone and hydrocodone have dominated the field of prescription opiates. However, all mu agonists induce significant side effects, including euphoric reward, which leads to abuse and addiction liability, severe physical withdrawal symptoms, constipation, and respiratory depression, which can lead to death from overdose.

Other opioid receptors have been examined in detail, but have not been found to be suitable for analgesic therapy.

High affinity delta agonists, such as SNC80, have a much more restricted analgesic profile, often cause convulsions and have not yet proven successful clinically [6,7] Delta agonists, however, do have significant antidepressant activity [8], a property that could be beneficial in a compound that has activity at each of the opiate receptors. Kappa agonists have potent antinociceptive activity in many animal models however they have proven to be dysphoric and psychotomimetic in animal models and in people [9-11], and therefore have never been approved for use in man. Consequently, mu full or partial agonists, with or without some additional component (e.g. acetaminophen), are the analgesics of choice. However, as noted, these agents have the most severe side effects and are the most addicting, leading to the current epidemic of prescription drug dependence.

Mu opioid receptor agonists cause euphoria and are addictive; kappa receptor agonists cause dysphoria

Opiate pharmacology is very mature, with decades of research from pharmaceutical companies and academia. Because of the known problems with mu opiates, researchers have been attempting to produce analogs with reduced side effects for over 100 years, well before the opiate receptors were discovered or subtypes were identified. In fact, it was the pharmacological actions of opiate analogs that led Martin and colleagues to classify opiate receptors into subtypes [12].

Once opiate receptor subtypes were identified and could be examined independently for both binding affinity and functional activity, researchers attempted to modify the receptor binding profiles and efficacy of novel ligands with the hope of reducing side effects. This effort resulted in the synthesis and evaluation of "mixed agonist/antagonists", such as nalorphine (a kappa agonist and mu antagonist); mu/kappa agonists, such as nalbuphine; partial mu agonists, such as buprenorphine; and kappa agonists such as spiradoline. Each of these compounds has analgesic activity in animal models and people, and each has their own problems [13]. Table 1 shows the major beneficial and detrimental actions of mu, kappa, and delta opioid agonists.

	Analgesia	**Reward**	**GI Motility**	**Respiration**	**Activity**	**Renal**	**Withdrawal**
Mu	Potent	Euphoria	Constipating	Respiratory depressant	Species dependent	Anti-diuretic	Severe
Kappa	Potent	Dysphoria	Little effect	No effect	Sedative	Diuretic	Mild
Delta	Mild to moderate	Mild reward	Constipating	No effect	Increase	Diuretic	Unknown

Table 1: Side effect profile of mu, kappa and delta agonists

Morphine and other **mu opiates**, although potent analgesics, cause severe constipation, respiratory depression (sometimes causing death by overdose), and of course are euphoric or rewarding, leading to severe abuse liability and addiction. In addition, once dependent, withdrawal is very severe, often preventing abstinence. Mu antagonists (e.g. naltrexone or nalorphine) also induce withdrawal if the patient or addict is dependent on opiates. **Kappa agonists** (e.g. nalorphine and spiradoline) can also have potent antinociceptive activity, however they have very little effect on GI motility or respiration, making these compounds much safer than mu agonists. In addition, once dependent, the abstinence syndrome is very mild compared to mu agonists. Not only do kappa agonists not induce euphoria, in fact they induce dysphoria and often psychotomimetic activity. For this reason, kappa agonists have not been successful clinical compounds [14,15]. If one could discover a kappa agonist without the negative psychological aspects, these could be ideal analgesics.

It has long been recognized that **mu partial agonists** have reduced side effects. In fact, the partial mu agonist buprenorphine is quite a good drug. It has a ceiling effect on both GI motility and respiratory depression, thereby reducing its side effect profile and increasing its therapeutic index [16]. It also has reduced physical dependence [17,18]. Nevertheless, it has sufficient mu agonist activity to be addicting in people, and as such is a Schedule III narcotic. By analogy, a kappa partial agonist would likely have reduced side effects as well, in this case less sedation and dysphoria. Furthermore, **a kappa partial agonist with some small amount of mu efficacy might have analgesic activity with the euphoria/dysphoria balanced out due to activation of both receptors**.

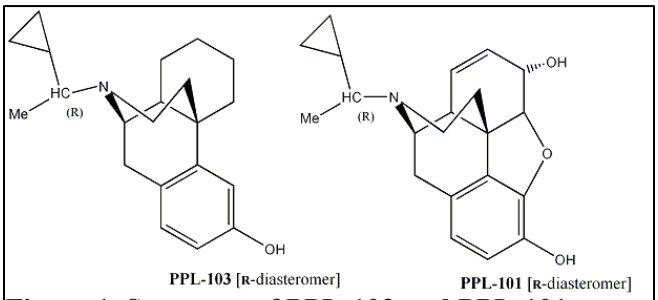


Figure 1. Structure of PPL-103 and PPL-101

Phoenix is developing a better alternative: PPL-103 – a mu/delta/kappa partial agonist/antagonist

In order to develop an opiate with reduced side effects, many investigators and pharmaceutical companies conducted structure activity relationship (SAR) studies on morphine and analogs. Early on it was determined that modification of the N-substituent of morphine could change the compound from an agonist into an antagonist. N-allyl or N-cyclopropylmethyl (N-CPM) substitution would lower efficacy at mu receptors and could lead to either agonist/antagonists such as nalorphine or antagonists such as naloxone. Several years later, when the opioid receptors were identified, it was determined that N-allyl and N-CPM also changed the binding profile, increasing binding affinity to the kappa receptor while maintaining mu binding [19].

