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Title:

NKTR-181: A Novel Mu-Opioid Analgesic with Inherently Low Abuse Potential

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List of Nonstandard Abbreviations

ADF, abuse-deterrent formulations
ANOVA, analysis of variance
BBB, blood-brain barrier
CHO, Chinese Hamster Ovary
CNS, central nervous system
IACUC, Institutional Animal Care and Use Committee
i.p., intraperitoneal

LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry

MOR, mu-opioid receptor

P-gp, P-glycoprotein

PK, pharmacokinetics

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ABSTRACT

The increasing availability of prescription opioid analgesics for the treatment of pain has been paralleled by an epidemic of opioid misuse, diversion, and overdose. The development of abuse-deterrent formulations (ADF) of conventional opioids such as oxycodone and morphine represents an advance in the field and has had a positive but insufficient impact, as most opioids are still prescribed in highly abusable, non-ADF forms, and abusers can tamper with ADF medications to liberate the abusable opioid within. The abuse liability of mu-opioid agonists appears to be dependent on their rapid rate of entry into the central nervous system (CNS) while analgesic activity appears to be a function of CNS exposure alone, suggesting that a new opioid agonist with an inherently low rate of influx across the blood-brain barrier could mediate analgesia with low abuse liability, regardless of formulation or route of administration. NKTR-181 is a novel, long-acting, selective mu-opioid agonist with structural properties that reduce its rate of entry across the blood-brain barrier compared with traditional mu-opioid agonists. NKTR-181 demonstrated maximum analgesic activity comparable to that of oxycodone in hot-plate latency and acetic acid writhing models. NKTR-181 was distinguishable from oxycodone by its reduced abuse potential in self-administration and progressive ratio break point models, with behavioral effects similar to those of saline, as well as reduced CNS side effects as measured by the modified Irwin test. The in vitro and in vivo studies presented here demonstrate that NKTR-181 is the first selective mu-opioid agonist to combine analgesic efficacy and reduced abuse liability through the alteration of brain-entry kinetics.
Introduction

Opioid analgesics have been used for centuries and remain one of the most effective treatments for severe pain. Drugs in this class activate specific opioid receptors in the central nervous system (CNS) and the periphery, leading to antinociceptive activity in animal models and pain relief in humans (Inturrisi, 2002; Dickenson and Kieffer, 2005; Whiteside et al., 2008). However, the use of opioid analgesics for the treatment of chronic pain remains problematic because of the narrow benefit/risk profile between analgesic efficacy and undesirable CNS adverse effects such as sedation and respiratory depression (Spetea et al., 2010). Furthermore, the euphorogenic and reinforcing effects of opioids carry with them a high risk of addiction and misuse (Melnikova, 2010). The development of a new pharmacotherapy that could take advantage of this analgesic pathway but with inherently lower abuse liability would be a major advance in the treatment of chronic pain.

There appears to be a relationship between the brain uptake rate and the abuse liability of traditional mu-opioid agonists such as oxycodone and morphine. Generally, the more rapidly a drug reaches efficacious levels in the brain to relieve pain, the more rapid is the onset of additional CNS effects experienced by the subject. This rapid onset of CNS effects can produce a feeling of euphoria, and a concomitant increase in drug likability, reinforcing behavior, and abuse liability (Farré, 1991; Marsch et al., 2001; Comer et al., 2009; Abreu et al., 2001; Webster et al., 2012; Webster and Smith, 2015).

Because the rate of mu-opioid receptor (MOR) occupancy by mu agonists depends on the rate at which these compounds cross the blood-brain barrier (BBB), strategies to slow the rate of mu-
agonist uptake by the brain may produce a beneficial cascade of effects limiting subjective feelings of euphoria, and ultimately, reducing addictive qualities (US Food Drug Admin, 2015). To date, pharmaceutical companies have concentrated on development of extended-release, abuse-deterrent formulations (ADF) that work to slow the intestinal absorption of traditional opioids, including oxycodone, hydrocodone, and morphine, into the bloodstream. However, in many instances, tampering can readily overcome ADFs. As a result, the liberated opioid enters the brain at a high rate and provides the reward sought by abusers.

Because abuse liability, but not analgesia, is dependent, at least in part, on CNS pharmacokinetics (PK), we hypothesized that separating these two effects could provide a superior solution to the problem. Our objective was to develop a next-generation opioid molecule with an inherently slow rate of influx across the BBB into the CNS that retains the pharmacodynamic activity of traditional mu-opioid agonists. This approach could yield an analgesic with low abuse potential independent of formulation and route of administration. Our efforts resulted in the discovery of NKTR-181, a novel, long-acting, and selective mu-opioid agonist with inherently low abuse potential currently in phase 3 development for the treatment of chronic pain. NKTR-181 not only demonstrated significant analgesic efficacy in patients with chronic low-back pain (manuscript in preparation), but this occurred concomitantly with reduced abuse potential in humans compared with oxycodone (Webster et al., 2017). Taken together, these clinical data suggest that NKTR-181 represents a potential paradigm shift in opioid therapy for chronic pain, providing opioid-induced analgesia with low abuse potential.
Here we report for the first time the chemical structure of NKTR-181 and compare the effects of NKTR-181 with those of traditional mu-opioid agonists, with respect to rate of brain uptake, receptor binding and function, PK, analgesic action, CNS adverse effects, and drug liking/reinforcing effects. Our preclinical findings demonstrate that NKTR-181 is a novel, selective mu-opioid agonist with analgesic efficacy and reduced abuse liability compared with traditional opioid-agonist pharmacotherapies.
Materials and Methods

Test articles. Studies were performed using NKTR-181 hydrochloride salt (Nektar proprietary compound), oxycodone hydrochloride (Sigma cat # 01378), hydrocodone bitartrate salt (Sigma cat # H4516), and morphine sulfate pentahydrate (Sigma cat # M8777). Formulations for dosing in animal studies were prepared in saline at appropriate dosing concentrations.

