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Nonclinical Safety Assessment of PF614: a Novel TAAP Prodrug of Oxycodone for Chronic Pain Indication

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Abstract

PF614, a novel trypsin activated abuse protection (TAAP) prodrug of oxycodone, is being studied as chronic pain analgesic with extended release and abuse resistant properties. A series of nonclinical safety studies were conducted to support PF614 introduction to clinical trials. Ames assays (PF614 and its metabolites), comet assay (PF614 ≤ 50 mg/kg/day oral gavage in rats) and micronucleus assay (PF614 ≤ 175 mg/kg/day oral gavage in rats) were negative. hERG assay IC_{50} for PF614 was ≥ 300 μM. PF614 (0.1 and 10 μM) showed a low permeability in Caco-2 cells (≤1.17 × 10–6 cm/s) and was not a P-gp or BCRP substrate or inhibitor. The mean percent unbound PF614 among all concentrations in plasma ranged from 91.2 to 98.4, 79.4 to 100, and 52.9 to 79.9% in rat, dog, and human, respectively. Also, PF614 was metabolically stable in rat, dog, and human hepatocytes with no metabolites identified. Safety pharmacology study in dog indicated moderately lower heart rate at ≥ 2 mg/kg oral gavage doses. Toxicity studies of PF614 in rat and dog with daily oral doses of 25 and 18 mg/kg, respectively, for 14 Days were well tolerated with favorable safety profile supporting its further clinical evaluation.

Keywords

PF614; Prodrug; Oxycodone

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Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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1. Introduction

Oxycodone, a semisynthetic opioid derived from the alkaloid thebaine (from opioid poppy seeds) has been used for about a century for the treatment of various forms of pain (Kalso, 2005; Wightman et al., 2012). Although considered as an excellent efficacious analgesic, oxycodone eventually develops tolerance and dependence, very similar to other scheduled drugs under opioid class (Mars et al., 2014). Patients often end up consuming oxycodone for long periods of time at higher doses to achieve desired analgesic effects, and to prevent drug withdrawal symptoms, they have to consume more drug leading to its addiction and abuse (Jones et al., 2011a, b; Mars et al., 2014). Lately, the usage of oxycodone has sharply risen in the United States (Cobaugh et al., 2014). Statistics show that prescription opioids cause more than twice as many overdose deaths as heroin (Centers for Disease Control and Prevention, 2015) and oxycodone was associated with majority of opioid related deaths amid all opioid class compounds (Hirsch et al., 2014). Most importantly, current opioid formulations are susceptible to simple extraction methods and abuse by crushing of tablets (Kirkpatrick et al., 2017).

A prodrug approach was introduced by scientists in nineteenth century with pioneering work by Felix Hoffmann at Bayer in 1897, who synthesized Aspirin (acetylsalicylic acid), a synthetic prodrug of salicylic acid with anti-inflammatory, antipyretic and analgesic properties (Fuster and Sweeney, 2011). Prodrug (parent drug), an inactive bioconvertible form of active drug, needs to undergo biotransformation and/or chemical transformation in-vivo to release the active drug to produce effects (Huttunen et al., 2011; Gudin and Nalamachu, 2016).

Use of a novel trypsin activation abuse protected (TAAP™) prodrug of oxycodone, PF614, with a distinct chemical structure appears to be advantageous over conventional approach of using active form of drug (oxycodone) for reasons, such as unique mechanism of bioactivation of prodrug to active form by intestinal trypsin, abuse resistance and extended release mechanism of oxycodone. The TAAP™ prodrug design of PF614 has been demonstrated preclinically by Kirkpatrick et al., 2017. PF614 was stable when exposed in-vitro to human plasma, saliva, liver microsomes or culinary enzyme preparations (Kirkpatrick et al. 2017). Also, it was stable at room temperature for 24 hours with ≥ 90 percent drug in prodrug form. At temperature of 80 °C for 1 hour or for 24 hours (in vinegar or vodka), showed, respectively, either no conversion to oxycodone from PF614 or produced a 6 percent conversion to oxycodone. In-vitro incubation with trypsin at 37 °C converted PF614 to oxycodone with a half-life of 4 hours. PF614's exposure to the central nervous system was 83-fold lower than oxycodone and it had a 6.5-fold reduced potency as a μ-opioid agonist. Single dose PK studies in dogs showed that after oral administration of PF614 (9.1 mg/kg), oxycodone was distributed into plasma with an extended-release profile (reduced Cmax; delayed Tmax). The TAAPTM prodrug design of PF614 has been demonstrated to be bioactivated only after oral administration to oxycodone, preventing potential drug abuse, further, release of oxycodone could not get accelerated by chewing or ex-vivo extraction methods (Sellers et al., 2014; Kirkpatrick et al., 2017).

Considering above properties of PF614 as a prodrug and a novel chemical entity, we performed series of in-vitro and in-vivo nonclinical safety studies in rats and dogs to support its clinical evaluation for chronic pain indication.

2. Methods and Materials

Test Article:

PF614 is off-white solid powder with chemical purity of 99.9% by HPLC. Based on chemical structure of PF614, hydroxyl is modified at hydroxyl oxygen as described previously (International Publication Number WO 2017/070576 Al).

BioAnalytical Assay: Blood samples from the study animals were collected in K2EDTA tubes and processed to plasma for toxicokinetic analysis. PF614 and oxycodone in acidified plasma samples were extracted by protein precipitation and analyzed using liquid chromatography (LC) with tandem mass spectrometric detection (MS/MS).

Animal Care and Use: All in-vivo study procedures were in compliance with applicable animal welfare acts and were approved by the Institutional Animal Care and Use Committee (IACUC).

Statistical Analysis: Analysis of variance and pairwise comparisons were used to analyze the Absolute body weight, Body weight change, Food consumption, Electrocardiographic data, Respiratory rate, Body temperature, Continuous clinical pathology values, Terminal body weight, Organ weight data, Functional observational battery evaluations (continuous data). Levene's test was used to test for equality of variances between groups. Where Levene's test was significant ($p < 0.05$), a rank transformation (to stabilize the variances) was applied before ANOVA was conducted. Where Levene's test was not significant ($p > 0.05$), ANOVA was conducted. If the group effect of the ANOVA was significant ($p < 0.05$), Dunnett's t-test was used for pairwise comparisons between each treated and control group. If the ANOVA was not significant ($p > 0.05$), no further analyses were conducted.