Compound	Mu	Delta	Kappa
	Ki (nM)	Ki (nM)	Ki (nM)
DAMGO	0.88 ± 0.07	300.0 ± 58.6	305.5 ± 46
DPDPE	503.6 ± 10.0	1.59 ± 0.08	>10,000
U69593	1,145 ± 335	>10,000	1.6 ± 0.26
morphine	1.1 ± 0.05	140.0 ± 1.5	46.9 ± 14.5
buprenorphine	1.5 ± 0.8	4.5 ± 0.4	0.8 ± 0.05
PPL-101	0.35 ± 0.04	3.97 ± 1.41	0.43 ± 0.11
PPL-103	0.36 ± 0.11	2.47 ± 0.105	0.29 ± 0.03

Table 2: Opioid receptor binding affinities of PPL-103 (α-methyl-CPM-morphinan), PPL-101 (D1) and standard opioids.

	Mu		Delta		Kappa	
Compound	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation
DAMGO	13.7 ± 5.2	100	>10,000		>10,00	
DPDPE	>10,000		1.3 ± 0.5	100	>10,000	
U69593	>10,000		>10,000		78.4 ± 8.8	100
morphine	16.0±1.0	97.6±1.0	412±12	78.1±0.9	575±81	24.9±1.9
buprenorphine	2.3 ± 1.7	19 ± 05	flat		flat	
PPL-101	0.3±0.09	12.2±2.9	39.6±6.30	22.4±5.83	15.2±2.5	62.6±0.33
PPL-103	4.30±2.1	22.6±0.05	9.01±2.64	39.8±3.9	2.99±0.92	41.7±5.0

Table 3: Functional activities of PPL-103, PPL-101 and standard opioids using the [^{35}S]GTPγS binding assay

While studying the importance of the N-substituent in morphine analogs for binding affinity and functional activity, Dr. John Lawson, founder of Phoenix PharmaLabs, introduced a new chiral center in morphine analogs by inserting a methyl group onto the alpha carbon in N-CPM morphine (**Figure 1**). This insertion restricted the rotation of the N-substituent and produced two diastereomers of α-methyl-CPM morphine: D1 (now named PPL-101) and D2 [20]. It turned out that PPL-101 has a favored conformation, high affinity for mu, delta, and kappa opioid receptors (**Table 2**), very weak partial agonist activity at mu receptors, with slightly higher efficacy at delta and kappa receptors [21] (**Table 3**). It has been tested many times in mice, rats, and monkeys for antinociceptive activity, addiction liability and other side effects and has demonstrated a particularly promising profile [22-25]. However, pharmaceutical companies have not been interested in further exploring the clinical utility of PPL-101, as it is now off patent. Accordingly, we continued SAR studies and came up with a set of new compounds, including PPL-103 (α-methyl-CPM-morphinan), a very close structural analog of PPL-101. As seen in **Tables 2** and 3, PPL-103 has high affinity at each of the opioid receptors and has partial agonist activity at each receptor [21]. As presented below, this profile yields a potent analgesic (three times more potent than PPL-101 and ~10 times more potent than morphine in the tail flick assay), with greatly reduced side effects and a greatly diminished risk of abuse liability, but without dysphoria.

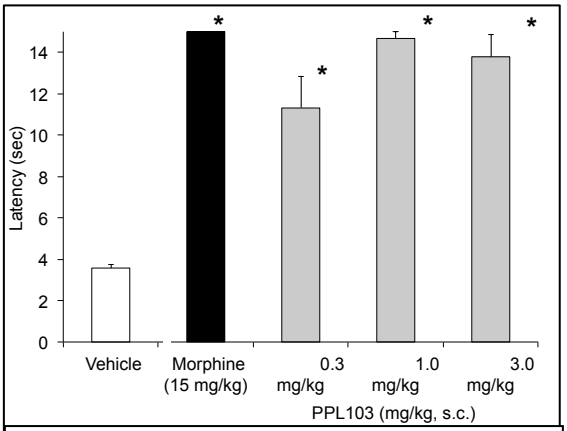


Figure 2: PPL-103 has potent anti-nociceptive activity in the tail flick assay in mice. n=10 mice per group. *p<0.05

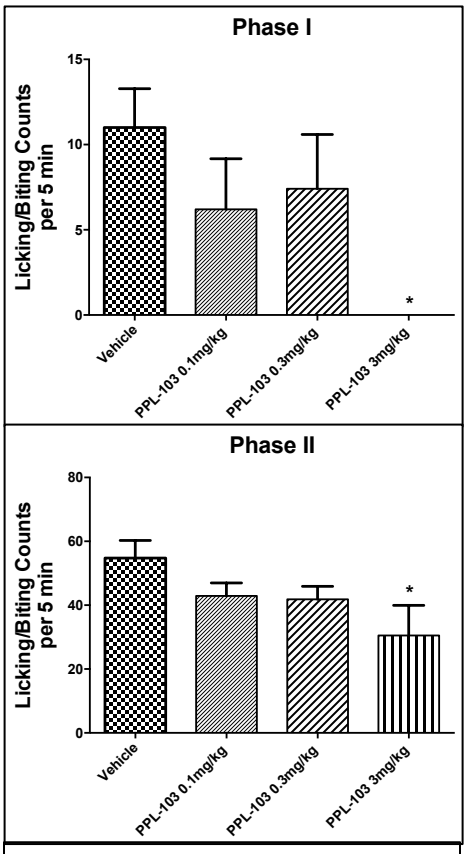


Figure 3. PPL-103 reduces pain in the formalin assay in rats. PPL-103 was administered orally. Formalin was administered to the paw 30 min after PPL-103 and painful responses were quantified over the following hour with phase I representing the first 10 min and Phase II the following 50 min.