In situ brain perfusion. Male Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) weighing 250–300 g were maintained on a 12-hour light/dark cycle with ad libitum food and water, housed two per cage. All testing was performed in accordance with the policies and recommendations of the Institutional Animal Care and Use Committee (IACUC) of the testing facility (Absorption Systems, LP, Exton, PA). On the day of study, each rat was anesthetized intraperitoneally with a ketamine HCl/xylazine HCl solution prior to implantation of a cannula into the left carotid artery. Perfusion was performed using a single time-point method, in which perfusate-containing control compounds (5.0 μM antipyrine and 50 μM atenolol) and test compound (at 10 or 100 μM) were infused into the cannula for 30 seconds by infusion pump. After 30 seconds, the perfusion was stopped, the cerebral tissue immediately removed from the skull, cut sagitally in half and placed into a chilled tube, frozen on dry ice, and stored at −60 to −80°C until analysis. Each left hemisphere was thawed and weighed for analysis. Methanol (20%) was added to each left hemisphere at 4.0 ml/g tissue, and the mixture homogenized by sonication (VirSonic Ultrasonic Cell Disruptor 100). Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) analytical methods were used to quantify analytes.
**Caco-2 permeability.** Caco-2 monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell® plates. The permeability assay buffer for the donor chambers was Hanks balanced salt solution containing 10 mM HEPES and 15 mM glucose at a pH of 7.4. The buffer in the receiver chambers also contained 1% bovine serum albumin. The dosing solution concentration of the test compounds was 10 μM in the assay buffer. In some studies, the assay buffer and dosing solution contained 100 μM verapamil. Cell monolayers were first preincubated for 30 minutes with assay buffer to presaturate P-glycoprotein (P-gp) sites with verapamil. After 30 minutes, the buffer was removed and the cell monolayers were dosed on the apical (A-to-B) or basolateral side (B-to-A) and incubated at 37°C with 5% CO₂ in a humidified incubator. After 2 hours, aliquots were taken from the receiver and donor chambers. Each determination was performed in duplicate. To ensure that no damage was inflicted to the cell monolayers during the flux period, we also measured the Lucifer Yellow flux for each monolayer. All samples were assayed by LC-MS/MS using electrospray ionization.

The apparent permeability, \( P_{\text{app}} \), and percent recovery were calculated as follows:

\[
P_{\text{app}} = \frac{dC_r}{dt} \times \frac{V_r}{A \times C_{\Lambda}}
\]

(1)

\[
\text{Percent Recovery} = 100 \times \frac{(V_r \times C_{r \text{ final}}) + (V_d \times C_{d \text{ final}}))}{(V_d \times C_N)}
\]

(2)

Where,

\( dC_r /dt \) is the slope of the cumulative concentration in the receiver compartment versus time in \( \mu M \ s^{-1} \).

\( V_r \) is the volume of the receiver compartment in cm³.

\( V_d \) is the volume of the donor compartment in cm³.

\( A \) is the area of the cell monolayer (1.13 cm² for 12-well Transwell®).

\( C_N \) is the nominal dosing concentration of the dosing solution in \( \mu M \).
CA is the average of the nominal dosing concentration and the measured 120-minute donor concentration in µM.

C_r final is the receiver concentration in µM at the end of the incubation period.

C_d final is the concentration of the donor in µM at the end of the incubation period.

The recovery typically was between 75% and 88%. This study was conducted at Absorption Systems.

**Rodent PK.** Test articles were dosed either as a single intravenous (tail vein) bolus injection or via oral gavage to overnight-fasted rats. Male Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) weighing between 240 and 280 g were maintained on a 12-hour light/dark cycle with ad libitum food and water. All testing was performed in accordance with the policies and recommendations of the IACUC of the testing facility (SRI International, Menlo Park, CA). For each time point, a minimum of three animals received either NKTR-181 or oxycodone at the indicated dose levels. At predetermined time points, blood was collected in K₂EDTA-coated microtainer tubes. For rat brain PK, animals were exsanguinated and then perfused via the left ventricle with 60 ml ice-cold saline to remove blood from the organ. Plasma was separated from blood after centrifugation (10,000 rpm for 5 min) and kept frozen at −70º C until analysis. Cerebral tissue was removed, weighed, frozen on dry ice, and stored at −70º C until analysis. Proteins from tissue homogenates and plasma samples (100 µL each) were removed by precipitation after adding 300 µL cold acetonitrile. Samples were then centrifuged and analyzed using a qualified LC-MS/MS method in the MRM mode. Chromatographic separation was performed using an optimized isocratic composition of 0.1% formic acid in 10 mM ammonium
acetate (NH$_4$Ac) as mobile phase A and 0.2% formic acid in 10:90 water:acetonitrile as mobile phase B.

Noncompartmental analysis was conducted using PhoenixWinNonlin (version 6.4; Pharsight, Mountain View, CA) to obtain PK parameters. Nominal doses and sampling times were used. Maximum plasma concentration ($C_{\text{max}}$) and time of observed maximum plasma concentration ($T_{\text{max}}$) were derived from the input dataset. The area under plasma concentration–time curves (AUC) were calculated using linear-log trapezoidal interpolation and sparse sampling mode and uniform weighing. Terminal half-life was used for extrapolation to infinity (AUC$_{\text{inf}}$). Clearance (CL), volume of distribution at steady state (V$_{ss}$), and terminal half-life ($t_{\frac{1}{2}}$) were determined for NKTR-181 and oxycodone. Percent absolute bioavailability was estimated from the dose-normalized AUC$_{\text{inf}}$ (p.o.)/AUC$_{\text{inf}}$ (i.v.) * 100.

The rate of in vivo uptake ($K_{\text{in}}$) was estimated following an intravenous bolus injection at 10 min postdose according to equation 3:

$$K_{\text{in}} = \frac{A_{\text{brain}}}{AUC_{\text{plasma}}}$$

(3)

Where $A_{\text{brain}}$ is the amount [ng] in the brain at 10 min and AUC$_{0–10, \text{plasma}}$ (min*ng/ml) is the area under the plasma curve from $t = 0$ to 10 min postdose.

