



INVESTIGATOR'S BROCHURE

PF-00446687

March 2007

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ABBREVIATIONS AND DEFINITION OF TERMS

ACTH	adrenocorticotrophic hormone
APD₅₀	action potential duration at 50% repolarisation
APD₉₀	action potential duration at 90% repolarisation
ANOVA	analysis of variance
AUC	area under the plasma concentration-time curve
AUC_(0-∞)	area under the curve from zero to infinity
AUC₍₀₋₂₄₎	area under the plasma concentration-time curve from 0 to 24 hours
AUC_(0-tlast)	area under the plasma concentration-time curve from 0 to the time of last measurement
AUC_(5min-24h)	area under the plasma concentration-time curve from 5 minutes to 24 hours
BP	blood pressure
AUEC	area under the effect curve
bpm	beats per minute
cAMP	cyclic adenosine monophosphate
CHO	chinese hamster ovary
CI	confidence interval
C_{max}	maximum plasma concentration
C_{max,u}	maximum unbound plasma concentration
CNS	central nervous system
CSF	cerebrospinal fluid
CYP	cytochrome P450
DDI	drug/drug interaction
dP/dt	change in ventricular pressure over the change in time
EC₅₀	median effective concentration
ECG	electrocardiogram
ED	erectile dysfunction
E_{max}	maximum response
FSAD	female sexual arousal disorder
FSD	female sexual dysfunction
FSH	follicle stimulating hormone
f_u	fraction of drug unbound
GABA	gamma amino butyric acid
GH	growth hormone
GLP	Good Laboratory Practice
HEK	human embryonic kidney
hERG	human ether-a-go-go-related gene
HPLC	high-performance liquid chromatography
IC₅₀	50% inhibitory concentration
I_{Kr}	hERG current
IN	intranasal
IV	intravenous
K_i	inhibition constant
LH	lutensising hormone
LV	left ventricular
MAP	mean arterial pressure

MC4	melanocortin 4
MED	male erectile dysfunction
msec	milliseconds
MSH	melanocyte stimulating hormone
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
PK	pharmacokinetic
PO	per os (orally)
QT	time from the beginning of the QRS complex to the end of the T wave in the electrocardiogram
QTc	QT interval, corrected for heart rate.
SC	subcutaneously
t_½	apparent terminal half-life
TK	toxicokinetic
TSH	thyroid stimulating hormone
t_{max}	time of occurrence of C _{max}
VSS	visual sexual stimulation

2. SUMMARY

PF-00446687 is a MC4 receptor agonist, intended for the treatment of female and male sexual dysfunction.

Studies examining the general safety pharmacological properties of PF-00446687 demonstrated that the compound was well tolerated and had little interaction with physiologically important receptors, binding sites, enzymes, or ion channels at relevant concentrations. PF-00446687 has been evaluated in 4 in vivo pharmacology models: proceptive female sexual behaviour (conscious rat), proerectile activity as a measure of male sexual function (conscious and anaesthetised rat), an emesis model (conscious ferret), and a cardiovascular model (conscious rat). These studies support the hypothesis that a centrally acting selective MC4 agonist could provide an improved therapeutic window in comparison with non-selective MC agonists.