PPL-103 is a potent analgesic with a reduced reward and side effect profile

PPL-103 has been determined to be a very potent analgesic in a **Tail Flick** test in mice, administered s.c. 20-60 minutes before application of radiant heat to the tail. It has exhibited an ED_{50} of less than 0.3 mg/kg (**Figure 2**), which is 10 times more potent than morphine (ED_{50} = 2.0 mg/kg). When tested in the presence of selective antagonists, PPL-103 antinociceptive activity was attenuated by both nor-BNI (kappa selective) and β-FNA (mu selective), indicating that analgesic activity seems to be mediated to some extent by both receptors. PPL-103 also has antinociceptive and anti-allodynic activity in other rodent models. In the formalin test, a measure of inflammatory pain, PPL-103 had an ED_{50} of approximately 3 mg/kg when administered orally (**Figure 3**). Even when administered orally, PPL-103 was almost as effective as morphine, which has an ED_{50} of 2.2 mg/kg when administered s.c. [26]. PPL-103 is also effective in chronic neuropathic pain models. As demonstrated in **Figure 4**, PPL-103 was fully effective at 3 mg/kg when

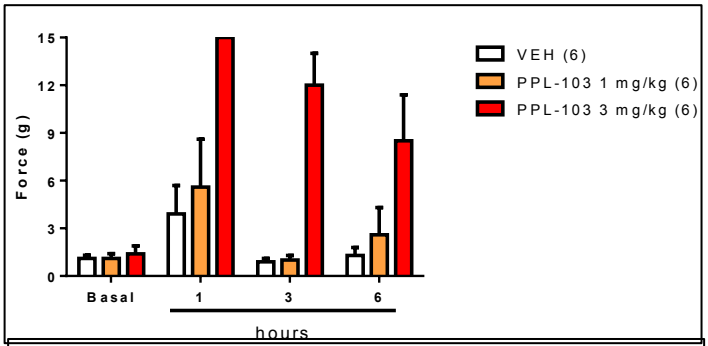


Figure 4. PPL-103 has antiallodynic activity in spinal nerve ligated rats. Von Frey filaments were used to measure allodynia in SNL rats. Measurements were taken 1, 3,and 6 h after i.p. PPL-103 administration.

inhibiting mechanical allodynia in spinal nerve ligated rats. We have demonstrated this to be equivalent to a 10mg/kg dose of morphine, in the same pain model [27]. As expected for a kappa agonist, PPL-103 was very effective in blocking pain in the acetic acid writhing test, a model of visceral pain. With an ED_{50} of approximately 0.1 mg/kg, PPL-103 was nearly 10 times more potent than morphine. (**Figure 5**).

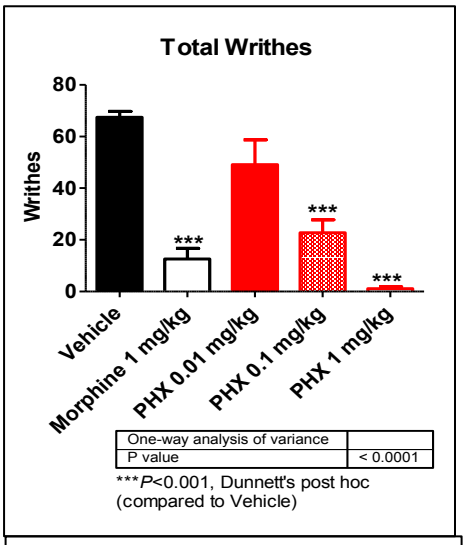


Figure 5. PPL-103 is very effective in the acetic acid writhing model of visceral pain after s.c. administration. Data shows total writhes in 20 min after i.p. injection of 0.9% acetic acid.

When tested for side effects, PPL-103 was significantly different than morphine, exhibiting the milder and more tolerable side effect profile more characteristic of kappa agonists, but without the dysphoria.

Constipation was quantified by measuring the rate that charcoal passes through the intestines. We have demonstrated that PPL-103 does not induce any decrease in the rate of charcoal transport through the intestines of mice at doses up to 50 mg/kg (250x its ED_{50} in the tail flick assay), and induces only a modest decrease at doses up to 70 mg/kg (350x its ED_{50} dose). By comparison, morphine caused a greater than 50% decrease at 10 mg/kg, only 5 times its ED_{50} in tail flick (**Figure 6**).

Respiratory Depression is one of the most severe side effects associated with morphine and other mu opioid receptor agonists; it is the leading cause of opioid overdose death. This side effect was measured at increasing doses in mice in the Comprehensive Lab Animal Monitoring System (CLAMS) for mice (n=10/group), as described in the legend to **Figure 7**. PPL-103 caused only a 25% decrease in respiratory rate at up to 30 mg/kg (150 times its tail flick ED_{50}) and was not lethal even at 70 mg/kg (350 times its ED_{50} dose) (**Figure 7**). Finally, LD_{50} was reached at about 500 times the ED_{50} dose. Similarly, in non-human primates, PPL-103 exhibited a 20 to 30% decrease in respiration rate at its ED_{50} dose but then plateaued at about that level as the dose increased. In the pharmacokinetic studies described below, monkeys did not die from overdose even at 500x its thermal analgesia ED_{50} dose.