**In vitro opioid receptor binding.** Competitive displacement studies were conducted using commercially available membranes prepared from Chinese Hamster Ovary (CHO) cells heterologously expressing recombinant human mu, delta, or kappa opioid receptors (PerkinElmer, Richmond, CA). [$^3$H]Naloxone, [$^3$H]DPDPE, and [$^3$H]Diprenorphine were used as the radioligands for mu, delta, and kappa receptors, respectively. Membranes containing the receptor of interest were incubated with increasing concentrations of test compound in the
presence of a single concentration of radioligand. The fixed concentration of the radioligand used was at the reported $K_d$ for the receptor. Serial dilutions of the test compound were prepared and each concentration was tested in triplicate. Nonspecific binding was measured in the presence of excess naloxone; this value was subtracted from the total binding to yield the specific binding at each test concentration. Calculations for saturation binding, 50% inhibition (IC$_{50}$), and $K_i$ were determined using published calculations (Cheng and Prusoff, 1973; Leslie, 1987; Motulsky and Christopoulos, 2004; McKinney and Raddatz, 2006). Data were analyzed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA).

**In vitro opioid receptor function.** In vitro intrinsic efficacy was evaluated by measuring the reduction of cAMP levels (inhibition of cAMP formation) in forskolin-stimulated CHO cells that heterologously express the cloned human mu, kappa, or delta opioid receptors (Nektar Therapeutics, San Francisco, CA). The cells were pretreated with buffer containing the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine and then with forskolin and varying concentrations of morphine, oxycodone, hydrocodone, or NKTR-181, with each concentration tested in triplicate. Inhibition of forskolin-stimulated cAMP formation was measured using a cAMP HiRange homogeneous time-resolved fluorescence assay that is based on the principle of a competitive immunoassay (Cisbio, Bedford, MA; Cat. #62AM6PEC). Results were calculated from the fluorescence ratio (665 nm/620 nm). The EC$_{50}$ was calculated using XLfit4 software (IDBS, Boston, MA)

**Acetic-acid writhing test.** The acetic-acid writhing test of visceral pain in the mouse was used to assess acute antinociceptive effects. Male Swiss Webster (CD-1) mice (Charles River
Laboratories, Hollister, CA) weighing ~20 g at the start of the study were housed five per cage and maintained on a 12-hour light/dark cycle with ad libitum food and water. All testing was performed during the light cycle and in accordance with the policies and recommendations of the IACUC of the testing facility (SRI International, Menlo Park, CA). Mice received NKTR-181 or oxycodone orally at doses of 0 (saline), 1, 3, 10, 30, or 100 mg/kg ($n = 10$/dose level). One hour after administration of test articles, mice were given an intraperitoneal (i.p.) injection of 0.5% acetic acid in water (10 ml/kg) to induce writhing. Writhing included the following behaviors: contractions of the abdomen, twisting and turning of the trunk, arching of the back, and extension of the hindlimbs. Naïve animals were randomly assigned to groups on day of dose and each observer was blinded to the treatment groups. After the i.p. injection, the animals were placed in clear plastic beakers and their behavior observed. Single writhing episodes were counted during a 20-minute period immediately after the i.p. injection. Immediately after the end of the behavioral study, animals were anesthetized with isoflurane and blood samples were taken. After blood sampling, animals were euthanized by cervical dislocation. Statistical significance for dose response was evaluated using one-way analysis of variance (ANOVA), Dunnett’s post-test with respect to saline using GraphPad Prism (GraphPad Software Inc., La Jolla, CA). The ED$_{50}$ was calculated using WinNonlin 5.2.1, Model 104 (Pharsight, Mountain View, CA)

**Hot-plate latency.** Analgesic efficacy to thermal nociception was evaluated using the hot-plate latency test. Male Swiss Webster (CD-1) mice (Charles River Laboratories, Raleigh, NC) weighing 16–20 g at the start of the study were housed 10 per cage and maintained on a 12-hour light/dark cycle with ad libitum food and water. For the rat time-course study, male CD IGS rats
(Charles River Laboratories, Raleigh, NC) weighing 101–125 g were received, housed four per
cage, and maintained on a 12-hour light/dark cycle with ad libitum food and water for 1 week
before the start of the study. All testing was performed during the light cycle and in accordance
with the IACUC of the testing facility (Howard Associates, LLC, Durham, NC). Compound
(NKTR-181 or oxycodone) was administered orally and 30 minutes later we measured hot-plate
latency (time to response by licking hind paw or jumping) after placement on a 55ºC hot plate. A
saline control was included and evaluated at 1 hour postdose. The time to lick or jump was
recorded; however, if no lick or jump occurred within 30 seconds, the animal was removed and
30 seconds recorded as response latency. Testers were blinded to the treatment groups.
Immediately after the last time point, the mouse was euthanized by CO₂ inhalation and samples
collected for PK analysis (not shown). Statistical significance for dose response was evaluated
using one-way analysis of variance (ANOVA), Dunnett’s post-test with respect to saline. Two-
way repeated measures ANOVA was used to evaluate time-course response. Statistical
significance was indicated where \( P < 0.05 \) (two-tailed test). The ED₅₀ was calculated with the
lower bound at the saline mean and the upper bound at the cutoff point, 30 seconds. Data were
analyzed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA).
**Modified Irwin test.** The modified Irwin test consisted of observations of CNS-mediated behavior (Roux et al., 2005). Naïve male Swiss Webster (CD-1) mice (Charles River Laboratories, Raleigh, NC) weighing 16–18 g at the start of the study were housed 10 per cage and maintained on a 12-hour light/dark cycle with ad libitum food and water. All testing was performed during the light cycle and in accordance with the policies and recommendations of the IACUC of the testing facility (Howard Associates, LLC). NKTR-181, oxycodone, or saline was administered orally and each mouse placed immediately in the observation chamber. At each time point, undisturbed observations were made for gross signs of hyper- or hypoactivity, Straub tail (rigidity in the tail that is commonly observed in mice administered with opioid agonists), hindlimb splay, tremor, and seizure. Then, each mouse was handled for evaluation of muscle tone (rigid or not), pinna reflex (presence or absence), righting reflex (intact or lost), and placing (whether or not the forepaws were extended when the mouse was placed near the surface). On the next day, each mouse was inspected for any gross physiological abnormality, and then immediately euthanized by CO2 inhalation.
Rotarod. Locomotor coordination was evaluated using the rotarod test. Male Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) weighing between 240 and 280 g were housed two to three per cage and maintained on a 12-hour light/dark cycle with ad libitum food and water. All testing was performed during the light cycle and in accordance with the policies and recommendations of the IACUC of the testing facility (SRI International). Naïve rats were trained to run on the treadmill for 2 consecutive days before the day of study, when trained animals were placed on the rotarod and baseline scores recorded. After baseline assessment, animals were randomized into groups and received oral doses of saline, oxycodone (10–100 mg/kg), or NKTR-181 (10–300 mg/kg). Then each rat’s performance on the rotarod was evaluated 30 and 60 minutes later. The tester was blinded to the treatment groups. The rod rotation was set at a constant speed of 4 rpm for 15 seconds then set to accelerate from 4 to 40 rpm over a 5-minute period. The time (in seconds) at which each animal stopped running and fell off the rotarod was recorded as the time spent on the rotarod. Animals that ran for 300 seconds were taken off the rotarod and 300 seconds was recorded. Statistical significance was evaluated using two-way repeated measures ANOVA with Bonferroni post-test with respect to saline. Statistical significance was indicated where $P < 0.05$ (two-tailed test). Data were analyzed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA).