Doses of PF-00446687, at 30 to 300 µg/kg SC, also produced no change in heart rate or blood pressure in a conscious telemetered rat. Although there was a concentration dependent block of hERG potassium current in vitro there were no significant changes in QTc at any dose level in vivo, which is consistent with observations at similar concentration ranges in the in vitro I_{Kr} assay. In the conscious dog, PF-00446687 at total plasma concentration of 49.75 ng/mL ($C_{max,u}$ 15.5 nM) resulted in minor reductions in blood pressure, small increases in heart rate and myocardial contractility. There were no changes on QTc. In ferrets, PF-00446687 did not induce emesis up to a C_{max} of 682.7 ng/mL ($C_{max,u}$ 178 nM). In rats, the IV dose of 10 mg/kg (C_{max} 976 ng/mL, $C_{max,u}$ 145.6 nM) produced a slight increase in respiratory rate (+12 breaths per min) and small decrease of tidal volume (0.07 mL). At a dose of 1 mg/kg, effects on respiratory rate were minor and unlikely to be of biological significance. From 1 mg/kg, dose dependent increases in urine volume and electrolyte secretion (Na^+ , K^+ , and Cl^-) were observed. However, urinary creatinine, osmolality and electrolytes (sodium, potassium, and chloride) were monitored in the first-in-human study and there were no dose-related changes of note. PF-00446687 was well tolerated centrally and there were no effects on locomotor activity up to doses of 1 mg/kg IV. The corresponding total C_{max} and free C_{max} were 90.8 ng/mL and 13.5 nM, respectively. At 10 mg/kg IV (C_{max} 976 ng/mL, $C_{max,u}$ of 145.6 nM), the lack of severity of CNS effects and the exposure approximately 17-fold (146 nM/8.3 nM) greater than the human exposure suggest that these CNS effects will unlikely be of clinical significance. Overall, the non-clinical data generated in rats and dogs indicate that PF-00446687 should be well tolerated in humans at therapeutic dose levels.

PF-00446687 was extensively metabolised in vitro in rat, dog, and human liver microsomes with mean apparent intrinsic clearance values of 60, 242, and 45 µL/min/mg protein, respectively. Following incubation with human hepatocytes, several metabolites of PF-00446687 were detected, including a mono-oxidised metabolite consistent with oxidation of the t-butyl, and an N-desalkyl metabolite consistent with the loss of the t-butyl. A similar metabolite profile was seen following incubation with hepatocytes of rats and dogs. No glucuronide or sulphate conjugates were detected in any of the hepatocyte extracts. In metabolism studies in vivo (rat), less than 1% of the parent compound was eliminated unchanged in the bile and <3% was excreted unchanged in the urine, suggesting that metabolism is likely to be the major route of clearance and that renal and biliary elimination of unchanged PF-00446687 are minimal. It is

expected that in man PF-00446687 will be primarily metabolized by CYP3A4, and inhibition of this enzyme is expected to result in an increase in PF-00446687 exposure.

In keeping with its physicochemistry, PF-00446687 combines significant solubility and membrane permeability. In the rat PF-00446687 freely penetrates into the CNS (as judged by CSF: free plasma ratio of approximately 1) and is also expected to penetrate the CNS in humans. In rats and dogs at low doses the oral exposure of PF-00446687 is limited by almost complete first pass extraction. This contrasts with the situation in humans where the hepatic clearance is assumed to be less than liver blood flow and adequate systemic exposure is obtained following oral dosing with PF-00446687.

General toxicology studies using intravenous administration have been conducted up to 1 month duration in rats and dogs. In single-dose IV toxicity studies, the highest non-lethal dose in mice and rats were 20 and 10 mg/kg, respectively. In IV repeated-dose studies, the NOAEL was considered to be 3 mg/kg in rats, (C_{max} 317 ng/mL, AUC 810 ng•h/mL) and 3 mg/kg in dogs (C_{max} 439 ng/mL, AUC 1100 ng•h/mL) for systemic effects. Death was noted in rats at 15 mg/kg and an episode of convulsions was noted at 9 mg/kg. In addition, 7.5 mg/kg induced minor clinical signs (stretching, yawning, urogenital licking and sporadic excessive grooming). In dogs, a spectrum of behavioral changes and reduction of body weight were noted at 10 mg/kg. Irritant effects at the IV injection site were observed in rats and dogs and were attributed to high injection concentration of PF-00446687. There was no evidence of irritation when applied topically to the hamster cheek pouch at concentrations up to 80 mg/mL. Oral gavage administration of PF-00446687 in rat did not produce irritation of gastrointestinal tract up to 2 mg/mL. Following oral administration for 4 days in rats, death preceded by CNS signs of toxicity was observed at 1000 mg/kg. From 100 mg/kg, slight decrease in food consumption was noted and associated with body weight decrease from 300 mg/kg (up to -21% at 1000 mg/kg, compared with control). Minor decrease in reticulocytes and slight increase in liver weight were noted from 100 mg/kg. In dogs convulsions were noted after a single oral dose of 300 mg/kg. From 100 mg/kg emesis, arched back with extended limbs and decreased body weight were observed. There was an increase in white blood cell parameters, globulin, protein and transaminase from 200 mg/kg.