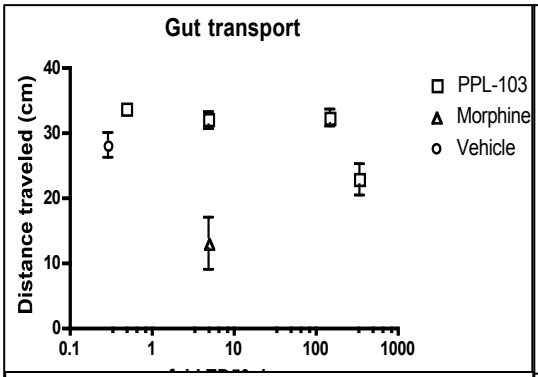


Figure 6. PPL-103 has no effect on intestinal transport. Transport was determined in mice (n=10) by measuring the distance charcoal pellets travelled in 1 h after oral administration. Results are presented in fold ED_{50} dose, based on an ED_{50} of 0.2 mg/kg for PPL-103 and 2.0 mg/kg in the tail flick assay.

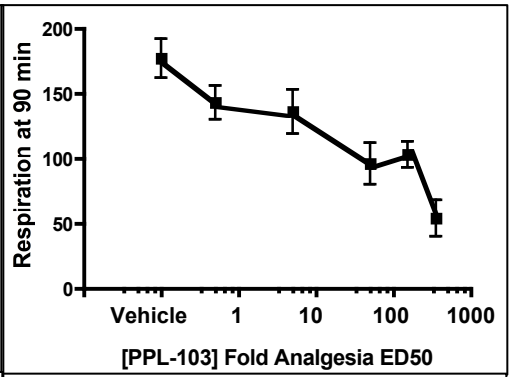


Figure 7. Effect of PPL-103 on respiration. Respiration was measured for 90 min after administration of PPL-103 in mice (n=10/group) using a CLAMS. Oxymax/CLAMS can simultaneously measure and record the following parameters: drinking volume, drinking licks, animal locomotive activity, diuresis, and respiration.

When **Locomotor Activity** was measured using CLAMS, PPL-103 induced a 50% decrease at doses of 1mg/kg, approx.-imately 5 times its analgesic dose (**Figure 8**). This is in contrast to morphine, which increases locomotor activity in mice at analgesic doses. As a decrease in locomotion is mediated by kappa receptor activity [28,29], these assays demonstrate the predominance of this receptor in PPL-103's activity and side effect profile.

PPL-103 was also tested in mice to determine whether it would induce a **Conditioned Place Preference or Aversion (CPP or CPA)**. The CPP paradigm has been used to measure the rewarding as well as the aversive properties of drugs of abuse. The CPP paradigm measures the incentive motivational properties of stimuli that become associated with drug effects through classical conditioning. The drug is administered in a distinct environment. In this case, daily injections for 6 consecutive days, 3 with drug and 3 with vehicle, while being

confined for 15 minutes to a drug-paired or vehicle-paired compartment of the CPP chamber. On the seventh day, the animals are allowed free access to both compartments and the time spent in each compartment is determined. After several pairings, the environment becomes associated with the effects of the drug, thereby acquiring incentive-motivational properties. Thus, the environment becomes a cue eliciting approach (*i.e.* CPP) or avoidance (*i.e.* CPA), depending on whether rewarding or aversive properties of the drug have been conditioned. The CPP paradigm offers several advantages including: (1) Both rewarding and aversive properties of drugs can be assessed using this procedure; (2) other behavioral measures such as locomotor activity can be assessed following acute and repeated drug administration; (3) nonspecific effects of the drug on motor and sensory systems do not influence the behavioral measure because animals are tested in a drug-free state; (4) this method allows for controlled drug doses, whereas with the self-administration paradigm the dose administered is dependent on the animal's rate of responding [30-33]. In this paradigm rewarding substances such as morphine, heroin, cocaine, nicotine, amphetamines and alcohol all induce a CPP, while aversive or dysphoric substances, such as the selective kappa agonist U50,488 induce a CPA.

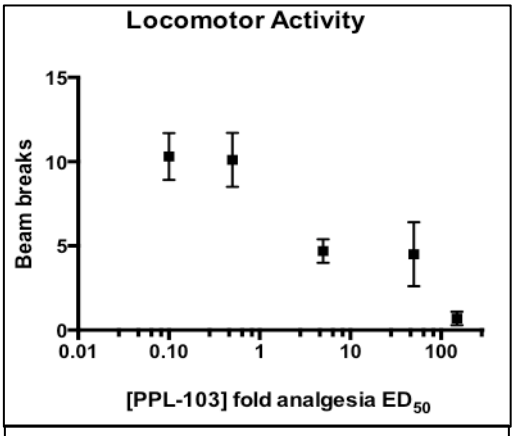


Figure 8. Effect of PPL-103 on locomotor activity in mice. Locomotor activity was determined using the CLAMS system, as described in the legend to Figure 7.

As expected for mu agonists, morphine elicited significant CPP at 15mg/kg (**Figure 9**). On the other hand, PPL-103 was expected to elicit CPA based on the apparent kappa-mediated actions described above. However, at each dose of PPL-103 tested, the treated animals spent time in the drug-paired compartment that was not significantly different than either vehicle or morphine. This result indicates that PPL-103 did not have a significant CPP in mice, though with additional numbers of mice significant differences between vehicle, PPL-103 and morphine might be determined. In any case, these studies indicate that **PPL-103 has less reward than morphine but is clearly not dysphoric like kappa agonists**.