2.1. Genetic Toxicology Evaluation

2.11. Ames Assay—PF614 and its fragments (PFR06112: amino acid fragment, and PFR06110: cyclic urea fragment) were evaluated for their ability to induce reverse mutations at the histidine locus in strains of Salmonella typhimurium (Salmonella; TA98, TA100, TA1535, and TA1537; University of California, Berkeley, CA), and at the tryptophan locus of Escherichia coli (E. coli) strain WP2uvrA (The National Collection of Industrial Bacteria, Torrey Research Station, Scotland, United Kingdom) in the presence or absence of an exogenous mammalian metabolic activation system (S9). Liver homogenate (S9) was prepared from male Sprague-Dawley rats injected intraperitoneally with Aroclor 1254 (200 mg/mL in corn oil) at 500 mg/kg, 5 days before sacrifice (Molecular Toxicology, Inc., Lot No. 3234 containing 37.5 mg/mL protein). A dose range finding study was performed with TA100 and WP2*uvr*A in the absence or presence of S9 at doses of 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 μg/plate to assess cytotoxicity of PF614 and its metabolites (PFR06112, and PFR06110). PF614 concentration verification of the 50 mg/mL samples

met acceptance criteria (±10%), the mean concentration result was 98.3% and the replicates were within 5.0% of their respective mean values. Considering no cytotoxicity observed with PF614 or PFR06110 except with PFR06112 (5000 μg/plate) in the absence of S9 with TA100 and WP2*uvr*A (data not shown), the mutagenicity assay was performed using same concentrations as dose range finding study. The assay design was based on ICH Guidelines S2 (R1) (ICH, 2011) and study specific procedure of the test facility.

2.12. Comet Assay—Since PF614 being a prodrug undergoes a bioactivation to oxycodone by intestinal trypsin when administered orally, its genotoxic potential in in-vivo comet assay was evaluated in liver and duodenum cells of rats. . Vehicle control (0.1 N Hydrochloric acid in reverse osmosis water) or PF614 formulations were administered for three consecutive days. Five male rats (Hsd:SD, Harlan, Frederick, MD) per group in groups 1 through 4 were dosed at a dose volume of 10 mL/kg by oral gavage for three consecutive days (Study Days 1, 2 and 3) at doses of 0, 10, 25 and 50 mg/kg/day respectively. Group 5 animals were dosed once with the positive control, ethyl methanesulfonate (EMS), approximately 3 to 4 hours prior to organs collection on Study Day 3. All animals were euthanized 3 to 4 hours after the last dose and liver and duodenum from each animal were collected. A portion of liver lobe and duodenum were used in preparation of cell suspensions and slides, which were analyzed for DNA damage. This assay was conducted according to the study-specific procedure of the test facility and available literature (Burlinson et. al., 2007; Smith et. al., 2008).

2.13. Micronucleus Assay—Micronucleus assay was included in the 14 Day Rat oral gavage GLP study to evaluate PF614 for in-vivo clastogenic activity and / or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocytes (PCEs) in CD® (SD) bone marrow. This assay was conducted according to the study-specific procedure of the test facility and available literature (Schmid, 1976). The positive control, cyclophosphamide, was used to validate the assay.

2.2. hERG Assay

PF614 was evaluated in-vitro for inhibitory effects on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for IKr, the rapidly activating delayed rectifier cardiac potassium current) (Redfern et al., 2003). The concentration-response relationship of the effect of PF614 (30 and 300 μM) on the hERG potassium channel current was evaluated at near-physiological temperature in stably transfected Human Embryonic Kidney Cells that express the hERG gene. This assay was conducted according to the study-specific procedure of the test facility and available literature (Kirsch et al., 2004).

2.3. P-gp and BCRP Transporters Assay

PF614 was evaluated as a potential substrate and or an inhibitor of human efflux transporters P-gp and BCRP when tested at concentrations of 0.1 and 10 μM using Caco-2 cells. This assay was conducted based on the study-specific procedure of the test facility and published literature (Fenner et al., 2009; Chimezie et al., 2016).

2.4. Plasma Protein Binding Assay in Rat, Dog, and Human

This in-vitro study was performed to determine the extent of binding of PF614 to rat, dog, and human plasma proteins. The protein binding of PF614 in rat, dog, and human plasma was determined by equilibrium dialysis for 8 hours at 37°C under an atmosphere of saturated humidity and 5% CO_2 at concentrations of 15, 100, 1000, 3000, and 10000 ng/mL. The assay was conducted based on the study-specific procedure of the test facility and published literature (Clarke et al., 2008).

2.5. Hepatocytes Metabolism Assay in Rat, Dog, and Human

The objective of this study was to determine the metabolic stability and metabolite profile of PF614 in-vitro using rat, dog, and human hepatocytes. Hepatocyte incubations were performed using viable and metabolically competent cryopreserved hepatocytes (750,000 viable cells per mL and 375,000 viable cells per well). PF614 (1 μM) was incubated with rat, dog, and human hepatocytes at 37°C in an atmosphere of 5% CO_2 for 0, 30, 60, and 120 minutes. In addition, PF614 (10 μM) was incubated with rat, dog, and human hepatocytes for 120 minutes. Samples were vortex mixed, stored on ice, and centrifuged at 1400 × g for 10 minutes at 4°C. Supernatants were removed from the hepatocytes pellets and stored in new tubes at approximately −20°C until analysis. Samples generated from incubations with PF614 (1 and 10 μM) were transferred to a 96-well plate and analyzed using the quantitative LC-MS/MS method. All incubations were conducted in triplicate. Metabolic activities of cryopreserved hepatocytes were verified by measuring 7-hydroxycoumarin, 7-hydroxycoumarin glucuronide, and 7-hydroxycoumarin sulfate. The assay was conducted based on the study specific procedure of the test facility and published literature (Zhang et al., 2016).

2.6. Rat 14 Day GLP Study

This study was performed in accordance with guidance on nonclinical safety studies (ICH/CDER, 2010). Rats (Crl:CD[SD], Charles River Laboratories, Raleigh, North Carolina) were administered once daily for 14 days via oral gavage at a volume of 10 mL/kg followed by 2 week recovery period. Study design (Table 1) included Group 1 (10 animals/sex in toxicity subgroup and 3 animals/sex in toxicokinetic subgroup) that received vehicle control (0.1 N hydrochloric acid in reverse osmosis water) only. Groups 2 through 4 (10 animals/sex in toxicity subgroup and 9 animals/sex in toxicokinetic subgroup) received PF614 in vehicle control at doses 10, 40 and 175 mg/kg, respectively. The doses were selected based on range finding study (results not provided). In Group 4, on Day 1, all females (19) in this group were found dead or were sacrificed due to PF614-associated moribundity. Males were on dosing holiday on Day 2 of the dosing phase. Beginning on Day 3 and continuing through the remainder of the dosing phase, surviving males were dosed once daily at dose 50 mg/kg. Group 7 (5 females in toxicity subgroup) was added to the study on Day 3 and received PF614 once daily at reduced dose of 50 mg/kg, beginning on Day 3 and continuing through the remainder of the dosing phase. In Group 3, animals were given dosing holiday on Day 2 of the dosing phase due to mortality. Beginning on Day 3 and continuing through the remainder of the dosing phase, animals were dosed once daily at reduced dose of 25 mg/kg. Vehicle control administered to Group 1 was prepared to match the vehicle composition for

Group 4 and Group 7. Toxicity animals in Groups 1 through 4 designated for recovery sacrifice (up to 5 animals/sex/group) underwent 2 weeks of recovery following the dosing phase. Blood samples were collected for toxicokinetic evaluations of PF614 and its metabolite oxycodone on days 1 and 14. Assessment of toxicity was based on mortality/moribundity, clinical observations, body weights, food consumption, ophthalmic examinations, functional observational battery (FOB) during predose and last week of dosing, plethysmography in last week of dosing, and clinical (on days 2, 7, 15 of dosing phase and day 15 of recovery phase) and anatomic (on day 15 terminal sacrifice in dosing phase and on day 15 recovery sacrifice in recovery phase) pathology. The FOB including body temperature, were conducted on five animals/sex/group in Groups 1 through 4 during the pre-dose and Week 2 of the dosing phases. Each animal was evaluated during handling (hand-held observations) and in an open field (open field observations), and were assessed for sensory reactivity to stimuli (elicited behaviors).