The genetic safety of PF-00446687 was assessed in a comprehensive series of in vitro and in vivo tests. There was no evidence of mutagenic activity in *Salmonella typhimurium* and *Escherichia coli* bacterial assays with and without the presence of S9 metabolic activation. PF-00446687 did not produce increases in structural chromosomal damage in cultured human lymphocytes under any of the testing conditions (3- and 24-hour tests without and 3-hour tests with metabolic activation). PF-00446687 did induce a significant increase in polyploid cells in cultured human lymphocytes under treatment conditions without metabolic activation. However, the lack of micronucleus induction in the in vitro (CHO cells) and in vivo bone marrow micronucleus assays in rats indicate that PF-00446687 does not appear to pose a genetic safety risk for clinical exposure in humans.

Two clinical studies in healthy male volunteers have been completed which investigated the pharmacokinetics, efficacy and safety of PF-00446687.

PK Summary: Following sublingual dosing, peak plasma concentrations were achieved within 2 to 8 hours, exposure increased supra-proportionally with increasing dose (<2-fold relative to dose), and terminal half-life was approximately 12 hours across the entire dose range. Following oral dosing, exposure was similar to that achieved for sublingual dosing at comparable dose levels. Urinary excretion was negligible with <2.5% dose excreted as parent over 48 hours after dosing. There were no discernible differences between the 195 mg dose of PF-00446687 and placebo for any of the pharmacodynamic markers evaluated (TSH, LH, FSH, GH and prolactin).

Safety Summary: Safety data are available from two protocols. A total of 64 subjects have received PF-00446687 (of which 52 have received 195 or 200 mg) and 63 subjects have received placebo in the two completed studies. The highest incidence of adverse events was due to low toleration locally for sublingual administration; reports included application site anaesthesia/irritation and hypoesthesia. The sublingual route is no longer being pursued. Across both studies, the most common treatment-related systemic adverse events were restlessness (6.3%), increased libido (3.1%), somnolence (3.1%), dizziness (3.1%); both nausea (6.3%) and abdominal pain (3.1%) were mostly reported with sublingual dosing. There have been no clinically meaningful changes in laboratory tests, vital signs or ECG parameters that could be attributed to study drug. There have been no discontinuations due to adverse events nor any serious adverse events in the PF-00446687 program.

Efficacy Summary: In a healthy male volunteer Rigiscan (penile plethysmography) study, the 200 mg dose of PF-00446687 demonstrated improvement over placebo during visual sexual stimulation. A second cohort demonstrated no evidence of a dose response (down to 3 mg); however, activity of the 200 mg dose was confirmed.

Interpretation of these results and a discussion of the potential risks associated with the administration of PF-00446687 are summarized in Section 7 of this Investigator's Brochure.

3. INTRODUCTION

PF-00446687 is a selective MC4 receptor agonist which is being evaluated for the treatment of both female and male sexual dysfunction.

Female Sexual Dysfunction is an overarching term, used to describe a group of related conditions, including disorders of arousal, desire, orgasm and sexual pain (American Psychiatric Association, 1994). It is a highly prevalent condition (the US National Health and Social Life survey in 1992 indicated that 43% of women had experienced symptoms in the previous 12 months). Symptoms often overlap and this contributes to the complexity of investigating FSD as, in addition to poorly understood pathophysiology, psychosocial, relational and pharmacological variables interact individually in each woman, giving rise to a range of possible presentations (Diamond et al., 2006).