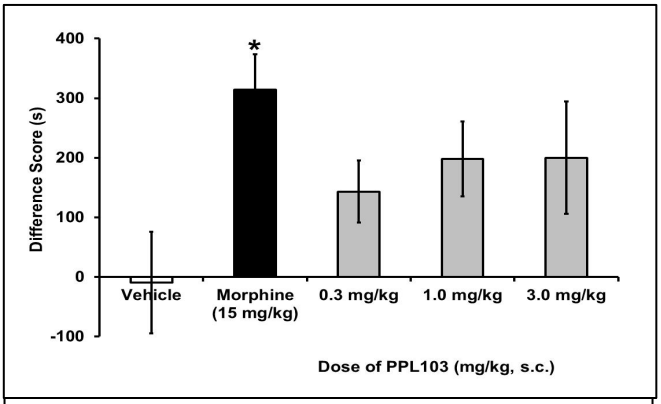


Figure 9. PPL-103 did not induce a significant CPP in mice (n=8), nor was it significantly different than morphine. Unlike morphine, PPL-103 induced a decrease in activity. *P<0.05, significantly different than vehicle control. +, significantly different than day 1 injection.

PPL-103 was also tested in the **self-administration paradigm in rats**. This assay is the gold standard for determining whether a compound is likely to be self-administered. **Research has shown that this study has a very high correlation to Human Abuse Liability (HAL) studies and other indications of the potential for abuse and addiction in humans [34].** In this experiment, rats pressed a lever which would deliver a dose of morphine through a jugular catheter using a fixed ration-1 (FR-1) schedule, meaning the rat would receive a single dose of morphine for each active lever press. After being trained to press for morphine, rats were switched to PPL-103 at 2 doses, both of which would be super-analgesic doses compared to morphine (due to the higher potency of PPL-103). As seen

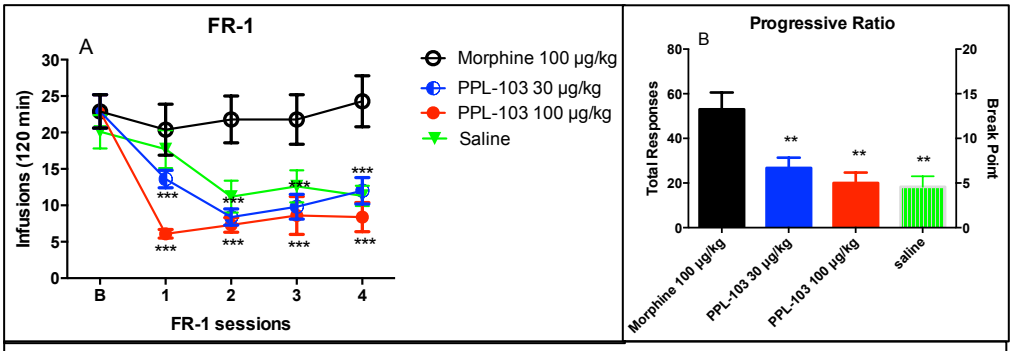


Figure 10. PPL-103 is not self-administered in rats. In both (A) Fixed and (B) Progressive ratio schedules, self-administration of PPL-103 was significantly different than morphine and similar to saline. **p<0.01, ***p<0.001

in **Figure 10**, when morphine was substituted with PPL-103, the rats did not press to a greater extent than saline. Rats were then tested on a progressive ratio schedule. In this experiment, the rats must press progressively more times to receive a reward. This measures motivation to work for the reward. In this experiment, as with the FR schedule, rats pressed for PPL-103 in a manner similar to saline. These studies clearly demonstrate that despite a trend toward reward in the CPP assay, rats were not interested in self-administering PPL-103. Based on these studies, there is a high level of confidence that PPL-103 will not be abused in people, but at the same time patients will be compliant because it is not aversive.

Finally, PPL-103 was tested in the **Single Dose Suppression assay** in monkeys (**Figure 11**). In this assay, PPL-103 was tested for its ability to block withdrawal signs in morphine-dependent rhesus monkeys n=3. In this test, PPL-103 substituted completely for morphine in doses ranging from 0.125 to 2.0 mg/kg, completely blocking morphine withdrawal for 6-9 h. It was also noted in these studies conduct ed by the Committee for Problems on Drug Dependence (CPDD) that PPL-103 induced an overt behavioral syndrome typically manifested in rhesus monkeys by selective kappa-opioid receptor agonists such as enadoline with kappa-like actions including sedation, ptosis and salivation at the higher doses. To quote the report: "Finally, it should be noted that kappa-opioid receptor agonists do not substitute for morphine in the SDS test." That is to say that PPL-103 is very unusual in that it has mostly kappa-like actions, but still can substitute for morphine in the SDS assay. Because of these unusual properties, PPL-103 also offers very promising use for addiction therapy as a preferred substitute for methadone and buprenorphine, since those drugs are, in and of themselves, addicting opiates.