2.7. Dog 14 Day GLP Study

This study was performed in accordance with guidance on nonclinical safety studies (ICH/CDER, 2010). Purebred beagle dogs (Covance Research Products, Cumberland, Virginia) were administered once daily for 14 days via oral gavage at a volume of 5 mL/kg followed by a 2 week recovery period. Study design (Table 2) included Group 1 that received vehicle control (0.1 N hydrochloric acid in reverse osmosis water) only. Groups 2 through 4 received PF614 in vehicle control at doses 2, 6 and 18 mg/kg, respectively. Vehicle control administered to Group 1 was prepared to match the vehicle composition for Group 4. Animals designated for recovery sacrifice (up to two animals/sex/group) underwent 2 weeks of recovery following the dosing phase. Assessment of toxicity was based on mortality/moribundity, clinical observations, body weight, food consumption, body temperature, ophthalmic examination, respiratory assessment, electrocardiogram (ECG) examinations during predose, dosing and recovery phases, and clinical (on days 2, 14 of dosing phase and day 14 of recovery phase) and anatomic (on day 15 terminal sacrifice in dosing phase and on day 15 recovery sacrifice in recovery phase) pathology. Blood samples were collected for toxicokinetic evaluations of PF614 and its metabolite, oxycodone on days 1 and 14.

2.8. Dog Cardiovascular Safety Pharmacology Study

This study was performed in accordance with Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, 2001, guidance for Industry: S7 A Safety Pharmacology Studies for Human Pharmaceuticals, to evaluate the potential cardiovascular effects of PF614 in telemetered animals by oral gavage. Four naïve male and four naïve female beagle dogs (Covance Research Products, Cumberland, Virginia) that had been surgically implanted with an electrocardiogram (ECG), blood pressure and temperature transmitter and allowed at least 40 days of recovery were given four doses via oral gavage of the vehicle control (0.1 N hydrochloric acid in reverse osmosis water) or 2, 6, or 18 mg/kg of PF614 at a dose volume of 5 mL/kg in a Latin square dosing design (Table 3). Assessment of cardiovascular function was based on qualitative and quantitative (PR, QT, and heart-rate corrected QT [QTc] intervals and QRS duration) ECG evaluation, as well as quantitative analysis of hemodynamic parameters (heart rate; arterial pulse pressure; and systolic, diastolic, and mean arterial pressures). Telemetry data were

continuously recorded for at least 90 minutes prior to dosing and through at least 49 hours postdose on each dosing day, and were analyzed and reported through 19 hours postdose. Assessment of general health of the dogs was based on mortality/moribundity, clinical observations, body weight, and body temperature.

3. Results

3.1. Genetic Toxicology Assays

3.11. Ames Assay—PF614, PFR06112 (amino acid fragment), and PFR06110 (cyclic urea fragment) did not produce positive increases in reverse mutations at doses of 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 μg/plate in any of the tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A in the presence or absence of S9 (triplicate plates per dose). All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met. These results indicated that PF614, PFR06112, and PFR06110 were negative in the Bacterial Reverse Mutation Assay.

3.12. Comet Assay—PF614 treated animals did not show significant increases in the % Tail DNA compared to the respective vehicle control (ANOVA, $p \leq 0.05$, Dunnett's post hoc analysis). The vehicle control % Tail DNA was within historical range and positive control had a statistically significant increase in % Tail DNA compared to the vehicle control indicating all criteria for a valid assay were met for liver and duodenum. Under the conditions of this study, PF614 via oral gavage, up to 50 mg/kg/day did not cause a significant increase in DNA damage in liver and duodenum relative to the concurrent vehicle control. Therefore, PF614 was concluded to be negative in the in-vivo comet assay (Table 4).

3.13. Micronucleus Assay—PF614 did not induce statistically significant ($p < 0.05$) increases in micronucleated PCEs at any dose examined (10, 40/25, and 175/50 mg/kg/day) in male or female animals. In addition, PF614 was not cytotoxic to the bone marrow (i.e. no statistically significant decreases in the PCE: NCE ratios) at any dose. Thus, PF614 was evaluated as negative in the rat bone marrow micronucleus assay (Table 5).

3.2. hERG Assay

PF614 inhibited hERG current by (Mean ± SEM) 3.6 ± 0.6% at 30 μM (n = 3) and 4.8 ± 0.1% at 300 μM (n = 3) versus 1.1 ± 0.4% (n = 3) in vehicle control. The IC_{50} for the inhibitory effect of PF614 on hERG potassium current was estimated to be greater than 300 μM. Even though the amount of inhibition observed was small, the hERG inhibition at all concentrations tested was statistically significant ($P < 0.05$) when compared to study specific vehicle control values. The mean inhibition observed at all concentrations was similar to the inhibition of comparable vehicle data, being within two standard deviations of this data, (Mean ± SD) 2.3% ± 1.3% (n = 15). The inhibition observed at all concentrations was very low compared to the inhibition of historical data for Terfenadine (positive control), (Mean ± SD) 80.5 ± 5.5% (n = 2279). Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by (Mean ± SD; n = 2) 84.1 ± 1.7% confirming the sensitivity of the test system to hERG inhibition.

3.3. P-gp and BCRP Transporters Assay

In this study, Caco-2 cells grown on 24-well transwell plates were used as the experimental model. PF614 was assessed at concentrations of 0.1 and 10 μM. It was found to have low apparent permeability in Caco-2 cells (≤1.17 × 10–6 cm/s) and was not a substrate or inhibitor of P-gp or BCRP transporter (Table 6).

3.4. Plasma Protein Binding Assay in Rat, Dog, and Human

PF614 was poorly bound to plasma proteins of rat, dog, and human. There was no apparent concentration dependence with respect to plasma protein binding in range of 15 to 10000 ng/mL PF614 in any species. The mean percent unbound PF614 among all concentrations in plasma ranged from 91.2 to 98.4, 79.4 to 100, and 52.9 to 79.9% in rat, dog, and human, respectively. Unbound percent PF614 was lower in human plasma than in rat and dog plasma (Table 7).