Abuse potential: self-administration and progressive ratio. Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) were received at approximately 7.5 weeks of age and studies were conducted at MPI Research, Inc. (Mattawan, MI). All animals were surgically implanted with chronic indwelling jugular catheters per Charles River Laboratories standard operating procedures, using specialized catheters provided by MPI Research, Inc. Animals were
housed individually and maintained on a 12-hour light/dark cycle with ad libitum food and water. One week after receipt, access to food was limited in order to maintain animals at 85% to 95% of the free-feeding weight during lever-pressing training, with supplemental feedings earned during training operant sessions, as assessed once during the study. During applicable operant sessions, animals received Dustless Precision Pellets, 45 mg, Rodent Grain-Based Diet (Bio-Serv, Flemington, NJ). All training and testing were conducted during the light cycle. Training and generalization were conducted as described in the literature (Gauvin et al., 2015). In brief, once animals were trained to respond consistently under the fixed-ratio 4 with food (four consecutive lever presses per food reward) then animals were transitioned to respond to cocaine infusion (0.56 mg/kg per infusion). Cocaine is a standard substance for initial reinforcement training in this model, and the particular history used to establish reinforcement behavior does not affect the later behavior shown in the test compounds (e.g., oxycodone and NKTR-181). Infusion training sessions were terminated after a maximum of approximately 1 hour in duration and a maximum of 10 rewards. After training, animals that consistently provided 10 responses for each cocaine infusion (FR10), with less than 20% day-to-day variation over 3 consecutive days, were used for the study.

Self-administration was evaluated during the testing and substitution sessions; the parameters reported for abuse liability indication are total number of infusions during the session. Exposures to saline or cocaine were considered test sessions, and exposures to the test compounds were considered substitution sessions. Test and substitution sessions were identical to training sessions except for the drug or drug dose that was self-administered, and no restriction was placed on the number of rewards attainable. Operant testing sessions were interspersed between cocaine
maintenance training sessions. In the substitution sessions, NKTR-181 was administered at 0.032, 0.1, 0.32, 1.0, and 3.2 mg/kg per infusion. Oxycodone was administered at 0.0032, 0.01, 0.032, and 0.1 mg/kg per infusion. All treatments were administered at a dose volume of less than 1 ml per infusion. Six animals were tested per dose level. Animals tested were exposed to each treatment condition once per day for three consecutive sessions.

The progressive ratio break points were determined by the highest number of responses emitted by the animal to receive a single drug delivery. In the progressive ratio schedule, the total number of lever-press responses required for each subsequent drug delivery was incremented using a logarithmic (base e) scale using the following equation:

\[
\text{Response requirement} = 5 \times e^{(\text{response increment} \times 0.2)} - 5
\]

After maintenance training session, a second substitution test was conducted under the progressive ratio schedule of drug delivery.

Data availability. All relevant data are available from the authors.
Results

**NKTR-181 has a reduced rate of brain entry relative to traditional opioid agonists.** We previously reported that derivatives of morphine-like (morphinan) scaffolds containing short-chain polyethylene glycol functional groups have altered oral bioavailability and reduced penetration across the BBB (Corsetti and Tack, 2015; Bui et al., 2016a; Bui et al., 2016b). We extended this work to screen a library of polymer-containing morphinan opioid agonists for their activity, bioavailability, and kinetics of BBB penetration. We selected NKTR-181 from these compounds as the first exemplar of this new class of opioid agonists with reduced influx into the CNS in vivo (Fig. 1).

Rates of brain uptake ($K_{in}$) for NKTR-181 and traditional opioids were determined by measuring relative concentrations in the brain after a 30-second, single perfusion at 10 or 100 μM in the rat (in situ brain perfusion, Table 1). Oxycodone, a readily brain-penetrant opioid, displayed consistent uptake values at both concentrations. Hydrocodone had reduced brain uptake compared with oxycodone, but was still measurable in the brain at appreciable amounts (507 ± 192 pmol/g). NKTR-181 had a reduced rate of brain entry compared with oxycodone and hydrocodone, measuring 71-fold less than oxycodone and 13-fold less than hydrocodone (Table 1).

The observed reduction in rate of brain entry for NKTR-181 compared with oxycodone in rats (Table 1) correlated with both reduced permeability and increased P-gp–mediated efflux, which we observed in experiments showing reduced flux across a Caco-2 cell monolayer (Table 2). Although verapamil 100 μM completely neutralized P-gp–mediated efflux across Caco-2
monolayers, it is noteworthy that the intrinsic permeability of NKTR-181 is approximately one order of magnitude less than that of oxycodone. Therefore, the rate of entry of NKTR-181 into the brain is expected to be considerably slower than that of oxycodone, even in the presence of physiologically attainable P-gp inhibitor plasma concentrations.

This reduced rate of brain uptake of NKTR-181 was replicated using rat PK, measuring brain concentrations after intravenous doses. $K_{in}$, determined by measuring brain concentrations over time, was more than 17-fold less for NKTR-181 than for oxycodone (0.007 versus 0.124 ml/min, respectively; Table 3). In addition, rat PK experiments showed that oral bioavailability was approximately nine-fold greater for NKTR-181 compared with oxycodone (34% versus 4%, respectively; Table 3).

**NKTR-181 has full functional activity and selectivity at the MOR.** NKTR-181 displayed higher binding affinity at the MOR than at the kappa and delta opioid receptors. At the MOR, the affinity of NKTR-181 was less than the affinities of traditional opioids (morphine and oxycodone), between 15- and 28-fold less potent. Traditional opioids exhibit full displacement of $[^3H]$naloxone as MOR agonists, and we observed the same result for NKTR-181 (Table 4).