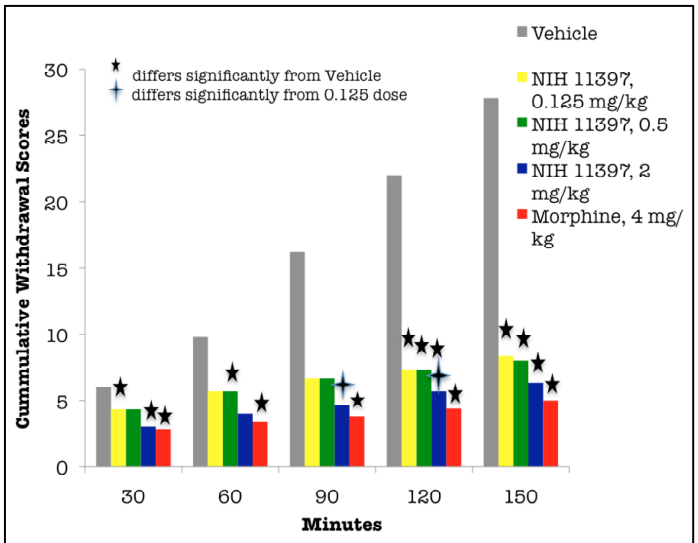


Figure 11. PPL-103 completely substitutes for morphine in the single dose suppression test in rhesus monkeys. Each dose blocked morphine withdrawal signs for 6-9 h. n=3.

PPL-103 as a cocaine abuse medication

It is known that the kappa receptor system is upregulated with chronic drug treatment, be it opiate, cocaine, or alcohol. As discussed previously, kappa receptor activation leads to stress and dysphoria. Because the kappa system is upregulated, the endogenous agonist dynorphin increases stress and anhedonia, inducing addicts to maintain drug levels to ward off withdrawal. A kappa partial agonist would be expected to reduce levels of stress to more basal levels without inhibiting the kappa system altogether and attenuate the actions of dynorphin upon withdrawal of the drug. This could be successful if the kappa agonist was not dysphoric in itself. A partial agonist would have less dysphoria, and a compound like PPL-103 that is a kappa partial agonist with a small amount of A ctivity, as we have demonstrated, is apparently not dysphoric. The value of a kappa opioid receptor partial agonist as a drug abuse medication has been proposed and explained in detail by our consultant Dr. Mary Jeanne Kreek and colleagues [36].

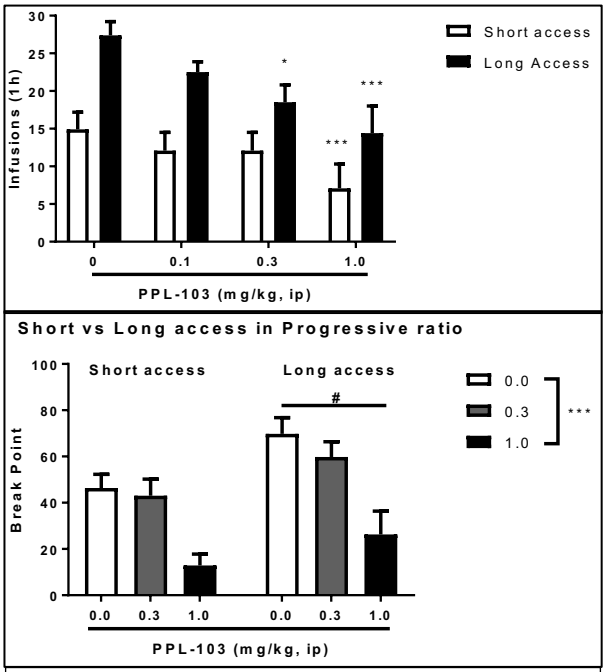


Figure 12. Effect of PPL-103 on A. Fixed ratio and B. progressive ratio cocaine self-administration. In fixed ratio and progressive ratio, PPL-103 is more effective in reducing cocaine self-administration in long-access animals. *p<0.05, ***p<0.001.

As seen in **Figure 12**, PPL-103 reduces cocaine self-administration in both fixed ratio (FR-1) and progressive ratio schedules of reinforcement. Furthermore, it works better on long access (6 h) rather than short access (1h) sessions. Long access self-administration is considered to be a model of

dependent animals. Perhaps more importantly, PPL-103, is very effective, at low doses, at blocking cocaine-prime induced reinstatement of cocaine seeking (**Figure 13**). PPL-103 was also effective in blocking cue-induced reinstatement, but it required 1.0 mg/kg for that effect (data not shown). These data clearly demonstrate that, in addition to great potential as an analgesic with very low abuse liability, PPL-103 has potential as the first cocaine abuse medication. Studies are ongoing to determine whether PPL-103 has potential as an opioid abuse medication.

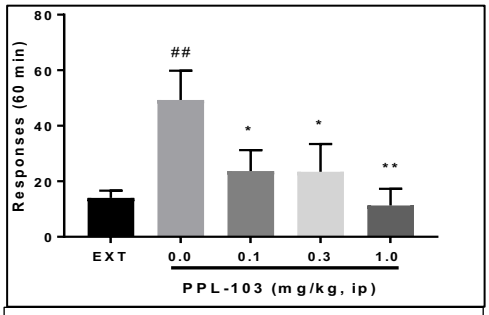
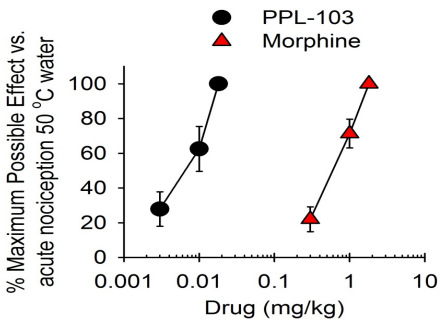


Figure 13. Effect of PPL-103 on cocaine-prime induced reinstatement. At very low doses PPL-103 blocks cocaine reinstatement. *p<0.05, **p<0.01, different than vehicle. ## p<0.01, different than extinction.