Female Sexual Arousal Disorder, one of the components of FSD, is characterised by both difficulty in becoming aroused and problems with the maintenance of arousal. It is expressed as a deficit of subjective excitement, or a lack of genital (lubrication/ engorgement/ sensation) responses and also as a combination of these. Definitions have recently evolved to differentiate between subjective, genital and combined subjective and genital sexual arousal disorder (Basson et al., 2004) and this reflects a growing understanding of how contributory elements may be distinct or interact with each other in the evolution of the difficulties with which women present clinically. Basson argues for five subtypes of FSAD (Basson, 2002) and asserts that each of these may be lifelong or acquire; these 5 sub-types of arousal disorder are generalised sexual, genital, missed, dysphoric and anhedonic. Pre-, peri-, and post-menopausal women all present with symptoms of FSAD, although physiological change, mostly associated with falling levels of oestrogen, becomes more prevalent with the onset of menopause, somewhat altering the profile of women seeking assistance.

Although it has been hypothesised that FSAD, owing to similar physiological factors to erectile dysfunction (ED), may be amenable to treatment with vasodilators or pharmacological agents that modulate the autonomic nervous system, clinical trials to evaluate the efficacy of sildenafil in FSAD (Berman et al., 2003; Basson et al., 2002) have found largely negative results for the use of type 5 phosphodiesterase inhibitors in FSAD. The consequent current lack of licensed treatments for this specific indication and for FSD in general, highlights the unmet medical need in this area, which continues to result in considerable personal and interpersonal distress.

Treatment of FSAD is primarily accounted for by psychosexual therapy, which incorporates different psychotherapeutic approaches and usually advocates both cognitive and behavioural change. In 2006, a testosterone patch (Intrinsa®) received regulatory approval within the EU for surgically menopausal women who have had hysterectomy and ovariectomy. There appears to be growing agreement (Diamond et al., 2006) that, as women complaining of chronically low arousal show vasocongestive responses to erotic stimuli which are comparable with controls (van Lunsen & Laan, 2004; Brotto et al., 2004), the incorporation of compounds with a central mechanism of action into the search for efficacious treatment of FSAD is of great importance.

Male erectile dysfunction is a condition that, in moderate or severe form, affects around 35% of men over the age of 40 (Feldman et al., 1994). Current treatment options include

phosphodiesterase type 5 inhibitors such as sildenafil, tadalafil, and vardenafil and these are effective in around 70% of patients. Although well tolerated, these drugs have dose-limiting side effects such as headache, facial flushing, and back pain. Furthermore, this class of drugs is contraindicated with nitrate therapy which is often used in coronary heart disease; MED and coronary heart disease frequently co-exist. There is a need for new agents with increased efficacy that are free of dose-limiting side effects and are available to a wider population of men.

The melanocortin family of related peptide neurotransmitters (MC1, MC2, MC3, MC4, and MC5) regulate a wide range of physiological actions. The endogenous ligand of the melanocortin receptors is α -MSH, a 13 amino acid peptide derived from pro-opiomelanocortin. That melanocortins can influence male and female sexual behaviour and penile erection in animals has been known for almost 40 years, and indices of erectile activity evoked by ACTH or α -MSH have been documented in a range of species from rodents to primates. A number of non-specific melanocortin agonists have been reported to elicit pro-sexual effects in the clinic. Melanotan-II has evoked spontaneous erections and heightened sexual desire in men with erectile dysfunction (Wessells et al., 2000(a) & (b)). Another non-selective agent, PT-141 (bremelanotide), is currently in Phase 2 development for both male and female sexual dysfunction, having reported positive effects in biomarker studies (Diamond et al., 2004; Diamond et al., 2006) and Phase 2. For both of these non-selective agents, nausea is dose-limiting with an incidence of approximately 15% (Wessells et al., 2000a; Wessells et al., 2000b; Diamond et al., 2004). In contrast to these non-selective agents, no emesis was observed with PF-00446687 in either the non-clinical models, or in the clinical studies to date, suggesting that selective MC4 receptor agonism could provide an improved therapeutic window in comparison with other melanocortin agonists.