Activation of $G_i$-coupled receptor, including opioid receptors, leads to inhibition of adenylate cyclase and decreased accumulation of cAMP (Seamon et al., 1981; Milligan and Kostenis, 2006). In forskolin-stimulated CHO cells heterologously expressing the human MOR, NKTR-181 produced a concentration-dependent inhibition of cAMP accumulation with an EC$_{50}$ value of $12.5 \pm 3.4 \mu$M (mean $\pm$ S.D.), as well as full agonist activity, with maximal efficacy at saturating
concentrations comparable to that of the other traditional mu agonists (Table 5). There was no measurable inhibition of cAMP accumulation with NKTR-181 when tested at the kappa and delta opioid receptors, at concentrations of up to 500 µM. These data demonstrate that NKTR-181 acts as a full agonist with high selectivity for MOR, and supports the hypothesis that NKTR-181 mediates its opioid-like effects in vivo by activation of endogenous MORs.

**NKTR-181 delivers full analgesic activity with durable effect.** To evaluate the centrally mediated analgesic activity via the MOR, we analyzed NKTR-181 in two typical models (writhing and hot-plate tests). As evaluated using the mouse acetic-acid writhing test, oral administration of NKTR-181 achieved complete inhibition of writhing response (as did oral administration of oxycodone), with an estimated ED$_{50}$ of 8.9 mg/kg at 1 hour post injection (Fig. 2). In the mouse hot-plate test, full analgesic efficacy was similar to that of oxycodone; maximal latency was achieved at the highest dose tested, 30 minutes after oral administration (Fig. 3).

For the time-course study, we evaluated NKTR-181 and oxycodone in mouse and rat hot-plate tests. For the mouse time-course study, data from the 30-minute time point were used to estimate ED$_{50}$ values (68 mg/kg for NKTR-181 and 2.3 mg/kg for oxycodone, Fig. 3), and hot-plate latency of that dose was measured at additional time points (0.5, 1, 2, 4, and 6 hours postdose). The response latency of NKTR-181 at 0.5-hour post dose persisted during the 6-hours test time (Fig. 4A). In addition, the effect of NKTR-181 and oxycodone was significantly different (two-way ANOVA $F[1, 9] = 31.04, P = 0.0003$). This durable analgesic effect was reproduced in the rat hot-plate test. NKTR-181 (170 mg/kg) had a significant analgesic effect up to 4 hours after a single dose (statistical significance to saline at 4 hours: $P = 0.0028$), whereas the analgesic action
of oxycodone (17 mg/kg) lasted for a maximum of 1 hour (statistical significance to saline at 1 hour: \( P = 0.0002 \); Fig. 4B).

**NKTR-181 demonstrates an acceptable CNS-mediated adverse event profile at efficacious analgesic doses.** Although mu agonists are useful in pain management, this class of drug is associated with severe adverse events, including sedation. Here we assessed CNS-mediated effects of NKTR-181 on mouse behavior using the modified Irwin test. Behavioral abnormalities were less prominent for NKTR-181 in both the number of incidents within a specific behavior and the variety of observed CNS-related behavioral responses compared with oxycodone. Incidence of Straub tail required a 300-mg/kg dose of NKTR-181—300-fold higher (158-fold higher molar equivalent dose) than oxycodone (Fig. 5).

In rat rotarod test, oxycodone showed impairment of motor coordination at 0.5 hour post dose even at the lowest dose tested, 10 mg/kg \( (P < 0.05) \) and the maximal impairment was reached at 30 mg/kg. However, NKTR-181 treatment resulted in moderate locomotor coordination defects compared with saline, at 0.5 hour post dose \( (P < 0.05) \) and at 1 hour post dose \( (P < 0.001) \) only at the highest tested dose, 300 mg/kg (Fig. 6).

**NKTR-181 demonstrates less abuse potential compared with oxycodone.** Administration of NKTR-181 showed no evidence of reinforcing behavior in rats in a well-established behavioral model of abuse potential. In an assessment of self-administration, NKTR-181 did not serve as a positive reinforcer of initiation or maintenance of lever press responding for test doses up to 1,000-fold higher than those needed to engender lever press responding by oxycodone (Fig. 7).
Direct intravenous infusions of NKTR-181 demonstrated an effect similar to that of saline, even at the highest concentrations tested, with a clear and distinct downward-staircase extinction pattern of lever-press responding on subsequent days. Despite high intravenous concentrations of NKTR-181 (>5,000 nmol/kg), the drug did not produce reinforcing effects in this assay. In contrast, oxycodone 9.1 nmol/kg maintained self-administration. The bell-shaped response observed with reduced self-administration at the highest concentration of oxycodone is common to all drugs of abuse. At the highest doses, direct motor effects of the drug begin to affect behavioral patterns of self-administration (Gauvin et al., 2015).

These lower rates of self-administration with NKTR-181 were also reflected in a reduced propensity or motivation to work increasingly harder to receive subsequent infusions of NKTR-181 in a progressive-ratio model (Fig. 8). Progressive-ratio “breakpoints” were used to determine the relative work (consecutive lever presses) an animal was willing to exert to receive a single dose of drug. This is a standard method for evaluating and comparing the relative reinforcing efficacy or hedonic valence of drugs, which directly correlates with their abuse liability (Panlilio and Goldberg, 2007). Unlike cocaine and oxycodone, for which rats were willing to perform a heightened number of lever presses to receive additional infusions, NKTR-181 displayed behavior similar to that seen with saline, even at milligram-per-kilogram doses 320-fold higher than the lower dose of oxycodone.
Discussion

Prescription opioid analgesics are an effective and necessary means of treating severe acute and chronic pain, but their abuse, misuse, and diversion have become an epidemic of national and international proportions (Manchikanti et al., 2012; Rudd et al., 2016). The speed at which traditional opioid agonists, such as oxycodone, enter the brain has a significant impact on abuse potential (Raffa et al., 2012). Current strategies to address abuse liability rely on extended-release formulations to slow the rate of systemic absorption through the gut, ultimately slowing brain absorption and reducing the impact of opioid-associated euphoria. However, once the active opioid agonist enters the circulation, any benefit achieved by the formulation is lost as the released opioid, whether from an extended- or immediate-release formulation, behaves the same: rapidly crossing the BBB and entering the CNS. Thus, extended-release formulations retain the potential to be misused when the opioid contained within them is recovered through tampering and/or introduced rapidly into the circulation by alternative routes of administration, such as insufflation or intravenous injection.