PPL-103
ED50: 0.006 mg/kg
95% CI: 0.003-0.016 mg/kg

Morphine
ED50: 0.64 mg/kg
95% CI: 0.43-0.94 mg/kg

Figure 14. Analgesic activity of PPL-103 in rhesus monkeys. PPL-103 and morphine were given subcutaneously and measured 1 h after drug administration. N=4.

Effects of PPL-103 in Non-Human Primates. Additional efficacy studies have been conducted in non-human primates (NHPs) that have been funded by our DoD grant to develop PPL-103. These studies are continuing under the direction of Dr. Mei-Chuan (Holden) Ko at Wake Forest University school of Medicine. Initial studies measured antinociceptive potency using the warm water tail withdrawal method in rhesus monkeys. These experiments determined that PPL-103 is a very potent analgesic with ED_{50} of 6 µg/kg. This is approximately 100 times more potent than morphine, which had an ED_{50} of 640 µg/kg (**Figure 14**). PPL-103 was even more potent in an inflammatory pain model, capsaicin-induced allodynia (**Figure 15**).

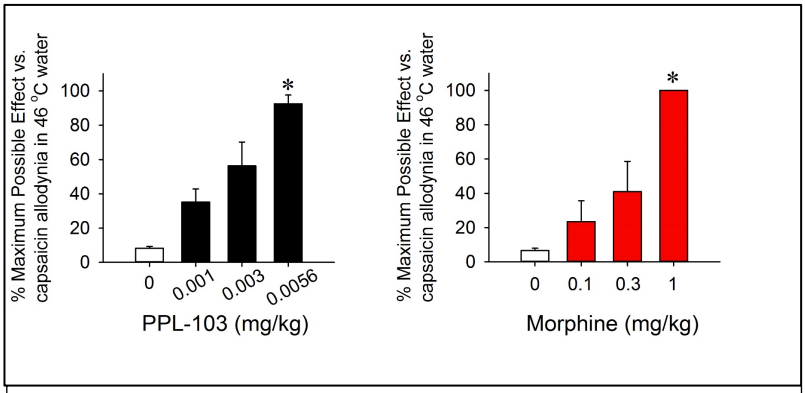


Figure 15. Effect of PPL-103 and morphine on capsaicin-induced thermal allodynia. Tail withdrawal was measured at a normally non-noxious temperature after application of capsaicin to the tail. * p<0.05 vs. vehicle n=4.

Addiction liability was also tested in NHPs. As seen in **Figure 16**, PPL-103 had a number of drug infusions that was slightly above but not significantly different than vehicle in self-administration studies conducted in the rhesus monkeys. This is in comparison to oxycodone, which was readily self-administered. This indicates that PPL-103 may have very mil d reinforcing effects. This is in contrast to most kappa agonists which are dysphoric and therefore unusable as analgesics.

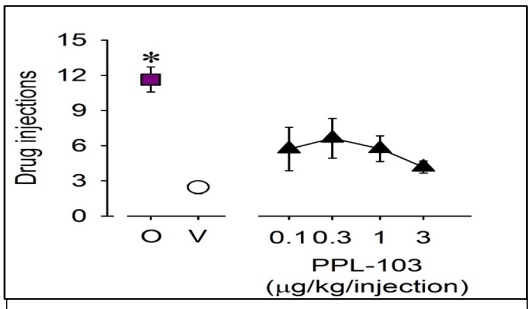
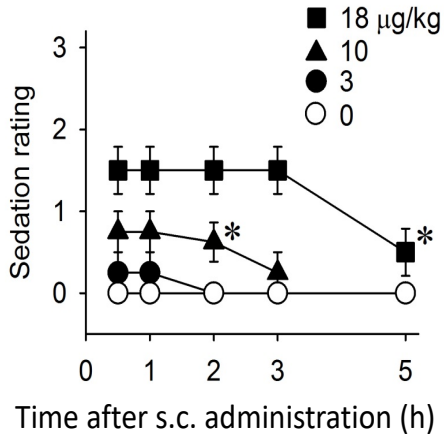


Figure 16. Self-administration studies of PPL-103 in rhesus monkeys (n=4) under a progressive-ratio schedule of reinforcement. O: Oxycodone, 3 ug/kg/inj; V: vehicle
* p < 0.05 for oxycodone vs. vehicle

There were mild side effects in the NHPs. As expected, PPL-103 produced some sedation. At the maximum analgesic dose of 18 mg/kg, PPL-103 produced sedation of approximately 1.5, on a scale of 0-6 (**Figure 17**). PPL-103 also induced a decrease in respiration in NHPs. As seen in **Figure 18,** PPL-103 caused a non-dose dependent decrease in both respiratory rate and minute volume at analgesic doses. Consistent with PPL-103 being a partial agonist, there seems to be a plateau effect whereby increasing the dose does not increase the respiratory depressant effect of PPL-103. This is also



Modified sedation rating scale

Grade	Sedation
0	No observable sedation; monkey is alert to environment
1	Monkey is attentive to ordinary movements of observer
2	Monkey responds to clapping noise in room
3	Monkey responds only to noises generated by knocking on its chair
4	Monkey responds only to clapping noise ~2 feet away from its head
5	Monkey responds only to touch
6	Monkey does not respond to touch

Figure 17 Sedation induced by PPL-103 and Sedation Scale

consisent with the marked lack of lethality of PPL-103 in both mice and NHPs. As previously discussed, in mice, there were no deaths at doses up to 350 times the analgesic EC_{50}, and in rhesus monkeys, PPL-103 did not cause death at 5 mg/kg, which is nearly 1000 times the analgesic EC_{50} value.