3.5. Hepatocytes Metabolism Assay in Rat, Dog, and Human

PF614 was found to be metabolically stable under the incubation conditions for 120 minutes in cryopreserved rat, dog, and human hepatocytes and no metabolites were identified (data not shown).

3.6. Rat 14 Day GLP Study

Exposure to PF614 and oxycodone increased in a dose dependent manner, however, the increases in peak concentration (C_{max}) and area under the concentration time curve ($AUC_{0–24}$) values were not consistently dose proportional (Table 8). Sex differences in PF614 C_{max} and $AUC_{0–24}$ values were generally less than 2-fold. Sex differences in oxycodone C_{max} and $AUC_{0–24}$ values were generally greater than 2-fold with females showing a higher exposure. No accumulation of PF614 or oxycodone for Group 2 (10 mg/kg/day) was observed after multiple dosing of PF614 in rats (due to a reduction in dose, Group 3 and 4 accumulation ratios were not reported). The oxycodone to PF614 ratios for the mean C_{max} and AUC0–24 ranged from 0.122 to 0.828 and from 0.847 to 8.78 respectively. PF614 was extensively converted to oxycodone in rats following oral gavage administration. Administration of PF614 resulted in several mortalities in group 4 (175 mg/kg/day). On Day 1, four males and all females (19) in group 4 were found dead or were sacrificed due to PF614 related moribundity. One male given 40 mg/kg/day and one female given 50 mg/kg/day were found dead following the first dose. Hypoactivity was the most common PF614 related clinical observation in animals sacrificed in moribund condition. Rigid stance, labored or audible respiration and /or hunched appearance were observed in some of these animals. The death or moribund condition of these animals was attributed more likely to PF614 associated central nervous system (CNS) effects, although no CNS microscopic correlates were present. Clinical pathology test results from four females given 175 mg/kg/day and sacrificed at an unscheduled interval on Day 1 of the dosing phase indicated that only two of these females had increased hepatic enzymatic activity including high aspartate aminotransferase (3053 and 1487 U/L), alanine aminotransferase (1042 and 840 U/L), which correlated with microscopic observation of slight centrilobular hepatocyte necrosis in liver. Significant increases in creatinine kinase (1167 and 6622) in these two

unscheduled sacrificed animals were considered non specific and could be due to struggling while dosing resulting into skeletal muscle stretching since these increases did not corroborate with cardiac or skeletal muscle histopathology. Noteworthy PF614 related microscopic findings in liver included centrilobular hepatocyte necrosis (minimal to moderate severity) that was observed in four females given 175 mg/kg/day and sacrificed on Day 1, with clinical pathology samples not available in 2 animals to correlate with hepatic activity. PF614 related urinary bladder dilatation was noted for all animals found dead or sacrificed on Day 1 or 4 of the dosing phase. Associated minimal to moderate hemorrhage and/or minimal neutrophil infiltrates were most often noted in the urinary bladder wall of four females given 175 mg/kg/day. Correlative macroscopic findings noted for some animals included large urinary bladder or large lumen, red luminal fluid, and/or red or dark red discoloration. During the dosing phase, hypoactivity was the most common PF614 related clinical observation in animals given 175 mg/kg/day; however, sporadic incidences of hyperactive behavior were noted in animals given < 50 mg/kg/day. Hyperactive behavior occurred transiently on 1 or 2 days of the dosing phase and was considered non-adverse. During the dosing phase, males given 175/50 and 40/25 mg/kg/day gained up to 72% or 46% less weight, respectively, relative to corresponding controls. Effect on body weight was considered adverse only in males given 175/50, due to the magnitude of difference relative to vehicle control and the associated mortality, after decreasing the dose to 50 mg/kg/day. Mean body weight of females given 50 mg/kg/day was lower (up to 11%) relative to controls during dosing phase. No PF614 related effects occurred on ophthalmology, food consumption, body temperatures, and FOB parameters (elicited behaviors, hand held observations, home cage observations, or open-field observations). All PF614 associated clinical chemistry changes were reversible and were of small in magnitude except moribund sacrificed high dose animals. No PF614 related changes were present in other clinical pathology parameters and no changes were noted in anatomic pathology parameters at terminal sacrifice (males given 175/50 mg/kg/day or females given 40/25 or 50 mg/kg/day) or at the recovery sacrifice. On Day 1 of the dosing phase, administration of PF614 at 175 mg/kg/day caused mild respiratory depression, including slightly lower tidal volume at 3 to 6 hours postdose (up to 12% lower than concurrent control) and lower respiration rate and minute volume at 24 hours postdose (22 and 15% lower for respiration rate and minute volume, respectively). The only exception was the marked change in all respiratory parameters (31, 37, and 55% lower tidal volume, respiration rate, and minute volume, respectively, relative to controls) in one animal given 175 mg/kg/day, that was sacrificed in moribund condition. In conclusion, PF614 was well tolerated up to 25 mg/kg/day dose with corresponding C_{max} and $AUC_{0–24}$ values of 61.7 ng/mL and 76.6 ng·hr/mL, respectively, in males and 77.2 ng/mL and 67.1 ng·hr/mL, respectively, in females on Day 14 of the dosing phase. In addition, TK parameter values for oxycodone were 26.1 ng/mL and 231 ng·hr/mL for C_{max} and AUC0–24, respectively, for males and 63.9 ng/mL and 320 ng·hr/mL for C_{max} and AUC_{0-}24, respectively, for females on Day 14 of the dosing phase.

3.7. Dog 14 Day GLP Study

Exposure to PF614 and oxycodone increased in a dose dependent manner from 2 to 18 mg/kg/day by oral gavage and the increases in mean C_{max} and $AUC_{0–24}$ values were generally dose proportional (Table 9). No sex differences were observed, as mean C_{max} and