In our approach we invented a next-generation opioid agonist, which, based on its physicochemical properties, has a significantly reduced rate of flux across the BBB compared with traditional opioid-agonist medicines. The addition of a polyethylene glycol functional group to a small molecule changes not only its oral availability, but also its distribution across the BBB. This feature is demonstrated by the activity of naloxegol (Movantik; Astra Zeneca), which is effectively restricted to peripheral tissues outside of the CNS (Corsetti and Tack, 2015; Bui et al., 2016a; Bui et al., 2016b). In NKTR-181, addition of a polyethylene glycol side chain to a
morphine-like (morphinan) pharmacophore confers a slower rate of brain uptake than traditional opioids, while retaining activity as a selective mu-opioid agonist.

NKTR-181 has a 17- to 70-fold lower rate of brain entry in rats as compared with oxycodone, which is the result of a combination of reduced permeability and increased P-gp–mediated efflux at the BBB. Importantly, no chemical or physical tampering method has yet been identified that can alter the NKTR-181 molecule to an active form that has a higher rate of entry into the CNS. In fact, to date, all efforts to chemically manipulate the molecule (Cone et al., 2013) so as to remove the polymer functional group have degraded the pharmacophore, rendering it inactive as a mu-opioid agonist (data not shown). In accordance with US Food and Drug Administration guidelines, these findings were confirmed by a third-party laboratory (Drugscan, Horsham, PA).

NKTR-181 is a full MOR agonist in vitro and achieved maximum analgesic efficacy in mouse models equivalent to treatment with oxycodone. In the mouse acetic-acid writhing model, 1-hour postdose NKTR-181 had an ED$_{50}$ 1.8-fold higher (molar equivalent) compared with oxycodone, while in the hot-plate latency assay at 0.5 hour post dose, the ED$_{50}$ was 16-fold higher (molar equivalent) for NKTR-181 compared with oxycodone. The unique physicochemical properties of NKTR-181 also impart an extended PK profile in the circulation compared with oxycodone, effectively eliminating the need for an extended-release formulation for NKTR-181 with twice-daily dosing. When dosed at its 0.5 hour postdose ED$_{50}$, NKTR-181 demonstrated a prolonged analgesic effect, for up to 6 hours. This result reflects the improved oral bioavailability and extended PK profile of NKTR-181 compared with oxycodone.
Most importantly perhaps, the nonanalgesic CNS effects of NKTR-181 are relatively mild at therapeutic (analgesic) dose levels. CNS-mediated adverse effects on mouse behavior, as measured using the modified Irwin test, were not observed at doses below 100 mg/kg and were very limited even at the 300-mg/kg dose. To put this observation into perspective, NKTR-181 had an ED$_{50}$ of 68 mg/kg at 0.5 hour in the mouse hot-plate latency assay, with substantial analgesic efficacy at 2 hours post dose (the same time point at which the modified Irwin test was conducted). These findings demonstrate that NKTR-181 has an acceptable CNS adverse effect profile at efficacious analgesic doses. In comparison, oxycodone had an ED$_{50}$ of 2.3 mg/kg at 0.5 hour post dose in the hot-plate latency assay, but in the modified Irwin test, also measured at 0.5 hour post dose, oxycodone showed CNS-mediated behavioral effects starting at 1.0 mg/kg. These data indicate that NKTR-181 has an improved therapeutic ratio for CNS adverse effects in mice compared with oxycodone. Despite higher peripheral exposures of NKTR-181, inhibition of gastrointestinal transit in mice was comparable to that observed with equianalgesic doses of oxycodone.

Consistent with our hypothesis, self-administered NKTR-181 did not produce any significant reinforcing effects even at concentrations exceeding 500-fold (molar equivalents) over those concentrations of oxycodone that maintained stable self-administration behavior. This was also reflected in the progressive ratio “break points” study (measuring the relative work an animal was willing to exert to receive NKTR-181) in which NKTR-181 showed predominantly low progressive ratio break points similar to saline, even at concentrations exceeding 170-fold (molar...
equivalents) those for oxycodone. These data demonstrate that NKTR-181 has reduced abuse potential in rats compared with oxycodone.

In understanding the abuse liability of the mu-agonist class of opioid drugs, it is important to consider the critical relationship between signal transduction through the MOR, which by inhibiting GABAergic interneurons in the ventral tegmental area increases dopamine release and activity of dopamine-responsive neurons in the reward centers (e.g., nucleus accumbens) of the CNS. Although we did not measure MOR occupancy or rate of dopamine release in this study, it is reasonable to speculate from the results of our behavioral data that the inherent PK properties of NKTR-181 may reduce its downstream effects on MOR occupancy and dopamine activity relative to traditional opioids. The consequence is that there is a reduction of the abuse potential of NKTR-181 and significant overall improvement of its pharmaceutical profile compared with traditional opioids. That these effects are influenced primarily by kinetic considerations is evidenced by the equivalent maximal analgesic efficacy of NKTR-181 and oxycodone.

The data that we have presented here are from analgesic and behavioral studies that were conducted only on male animals. However, it has been reported in both animals and humans that the analgesic potency of opioids is affected by sex and gender (Kest et al, 2000). Therefore, further evaluation on female animals would need to be conducted to generalize these data for all animals (Greenspan et al., 2007). Most importantly, these preclinical data for NKTR-181 have translated well in the clinic for both men and women. Thus, phase 1 and human abuse potential studies showed slower onset of CNS effects, e.g., miosis (comparing times between peak plasma concentration and maximal pupil constriction) and minimal drug likability (Webster et al., 2017).
Furthermore, analgesic efficacy characteristic of long-acting mu-opioid agonists was recently demonstrated by NKTR-181 in a pivotal human study of chronic low-back pain (manuscript in preparation).