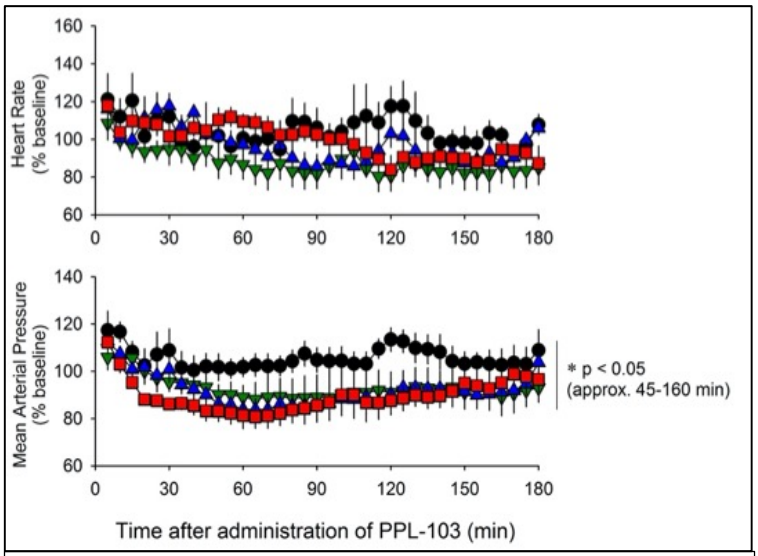


Figure 18. Respiratory effects of PPL-103 in rhesus monkeys

PPL-103 shows favorable preliminary pharmacokinetic and safety properties

Phoenix PharmaLabs has contracted additional studies to further examine some pharmacokinetic and safety aspects of PPL-103. Although unstable in rat microsomes, PPL-103 was very stable in human liver microsomes, with a half-life of 173 minutes. PPL-103 was found to have low to moderate plasma protein binding with 58.8% and 75% protein bound in rat and human plasma, respectively. It had very little inhibition of cytochrome P450 (CYP), with an IC_{50} of greater than 50µM for CYP 3A4 and 1.9µM for CYP 2D6. PPL-103 was found to be highly permeable in the CaCo2 assay (greater than10^{-6} cm/s), indicating oral availability, however it was not a substrate for P Glycoprotein, with an efflux ratio of less than 3.

Pharmacokinetic parameters were studied in rats and monkeys. PPL-103 had low oral availability in rats, probably due to high IV clearance, consistent with instability in rat liver microsomes. PPL-103 had higher oral availability in monkeys. It had a C_{max} of 70.8ng/ml (230nM), occurring from 2-8h post oral administration of 5mg/kg PPL-103. Half-life at this very high dose was approximately 8h. This high dose induced sedation and a decrease in body temperature in the monkeys. Although PPL-103 can reach high plasma concentrations after oral administration, the oral bioavailability of the API is probably not sufficient for general use without formulation. First in human studies will be conducted with i.v. administration. Later on formulations will be examined for oral, transdermal or sublingual administration. Since subcutaneous bioavailability appears to be very good, ultimate administration by transdermal patch appears to be likely.

In a preliminary safety evaluation, PPL-103 was found to be a poor inhibiter of hERG channel with an IC_{50} of 2.8 µM, indicating that, based upon the Cmax, it is unlikely to induce a cardiac event in individuals with long QT syndrome at analgesic doses.

In summary, these data suggest that: (1) PPL-103 will get into the brain after oral administration, (2) it has a long

half-life, (3) it does not inhibit two important CYP enzymes, and (4) it does not inhibit hERG until reaching high concentrations.

Impact

Taken together, these *in vitro* pharmacology and behavioral studies indicate that PPL-103 is a kappa and mu partial agonist with potent analgesic activity that appears to be mediated by both kappa and mu opioid receptors. Side effects appear to be kappa-mediated, with little to no constipation or respiratory depression. In the CPP/CPA study in mice together with the self-administration study in rats, PPL-103 demonstrated that it produces little or no euphoria, but nevertheless is not dysphoric like other kappa agonists. Despite the kappa mediated actions, PPL-103 substitutes for morphine and blocks morphine withdrawal, suggesting that it could be given to morphine-naive or morphine-dependent patients without inducing withdrawal. This combined profile is not present in any clinically available compound, and even in the literature has only been demonstrated for its close congener, PPL-101.

Based upon all of these efficacy and initial pharmacokinetic and safety evaluations, Phoenix PharmaLabs is anxious to take PPL-103 through the next steps required to initiate clinical trials. Those next steps correspond to the experiments described in this application. These will include all safety pharmacology required to file an IND and begin clinical trials as an analgesic in humans. The steps required are described in detail below. We currently have a team of experts advising us on the clinical trials required for this type of compound. After the safety toxicology is completed and an IND has been filed, we intend to move quickly into the clinical evaluation of PPL-103 for acute and chronic pain.

As discussed above, there are no other compounds with the *in vitro* and *in vivo* profile of PPL-103. We strongly believe that a compound with this profile, a partial agonist at kappa, mu, and delta receptors, with very low efficacy at mu, will act as a potent analgesic in humans without the normal opiate side effects, including constipation, respiratory depression, and addiction liability.

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