$AUC_{0–24}$ values were generally within 2-fold for PF614 and oxycodone. Accumulation of oxycodone was observed after multiple dosing of PF614 in dogs, indicating a potential change in absorption and / or disposition of PF614 after multiple dosing. The mean C_{max} and AUC0–24 oxycodone to PF614 ratios ranged from 0.181 to 0.913 and from 0.797 to 3.53, respectively. PF614 is extensively converted to oxycodone in dogs following oral gavage administration of PF614. No PF614 related deaths occurred. On Day 8 of the dosing phase, the accidental death of one female given 2 mg/kg/day was the result of gavage error and was confirmed by microscopic observations of hemorrhage in lungs. PF614 related clinical observations during the dosing phase included abnormal feces (yellow, mucoid, liquid, and /or nonformed), which occurred only in treated animals or occurred at a higher frequency relative to vehicle control during the dosing phase in animals given >2 mg/kg/day and was noted sporadically during recovery phase in male given 18 mg/kg/day. Thin appearance of one male given 6 mg/kg/day during the dosing phase correlated with low food consumption and body weight loss (5% of Day 1 body weight), noted during the dosing phase and was considered PF614 related. Vomitus containing food, struggling during dosing, and excessive salivation were secondary to PF614 administration and occurred occasionally in some animals given PF614. These observations were considered non-adverse because none of these had effect on the overall health of the animals. PF614 related body weight loss (up to 6%) occurred in animals given 18 mg/kg/day. Body weight changes were considered PF614 related but not adverse during the dosing phase because they did not result in a statistically significant decrease in mean body weight relative to vehicle control. PF614 related lower average food consumption was noted for animals given 18 mg/kg/day during certain intervals of the dosing phase, which correlated with overall body weight loss noted for these animals during the dosing phase. Effect on food consumption was transient during the dosing phase and was not adverse. No remarkable ophthalmic observations and no PF614 related effects on body temperature or respiratory parameters (auscultation, respiratory rate, clinical observation of respiratory pattern) occurred. Heart rate data (Table 10) showed that, at 1 hour postdose on Day 12 of the dosing phase, a statistically significant lower mean heart rate was noted in mid and high dose males, relative to control males (133 bpm). Mean heart rates were 93 bpm (−21 bpm from predose) and 96 bpm (−34 bpm from predose), in mid and high dose males, respectively. These heart rate effects were not evident by Day 9 of the recovery phase. No statistically significant changes in heart rate were observed in males given 2 mg/kg/day or females given any dose of PF614. No PF614 related changes in other electrocardiography parameters (PR interval, QRS duration, QT interval, or corrected QT [QTc] interval) were observed on Day 12 of the dosing phase in animals given any dose. No PF614 related electrocardiography changes occurred on Day 9 of the recovery phase. A minimally PF614 related decrease in mean absolute reticulocyte count in males given 18 mg/kg/day, observed on Day 14 of the dosing phase, had reversed by Day 14 of the recovery phase. Administration of PF614 had no effect on coagulation, clinical chemistry, urinalysis, or urine chemistry test results. No PF614 related microscopic findings, macroscopic findings, or organ weight effects were present at the terminal or recovery sacrifice. In conclusion, PF614 was well tolerated up to 18 mg/kg/day, the highest dose tested with corresponding C_{max} and $AUC_{0–24}$ values of 924 ng/mL and 1800 ng·hr/mL, respectively, in males and 876 ng/mL and 1460 ng·hr/mL, respectively, in females on Day 14. In addition, TK parameter values for oxycodone were 162 ng/mL and 1480 ng·hr/mL for

C_{max} and AUC0–24, respectively, for males and 209 ng/mL and 2140 ng·hr/mL for C_{max} and AUC0–24, respectively, for females on Day 14 of the dosing phase.

3.8. Dog Cardiovascular Safety Pharmacology Study

All dogs survived until study termination on Day 14 of the dosing phase and were returned to the stock colony. PF614 related clinical observations consisted of a low incidence of vomitus and nonformed feces. No PF614 related changes in body weight occurred. Any of the qualitative ECG abnormalities observed on study including non-conducted cp-waves and a junction escape complex were considered incidental and not attributed to PF614 administration due to their occurrence in predose phase. No PF614 related changes in QTc interval, QRS duration, or PR interval occurred. Administration of PF614 reduced heart rate at ≥2 mg/kg in both males and females (table 11). The magnitude of the PF614 related differences in heart rate were not consistently dose dependent, especially in females the greatest effect occurred at the mid dose of 6 mg/kg, but the duration of the effect did increase with dose. Reductions in heart rate were greatest through 6 hours postdose. At 2 mg/kg, PF614 related changes in heart rate were resolved by 9 hours postdose. At 6 and 18 mg/kg, the effect persisted through 19 hours postdose. However, the magnitude of the difference was less at 19 hours postdose, indicating partial reversal of the effect.

In addition to the primary effect of lower heart rate, other PF614 associated changes included increases (systolic) / decreases (diastolic) in blood pressure (small in magnitude, inconsistently statistically significant, and sometimes transient in nature) and decreases in body temperature (inconsistently statistically significant). In summary, PF614 related noteworthy finding included moderately lower heart rate at ≥ 2 mg/kg, changes in heart rate at ≥6 mg/kg persisted through the end of the collection period (19 hours postdose), but demonstrated partial recovery and were considered an anticipated pharmacological effect of PF614.

4. Discussion

In these studies, PF614 has been evaluated for non-clinical safety to support its introduction to clinical trials for chronic pain indication Considering characteristics of PF614 as a prodrug and a novel chemical entity, we conducted a series of in-vitro studies (plasma protein binding, P-gP/BCRP transporters, Hepatocytes metabolism, Ames, Micronucleus, Comet, hERG assay) and in-vivo studies (rat and dog GLP toxicity and dog safety pharmacology studies) to evaluate safety profile of PF614 (designed for extended-release TAAP™ prodrug technology). For toxicology studies, daily oral dosing for 14 day duration was selected because the phase I clinical trials were proposed with this dosing regimen.

Oxycodone was tested negative in Ames assay, positive for mouse lymphoma assay in presence of metabolic activation, weakly positive for human peripheral blood lymphocyte in-vitro chromosomal aberration assay, and was tested negative in in-vivo bone marrow micronucleus assay in mice (Product MSDS for OxyContin® Oxycodone HCl Controlled Release Tablets, 10 mg, Purdue Pharma L.P., 2008, 2010). PF614, and its fragments; PFR06112 (amino acid fragment), and PFR06110 (cyclic urea fragment) were tested negative in Ames Assay and PF614 administration by oral gavage, up to 50 mg/kg/day

resulted in negative in-vivo comet assay. PF614 neither induced statistically significant increases in micronucleated PCEs nor it was cytotoxic to bone marrow at 10, 40/25, and 175/50 mg/kg/day oral gavage doses in both male and female rats.

When compared to oxycodone, PF614 unveiled different outcomes of genotoxicity tests plausibly due to its nature as inactive prodrug and in-vitro conditions for Ames could not mimic the in-vivo conditions in mammals involving intestinal trypsin catalyzed bioactivation of PF614 to oxycodone.

Fanoe et al. 2009 found that, among chronic nonmalignant pain patients treated with long-term oxycodone, higher doses (400 – 600 mg) were associated with longer QTc. Also, oxycodone inhibited hERG channels expressed in HEK293 cells (IC_{50} = 171 uM) with very low affinity compared to IC_{50} value of greater than 300 μM projected for the inhibitory effect of PF614 on hERG potassium currents in present project. PF614 associated reduction in heart rate at 2, 6 and 18 mg/kg PO doses is in conformance with cardiovascular effects of oxycodone (Fanoe et al. 2009) suggesting that prodrug had undergone bioactivation to oxycodone to manifest lower heart rate effect.

Affinity of opioids to P-glycoprotein (P-gp; ABCB1) plays a key role in affecting the transport, uptake, and PK/PD of many opioid compounds for being P-gp substrates (Zong and Pollack 2000; Dagenais et al. 2014). In rats, it has been demonstrated that oxycodone is a P-gp substrate and induces overexpression of P-gp to affect tissue distribution of chemotherapeutic agent such as paclitaxel (Hassan et al. 2007). As expected and contrary to outcomes of previous literature on oxycodone, PF614 was found to have low apparent permeability in Caco-2 cells (≤1.17 × 10–6 cm/s) at doses of 0.1 and 10 μM and was not a substrate or inhibitor of P-gp or BCRP transporters.