In summary, we have demonstrated that NKTR-181 is a long-acting, selective mu-opioid agonist with analgesic efficacy comparable to that of oxycodone, but with a significantly reduced rate of CNS uptake, independent of gut absorption rate. Significantly reduced effects on drug reinforcement and abuse potential were consistent with growing scientific evidence suggesting that rates of CNS uptake and reinforcement behavior contribute to drug addiction. NKTR-181 represents the first example of an effective opioid analgesic with substantially reduced abuse liability due to reduced rate of CNS uptake, which is entirely dependent on its inherent structural properties, rather than extended-release ADF.
Acknowledgments

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Authorship Contributions

Participated in research design: Choi, Evans, Gursahani, Pfeiffer, Gauvin, Riley, Riggs, Gogas, Doberstein.

Conducted experiments: Choi, Ali, Evans, Gursahani, Hennessy, Kim, McWeeney, Pfeiffer, Quach, Gauvin, Gogas.

Contributed new reagents or analytic tools: Pfeiffer, Riley, Riggs.

Performed data analysis: Choi, Rubas, Ali, Gursahani, Hennessy, Kim, Pfeiffer, Quach, Gauvin, Riley, Riggs, Gogas.

Wrote or contributed to the writing of the manuscript: Miyazaki, Choi, Rubas, Anand, Evans, Pfeiffer, Gauvin, Riggs, Gogas, Zalevsky, Doberstein.
References


Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem Pharmacol* **22**:3099–3108.


Footnotes

a) Unnumbered footnote providing the source of financial support.

This research was supported by Nektar Therapeutics (San Francisco, CA).

b) Unnumbered footnote providing thesis information, citation of meeting abstracts where the work was previously presented, etc.


c) The name and full address and e-mail address of person to receive reprint requests.

Reprint requests: Nektar Therapeutics, 455 Mission Bay Blvd South, San Francisco CA 94158
d) Numbered footnotes, using superscript numbers, beginning with those (if any) to authors’ names and listed in order of appearance.

¹These authors contributed equally to this manuscript.
Figure legends

Fig. 1. Chemical structure of NKTR-181.

Fig. 2. Mouse acetic acid writhing dose response for oxycodone (A) and NKTR-181 (B) at 1 hour post dose. Data represent mean ± S.E.M. values (n = 10). Statistical significance was determined using Dunnett’s post-test with respect to saline (*P < 0.05, **P < 0.01, ***P < 0.001). Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1.

Fig. 3. Increase in mouse hot-plate latency following administration of oxycodone or NKTR-181. Dose response at 30 minutes following oral administration of oxycodone (A) and NKTR-181 (B). Data represent mean ± S.E.M. values (n = 10). Statistical significance was determined using Dunnett’s post-test with respect to saline (**P < 0.01, ***P < 0.001). Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1.

Fig. 4. Time course of latency to respond following single administration of oxycodone or NKTR-181 in the mouse (A) and rat (B) hot plate test. Measurements were taken at 0.5, 1, 2, 4, and 6 hours post dose. Statistical significance was determined using two-way ANOVA, Dunnett’s post-test with respect to saline (*P < 0.05, **P < 0.01, ***P < 0.001). Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1 (n = 10/group).

Fig. 5. Modified Irwin test measuring CNS-mediated behavioral responses following oral administration of oxycodone or NKTR-181. Numbers indicate mice displaying each behavior at a respective dose (n = 2/group; green = no mice, yellow = 1 mouse, red = 2 mice).
maximal behavioral observation was averaged across behaviors. Earliest observations made were 0.5 h for oxycodone and 2 h for NKTR-181. Definitions of each category: muscle tone (rigid or not), Straub tail (tail is rigid and erect), loss pinna (absence of ear reflex), loss right (loss of righting reflex), and loss place (loss of forepaw extension when mouse is placed near a surface).

Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1. Molar conversions of doses tested: 1 (oxycodone 2.8 µmol/kg, NKTR-181 1.6 µmol/kg), 3 (oxycodone 8.5 µmol/kg, NKTR-181 4.7 µmol/kg), 10 (oxycodone 28 µmol/kg, NKTR-181 16 µmol/kg), 30 (oxycodone 85 µmol/kg, NKTR-181 47 µmol/kg), 100 (oxycodone 280 µmol/kg, NKTR-181 160 µmol/kg), and 300 mg/kg (oxycodone 850 µmol/kg, NKTR-181 470 µmol/kg).

**Fig. 6.** Effect of orally administered oxycodone (A) and NKTR-181 (B) on motor coordination in rats. Oxycodone and NKTR-181 were evaluated using the rotarod test at baseline (prior to dose) and 0.5 and 1 hour post dose. Duration of running was measured as time spent on the rotarod (seconds). Data represent mean ± S.E.M. values (n = 5). Statistical significance determined with two-way ANOVA, Bonferroni post-test with respect to saline (*P < 0.05, ***P < 0.001, ****P < 0.0001). Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1. Molar conversions of doses tested are as follows: 10 (oxycodone 28 µmol/kg, NKTR-181 16 µmol/kg), 30 (oxycodone 85 µmol/kg, NKTR-181 47 µmol/kg), 100 (oxycodone 280 µmol/kg, NKTR-181 160 µmol/kg), and 300 mg/kg (NKTR-181 470 µmol/kg); n = 10/group.

**Fig. 7.** Three-day substitution test with NKTR-181 (A) or oxycodone (B). NKTR-181 showed no positive reinforcing effects in the three-day substitution test in rats, whereas oxycodone showed increasing reinforcing effects with increasing concentrations, which was reduced at highest
concentrations. Bars represent the group means of total session infusions earned during the sequence of three-day substitution tests conducted with the indicated unit doses of NKTR-181. Each bar represents the mean of 6 rats per group. Inset graph in (A) shows stable cocaine reinforcing behavior compared with saline. Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1. Molar conversions of doses tested are as follows: 0.0032 (oxycodone 9.1 nmol/kg), 0.01 (oxycodone 28 nmol/kg), 0.032 (oxycodone 91 nmol/kg, NKTR-181 51 nmol/kg), 0.1 (oxycodone 280 nmol/kg, NKTR-181 160 nmol/kg), 0.32 (NKTR-181 510 nmol/kg), 1.0 (NKTR-181 1,582 nmol/kg), and 3.2 mg/kg (NKTR-181 5,100 nmol/kg).

**Fig. 8.** NKTR-181 displays behavior comparable to saline in the progressive ratio break point study. Doses of infusion were as follows: cocaine (COC) (0.56 mg/kg/infusion), oxycodone (OXY) (0.01 and 0.032 mg/kg/infusion), and NKTR-181 (0.032, 0.1, 0.32, 1.0, and 3.2 mg/kg/infusion). Values are expressed as mean ± S.E.M. Ratio of molar equivalent doses for NKTR-181 to oxycodone is 1.8 to 1. Molar conversions of doses tested are as follows: 0.01 (oxycodone 28 nmol/kg), 0.032 (oxycodone 91 nmol/kg, NKTR-181 51 nmol/kg), 0.1 (NKTR-181 160 nmol/kg), 0.32 (NKTR-181 510 nmol/kg), 1.0 (NKTR-181 1,582 nmol/kg), and 3.2 mg/kg (NKTR-181 5,100 nmol/kg).
Tables

TABLE 1

Rate of brain entry (K_{in}) following intravenous delivery of test article in anesthetized rats (n = 4/group), in an in situ brain perfusion model

Data are represented as means ± S.E.M.

<table>
<thead>
<tr>
<th>Test Article</th>
<th>K_{in, perfusion} (ml/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 μM</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>0.091 ± 0.034</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>0.497 ± 0.121</td>
</tr>
<tr>
<td>NKTR-181</td>
<td>0.007 ± 0.005</td>
</tr>
</tbody>
</table>

ND, not determined.
TABLE 2

Apparent permeability in Caco-2 cells with compounds tested at 10 µM in assay buffer, with or without verapamil 100 µM

Apparent permeability (Papp) and efflux ratio are reported.

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Papp A-to-B (10–6 cm/s)</th>
<th>Papp B-to-A (10–6 cm/s)</th>
<th>Efflux Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxycodone</td>
<td>32.8</td>
<td>31.1</td>
<td>1</td>
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<tr>
<td>Oxycodone + verapamil 100 µM</td>
<td>33.1</td>
<td>26.6</td>
<td>0.8</td>
</tr>
<tr>
<td>NKTR-181</td>
<td>0.87</td>
<td>8.55</td>
<td>9.8</td>
</tr>
<tr>
<td>NKTR-181 + verapamil 100 µM</td>
<td>3.07</td>
<td>2.84</td>
<td>0.8</td>
</tr>
</tbody>
</table>
TABLE 3

Plasma pharmacokinetics (data represented as means ± S.D.) following a bolus i.v. (1 mg/kg) and p.o. (5 mg/kg) administration to Sprague Dawley rats (n = 3/group)

Intravenous parameters include total clearance (CL), volume of distribution (Vss), half-life (t\(\frac{1}{2}\)), and brain uptake over time (K_in). Oral parameters include maximal plasma concentration (C_max), area under the curve (AUC), time to maximal plasma concentration (T_max), and percent oral availability (F).

<table>
<thead>
<tr>
<th>Test Article</th>
<th>i.v.</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Article</td>
<td>CL (ml/min/kg)</td>
<td>Vss (L/kg)</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>49.2 ± 7.1</td>
<td>1.72 ± 0.25</td>
</tr>
<tr>
<td>NKTR-181</td>
<td>59.3 ± 8.1</td>
<td>4.19 ± 0.70</td>
</tr>
</tbody>
</table>
TABLE 4

Receptor binding affinity of traditional mu-opioid agonists (morphine and oxycodone) and NKTR-181 at mu, delta, and kappa opioid receptors

<table>
<thead>
<tr>
<th></th>
<th>Mu (nM)</th>
<th>Delta (nM)</th>
<th>Kappa (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>8.40 ± 1.3</td>
<td>4,300 ± 500</td>
<td>118 ± 25</td>
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<tr>
<td>Oxycodone</td>
<td>16.0 ± 1.1</td>
<td>7,680 ± 1,200</td>
<td>43,000 ± 1,800</td>
</tr>
<tr>
<td>NKTR-181</td>
<td>237 ± 31</td>
<td>4,150 ± 870</td>
<td>&gt; 100,000</td>
</tr>
</tbody>
</table>
### TABLE 5

Functional activity and EC$_{50}$ for inhibition of cAMP formation of morphine, oxycodone, and NKTR-181 at mu, kappa, and delta opioid receptors

<table>
<thead>
<tr>
<th></th>
<th>Mu</th>
<th>Delta</th>
<th>Kappa</th>
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</thead>
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<tr>
<td>Morphine</td>
<td>0.0285 ± 0.006</td>
<td>ND</td>
<td>0.624 ± 0.15</td>
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<tr>
<td>Oxycodone</td>
<td>0.48 ± 0.19</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>NKTR-181</td>
<td>12.5 ± 3.4</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

ND, not determined.
Figures

Fig. 1.

*Figure 1.* Chemical structure of NKTR-181.
Fig. 2.

A

No. of writhes

Oxycodone (mg/kg, p.o.)

B

No. of writhes

NKTR-181 (mg/kg, p.o.)
Fig. 3.

A

Latency (s)

\[
\begin{array}{cccccccc}
0 & 0.03 & 0.1 & 0.3 & 1 & 3 & 10 & 17 & 100 \\
\end{array}
\]

Oxycodone (mg/kg, p.o.)

B

Latency (s)

\[
\begin{array}{cccccccc}
0 & 0.1 & 0.3 & 1 & 3 & 10 & 30 & 100 & 300 \\
\end{array}
\]

NKTR-181 (mg/kg, p.o.)
Fig. 4.

A

![Graph A]

B

![Graph B]
Fig. 5

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Oxycodone</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>NKTR-181</th>
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<tr>
<td></td>
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<td>Straub Tail</td>
<td>Loss Pinna</td>
<td>Loss Right</td>
<td>Loss Place</td>
<td>Muscle Rigid</td>
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</tbody>
</table>
Fig. 6

A

Time spent on rotarod (s)

Time Post-Dose (h)

Baseline 0.5 1.0

Saline Oxycodone 30 mg/kg Oxycodone 10 mg/kg Oxycodone 100 mg/kg

B

Time spent on rotarod (s)

Time Post-Dose (h)

Baseline 0.5 1.0

Saline NKTR-181 10 mg/kg NKTR-181 100 mg/kg NKTR-181 30 mg/kg NKTR-181 300 mg/kg
Fig. 7.
Fig. 8.