Poyhia and Seppala (1994) found that, in human plasma, in-vitro protein binding of oxycodone was 38% after using ultrafiltration based protein-binding assay. Protein binding assay for PF614 showed a poor binding of PF614 to plasma proteins without concentration dependence (15 to 10000 ng/mL) in rat, dog or human. The pattern of previous human plasma protein binding data for oxycodone is similar to the one obtained for PF614 in current project.

Incubation of human hepatocytes for 240 minutes with 0.1, 1 and 10 μM oxycodone yielded noroxycodone as a major metabolite whereas, ketoconazole (1 μM) markedly inhibited oxycodone metabolism emphasizing importance of CYP_3A_4 (Korjamo et al. 2012). Contrary to oxycodone, PF614 appeared to be metabolically stable under the incubation conditions for 120 minutes, in cryopreserved rat, dog, and human hepatocytes with no metabolites identified. The obvious reason for no detection of PF614 metabolites is the lack of an intestinal trypsin in hepatocytes required to catalyze PF614 bioactivation to oxycodone.

PF614 GLP study in rat showed high mortality / moribundity in high (19 females) and mid (1 male and 1 female) doses. The high acute mortality observed in female rats vs male rats could be due to higher exposure to PF614 and oxycodone. Although, hypoactivity was the most common PF614 related clinical observation in animals sacrificed in moribund condition, rigid stance, labored or audible respiration and/or hunched appearance were also

observed in some animals. The death or moribund condition of these animals was attributed to PF614 associated central nervous system (CNS) effects leading to respiratory/cardiac arrest without CNS histopathology and liver failure. Oxycodone is known to produce respiratory depression and bradycardia. High doses of oxycodone may lead to respiratory and cardiac arrest. Body weight loss (high and mid dose groups), respiratory depression (high dose group), liver and urinary bladder histopathology (with higher incidence in high dose group) were considered PF614 associated. In case of dog, PF614 related clinical observations included abnormal feces (yellow, mucoid, liquid, and /or nonformed), which occurred in mid and high doses. Low food consumption with thin appearance and body weight loss was observed in one mid dose male and was considered PF614 related. Body weight changes in PF614 treated groups were not statistically different compared to control. No PF614 related effects on respiratory parameters (auscultation, respiratory rate, clinical observation of respiratory pattern) were observed. Also, no PF614 related macroscopic or microscopic findings or organ weight effects were noticed in dog.

PF614 single oral gavage dose values for PK parameters (C_{max} and $AUC_{0\text{-}t}$) in dog were comparable with previous results (Kirkpatrick et al., 2017). In general, PF614 and oxycodone PK parameters values were higher in dogs compared to rats suggesting more prodrug conversion and/or more intestinal absorption in dogs than rats. Furthermore, dog tends to tolerate higher exposure to PF614 and oxycodone as compared to rat..

Oxycodone toxicity data of 10 (in rat), 28 Day, and 3 months (in rat and dog) studies (Product MSDS for OxyContin® Oxycodone HCl Controlled Release Tablets, 10 mg, Purdue Pharma L. P., 2008, 2010) resemble clinical signs of PF614 such as labored breathing (rat) and salivation (dog) supporting the prodrug bioactivation.

5. Conclusion

The studies demonstrated favorable safety profile of PF614 with the well tolerable doses in rat and dog of 25 and 18 mg/kg, respectively, when given daily orally for 14 Days indicating PF614 is a promising prodrug for chronic pain indication.

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References

Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, Escobar P, Honma M, Kumaravel TS, Nakajima M, Sasaki YF, Thybaud V, Uno Y, Vasquez M, Hartmann A, 2007 Fourth International Workgroup on Genotoxicity testing: results of the in vivo Comet assay workgroup. Mutat. Res 627(1), 31–35. [PubMed: 17118697]

Centers for Disease Control and Prevention. Underlying cause of death. National Center for Health Statistics, Hyattsville, MD 1999–2013, 2015.

Chimezie C, Ewing A, Schexnayder C, Bratton M, Glotser E, Skripnikova E, Sá P, Boué S, Stratford RE Jr., 2016 Glyceollin effects on MRP2 and BCRP in Caco-2 cells, and implications for metabolic and transport interactions. J. Pharm. Sci 105(2), 972–981. [PubMed: 26296158]

Clarke HJ, Gregoire F, Ma F, Martin R, Zhao S, Lavan BE, 2008 Cross-species differential plasma protein binding of MBX-102/JNJ39659100: A novel PPAR-gamma agonist. PPAR Res. 2008, 465715. [PubMed: 18815616]

Cobaugh DJ, Gainor C, Gaston CL, Kwong TC, Magnani B, McPherson ML, Painter JT, Krenzelok EP, 2014 The opioid abuse and misuse epidemic: implications for pharmacists in hospitals and health systems. Am. J. Health Syst. Pharm 71, 1539–1554. [PubMed: 25174015]

Dagenais C, Graff CL, Pollack GM, 2014 Variable modulation of opioid brain uptake by P-glycoprotein in mice. Biochem Pharmacol. 67, 269–276.

Fanoe S, Jensen GB, Sjøgren P, Korsgaard MP, Grunnet M, 2009 Oxycodone is associated with dose-dependent QTc prolongation in patients and low-affinity inhibiting of hERG activity in-vitro. Br. J. Clin. Pharmacol 67(2), 172–179. [PubMed: 19159406]

Fenner KS, Troutman MD, Kempshall S, Cook JA, Ware JA, Smith DA, Lee CA, 2009 Drug-drug interactions mediated through P-glycoprotein: clinical relevance and in vitro-in vivo correlation using digoxin as a probe drug. Clin. Pharmacol. Ther 85(2), 173–181. [PubMed: 18987624]

Fuster V, Sweeny JM, 2011 Aspirin: a historical and contemporary therapeutic overview. Circulation. 123(7), 768–778. [PubMed: 21343593]

Gudin JA, Nalamachu SR, 2016 Overview of prodrug technology and its application for developing abuse-deterrent opioids. Postgrad. Med 128 (1), 97–105. [PubMed: 26615852]

Hassan HE, Myers AL, Lee IJ, Coop A, Eddington ND, 2007 Oxycodone induces overexpression of P-Glycoprotein (ABCB1) and affects paclitaxel's tissue distribution in sprague dawley rats. 96(9), 2494–2506.

Hirsch A, Proescholdbell SK, Bronson W, Dasgupta N, 2014 Prescription histories and dose strengths associated with overdose deaths. Pain Med. 15, 1187–1195. [PubMed: 25202775]

Huttunen KM, Raunio H, Rautio J, 2011 Prodrugs--from serendipity to rational design. Pharmacol. Rev 63(3), 750–771. [PubMed: 21737530]

ICH/FDA/CDER: Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. 2010.

International Conference on Harmonisation (ICH). S2 (R1): Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. 2011.

Jones JD, Sullivan MA, Manubay J, Vosburg SK, Comer SD, 2011a The subjective, reinforcing, and analgesic effects of oxycodone in patients with chronic, non-malignant pain who are maintained on sublingual buprenorphine/naloxone. Neuropsychopharmacology. 36, 411–422. [PubMed: 20980992]

Jones JD, Vosburg SK, Manubay JM, Comer SD, 2011b Oxycodone abuse in New York City: characteristics of intravenous and intranasal users. Am. J. Addict 20, 190–195. [PubMed: 21477046]

Kalso E, 2005 Oxycodone. J. Pain Symptom Manage. 29 (Suppl), S47–S56. [PubMed: 15907646]

Kirkpatrick DL, Schmidt WK, Morales R, Cremin J, Seroogy J, Husfeld C, Jenkins T, 2017 In vitro and in vivo assessment of the abuse potential of PF614, a novel BIOMD™ prodrug of oxycodone. J. Opioid Manag 13(1), 39–49. [PubMed: 28345745]

Kirsch GE, Trepakova ES, Brimecombe JC, Sidach SS, Erickson HD, Kochan MC, Shyjka LM, Lacerda AE, Brown AM, 2004 Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. J. Pharmacol. Toxicol. Methods 50, 93–101. [PubMed: 15385083]

Korjamo T, Tolonen A, Ranta VP, Turpeinen M, Kokki H, 2011 Metabolism of oxycodone in human hepatocytes from different age groups and prediction of hepatic plasma clearance. Front Pharmacol. 2, 87. [PubMed: 22291644]

Mars SG, Bourgois P, Karandinos G, Montero F, Ciccarone D, 2014 "Every'never' ever said came true": transitions from opioid pills to heroin injecting. Int. J. Drug Policy. 25, 257–266. [PubMed: 24238956]

Poyhia R, Seppala T, 1994 Liposolubility and protein binding of oxycodone in vitro. Pharmacol. Toxicol 74(1), 23–27. [PubMed: 8159633]

OxyContin® Product MSDS, Purdue Pharma L. P, 2008, 2010.

Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PK, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG, 2003 Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovascular Research. 58 (1), 32–45. [PubMed: 12667944]

Schmid W, 1976 The micronucleus test for cytogenetic analysis. Chemical Mutagens. 4, 31.

Sellers EM, Shram MJ, Schoedel KA, 2014 The US FDA draft guidance for developing abuse-deterrent opioid analgesics: 2014 and beyond. Pharmaceut. Med 28: 317–327.

Smith CC, Adkins DJ, Martin EA, O'Donovan MR, 2008 Recommendations for design of the rat comet assay. Mutagenesis. 23(3), 233–240. [PubMed: 18326531]

Department of Health US, Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. Guidance for Industry: S7 A safety pharmacology studies for human pharmaceuticals. 2001.

Wightman R, Perrone J, Portelli I, Nelson L, 2012 Likeability and abuse liability of commonly prescribed opioids. J. Med. Toxicol 8, 335–340. [PubMed: 22992943]

WO 2017/070576 Al. 2017 International application published under the patent cooperation treaty.

Zhang J, He K, Cai L, Chen YC, Yang Y, Shi Q, Woolf TF, Ge W, Guo L, Borlak J, Tong W, 2016 Inhibition of bile salt transport by drugs associated with liver injury in primary hepatocytes from human, monkey, dog, rat, and mouse. Chem. Biol. Interact 255, 45–54. [PubMed: 27000539]

Zong J, Pollack GM, 2000 Morphine antinociception is enhanced in mdr1a gene-deficient mice. Pharm. Res 17, 749–753. [PubMed: 10955852]

Highlights:

- PF614, a novel TAAP, is a potential prodrug of oxycodone.
- Ames, comet and micronucleus assay were negative, hERG IC_{50} was ≥ 300 μM.
- Low permeability in Caco-2 cells and not a P-gp or BCRP substrate or inhibitor.
- Metabolically stable in rat, dog, and human hepatocytes.
- Oral doses of 25 and 18 mg/kg in rat and dog, respectively, were well tolerated.

Table 1:

Rat GLP study

Group	Dose (mg/kg/day)	Core group Dosing animals (M+F)	Core group Recovery animals (M+F)	TK group animals (M+F)
1	0	10 (5+5)	10 (5+5)	6 (3+3)
2	10	10 (5+5)	10 (5+5)	18 (9+9)
3	40/25	10 (5+5)	10 (5+5)	18 (9+9)
4/7	175/50	15 (5+10)	10 (5+5)	18 (9+9)

Table 2:

Dog GLP study

Group	Dose (mg/kg/day)	No. of Animals Dosing (M+F)	No. of Animals Recovery (M+F)
1	0	6 (3+3)	4 (2 + 2)
2	2	6 (3+3)	4 (2 + 2)
3	6	6 (3+3)	4 (2 + 2)
4	18	6 (3+3)	4 (2 + 2)

Table 3:

Dog Safety Pharmacology Study

Animals (4M and 4F)	Day 1	Day 4	Day 7	Day 10
1	Low[a]	Vehicle Control[a]	High[a]	Mid[a]
2	Mid	High	Vehicle Control	Low
3	High	Low	Mid	Vehicle Control
4	Vehicle Control	Mid	Low	High

[a]Dose: Vehicle control: 0 mg/kg, Low: 2 mg/kg, Mid: 6 mg/kg, High: 18 mg/kg

Table 4:

In-Vivo Comet Assay in Rat following oral gavage administration of PF614

Group	Dose[a] (mg/kg/day)	No. of Male Rats	% Tail DNA in Liver Cells (Mean ± SD)	% Tail DNA in Duodenal Cells (Mean ± SD)
Vehicle Control	0	5	0.46 ± 0.24	3.92 ±1.52
Test Article:				
PF614	10	5	0.44 ± 0.28	4.04 ± 0.63
PF614	25	5	0.27 ±0.18	3.90 ±0.50
PF614	50	5	0.24 ±0.10	3.73 ±2.54
Positive Control (EMS[b])	200	5	11.17 ± 2.69*	19.62 ±2.30*

[a]Dose volume was used at 10 mL/kg/day,

[b]Ethyl methanesulfonate

Table 5:

Micronucleus Assay – Summary Table

Group	Dose[a]	Harvest Time (Hr)	% Micronucleated PCEs Mean ± SD		Ratio PCE:NCE Mean ± SD	
			Male	Female	Male	Female
Controls						
Vehicle	10mL/kg/day	24	0.09 ± 0.07	0.09 ± 0.10	0.72 ± 0.11	0.65 ± 0.16
Positive	CP[b] 60 mg/kg	24	1.01 ± 0.46*	0.81 ± 0.24*	0.47 ± 0.22	0.33 ± 0.04*
PF614	10mg/kg/day	24	0.07 ± 0.08	0.08 ± 0.06	0.66 ± 0.12	0.74 ± 0.13
	40/25 mg/kg/day	24	0.06 ± 0.04	0.14 ± 0.07	0.71 ± 0.20	0.81 ± 0.13
	175/50 mg/kg/day	24	0.18 ± 0.06	0.09 ± 0.08	0.72 ± 0.13	0.73 ± 0.12

[a]Dose volume was used at 10 mL/kg/day

[b]Cyclophosphamide

Table 6:

Apparent permeability and efflux ratios of PF614 incubated with Caco-2 cells in the absence and presence of zosuquidar or Ko143 at 37°C for 2 hours

		Permeability (x 10^{-6} cm/s)		
Treatment	**Inhibitor**	**A to B Mean ± SD**	**B to A Mean ± SD**	**Efflux Ratio**
PF614 (0.1 μM)	Solvent	0.553 ± 0.132	1.17 ± 0.276	2.12
PF614 (0.1 μM)	Zosuquidar (2 μM)	0.596 ± 0.470	0.870 ± 0.167	1.46
PF614 (0.1 μM)	Ko143 (1 μM)	0.263 ± 0.107	0.807	3.07
PF614 (10 μM)	Solvent	0.303 ± 0.330	0.173 ± 0.00609	0.571
PF614 (10 μM)	Zosuquidar (2 μM)	0.246 ± 0.215	0.135	0.551
PF614 (10 μM)	Kol43 (1 μM)	0.0511	0.0619 ±0.0547	1.21

Table 7:

Percent bound, unbound, and recovered PF614 in rat, dog, and human plasma after fortification of PF614 at various concentrations and dialysis at 37°C for 8 hours

Species	Concentration (ng/mL)	% PF614 Bound Mean ± SD	% PF614 Unbound Mean ± SD	% PF614 Recovered Mean ± SD
Rat	15	8.8 ± 17.6	91.2 ± 17.55	95.7 ± 16.8
	100	7.7 ± 9.0	92.3 ± 9.0	105 ± 3.81
	1000	2.6 ± 3.4	97.4 ± 3.42	114 ± 5.26
	3000	1.6 ± 1.8	98.4 ± 1.84	108 ± 2.93
	10000	6.4 ± 4.3	93.6 ± 4.24	108 ± 3.62
Dog	15	20.6 ± 27.8	79.4 ± 27.79	136 ± 18.4
	100	6.3 ± 3.6	93.7 ± 3.59	120 ± 4.42
	1000	2.3 ± 2.6	97.7 ± 2.60	113 ± 4.36
	3000	0.0 ± 0.0	100 ± 0.0	120 ± 4.80
	10000	4.5[a]	95.5[a]	115[a]
Human	15	37.9 ± 15.5	62.1 ± 15.45	137 ± 18.9
	100	47.1 ± 2.8	52.9 ± 2.80	144 ± 3.87
	1000	28.0 ± 15.5	72.0 ± 15.57	121 ± 3.77
	3000	20.1 ± 0.9	79.9 ± 0.92	113 ± 14.3
	10000	24.6 ± 14.1	75.4 ± 14.15	130 ± 8.04

[a]No standard deviation calculation was possible due to data not used

Table 8:

Summary of the Mean PF614 and Oxycodone C_{max} and $AUC_{0–24}$ in Rat Plasma on Day 1 and 14

Interval (Day)	PF614 Dose (mg/kg/day)	Sex	PF614C_{max} (ng/mL)	PF614$AUC_{0–24}$ (ng·hr/mL)	Oxycodone C_{max} (ng/mL)	Oxycodone $AUC_{0–24}$ (ng·hr/mL)
1	10	M	14.5	25.6	5.40	41.3
		F	12.4	20.4	16.4	98.5
	40	M	55.2	85.5	18.6	217
		F	167	88.7	66.4	779
	175	M	261	563	85.9	982
		F	710	NR[a]	438	NR[a]
14	10	M	38.3	37.4	4.68	45.8
		F	27.9	23.3	18.9	108
	25	M	61.7	76.6	26.1	231
		F	77.2	67.1	63.9	320
	50	M	141	193	45.4	458

[a]Not reported due to an incomplete profile

Table 9:

Summary of the Mean PF614 and Oxycodone C_{max} and $AUC_{0–24}$ in Dog Plasma on Day 1 and 14

Interval (Day)	PF614Dose (mg/kg/day)	Sex	PF614C_{max} (ng/mL)	PF614$AUC_{0–24}$ (ng·hr/mL)	Oxycodone C_{max} (ng/mL)	Oxycodone $AUC_{0–24}$ (ng·hr/mL)
1	2	**M**	23.5	41.9	21.1	133
		F	39.2	81.7	22.6	110
	6	M	108	236	50.5	310
		F	93.6	233	44.9	347
	18	M	367	1320	66.6	748
		F	484	1590	157	1210
14	2	M	50.8	65.0	28.9	197
		F	63.6	93.0	45.1	191
	6	M	220	366	130	646
		F	135	254	57.9	552
	18	M	924	1800	162	1480
		F	876	1460	209	2140

Table 10:

Heart Rate – Dogs/Males (beats/minute) post PF614 multi-dose

Dose (mg/kg/day)		Predose Phase Day 5	Dosing Phase (Day 12, 1 hour postdose)	Recovery Phase Day 9
0	Mean	131	133	119
	SD	23.9	18.3	11.3
	N	5	5	2
2	Mean	126	105	121
	SD	21.8	23.1	14.6
	N	5	5	2
	P(v1)	-	0.1182	-
6	Mean	114	93*	125
	SD	17.8	10.8	14.1
	N	5	5	2
	P(v1)	-	0.0179	-
18	Mean	130	96*	124
	SD	15.4	27.1	12.7
	N	5	5	2
	P(vl)	-	0.0302	-
	P(Overall)	-	0.0272	-

Table 11:

Heart Rate - Differences from Control Values in Dogs (3 through 19 hours post PF614 single dose)

	Hours Postdose											
	Block 2 Overall	Block 2				Block 3 Overall	Block 3					
	3–6	3	4	5	6	9–19	9	11	13	15	17	19
Dose (mg/kg)	bpm											
Males												
2	−5 (−4%)	−12	1	−3	−4	−3 (−4%)	−7	−7	1	−2	−2	0
6	−14 (−12%)*	−24	−15	−6	−7	0 (0%)	−8	0	−1	4	3	0
18	−32 (−28%)*	−41	−34	−26	−23	−11 (−15%)	−25*	−16	−9	−9	−4	−5
Females												
2	−17 (−17%)	−19	−20	−8	−22	−5 (−7%)	−7	−4	−7	−1	−5	−2
6	−35 (−34%)	−39	−35	−30	−37	−9 (−13%)*	−15	−10	−12	−5	−4	−2
18	−21 (−21%)	−31	−20	−11	−22	−11 (−16%)*	−16	−13	−14	−6	−7	−6