Clinical Plan

The forward clinical plan for PF-00446687 is aimed at establishing proof of concept in both FSD and MED populations. In support of this, multiple dose safety, toleration and pharmacokinetic studies will be conducted as well as studies to address potential drug-drug interaction risks. Additional biomarker studies, in both males and females, will be conducted to continue to refine the therapeutic window in advance of the proof-of-concept studies. It is expected that clinical pharmacology studies will be conducted in approximately 100 volunteers and that biomarker studies will also be conducted in approximately 100 patients. The proof-of-concept studies in both FSD and MED population are expected to comprise of approximately 50 patients each in a cross-over design with all subjects being exposed to study drug.

References

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Diamond LE, Earle DC, Heiman JR, et al. An Effect on the Subjective Sexual Response in Premenopausal Women with Sexual arousal Disorder by Bremelanotide (PT-141), a Melanocortin Receptor Agonist. *J Sex Med*. 2006;3(4):628-38.

Van Lunsen RH, Laan E. Genital vascular responsiveness and sexual feelings in midlife women: Psychophysiologic, brain, and genital imaging studies. *Menopause*. 2004;11(6 pt 2):741-8.

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Wessells H, Gralnek D, Dorr R, et al. Effect of an alpha-melanocyte stimulating hormone analog on penile erection and sexual desire in men with organ erectile dysfunction. *Urology* 2000;56(4):641-6. (b).

4. PHYSICAL, CHEMICAL, & PHARMACEUTICAL PROPERTIES & FORMULATION

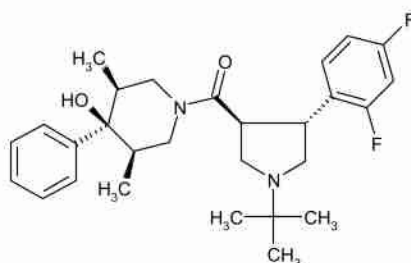
PF-00446687 has been manufactured both as the free base and as a hydrochloride salt. PF-00446687 has been used to produce a sublingual and oral solution. The hydrochloride salt has been used to produce an oral solution.

PF-00446687 free base has been previously used in regulatory non-clinical toxicology studies and in clinical studies. Future studies will use the hydrochloride salt form.

Laboratory Code: PF-00446687

Molecular Weight: 470.59 Daltons

Chemical Structure:



IUPAC Name: (3*R*,4*R*,5*S*)-1-([(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl)-4-hydroxy-3,5-dimethyl-4-phenylpiperidine

Molecular Formula: C₂₈H₃₆F₂N₂O₂

Physical Description: White to pale-coloured solid

Drug Substance Storage Condition: Store at temperatures between 2 and 8°C.

Formulations

Sublingual Solution

A known quantity of PF-00446687 drug substance is dissolved in an appropriate aqueous diluent to provide the extemporaneously prepared sublingual solution. This solution is filled into a suitable vessel for sublingual administration. The sublingual solution should be stored at temperatures between 15 and 25°C.

Oral Solution

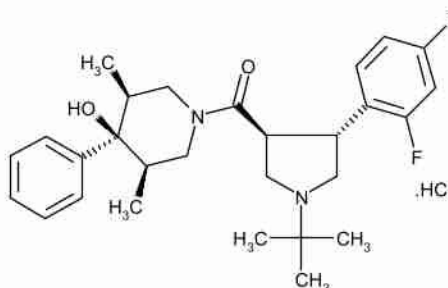
A known quantity of PF-00446687 drug substance is dissolved in an appropriate volume of a suitable aqueous diluent to provide an extemporaneously prepared oral solution. Appropriate volumes of this oral solution are dispensed into a suitable vessel for oral administration. The PF-00446687 oral solution should be stored at temperatures between 2 and 8°C.

PF-00446687-01 (Hydrochloride Salt)

Laboratory Code: PF-00446687-01

Molecular Weight: 507.07 Daltons

Chemical Structure:



IUPAC Name: (3*R*,4*R*,5*S*)-1- {[(3*S*,4*R*)-1-*tert*-Butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}-4-hydroxy-3,5-dimethyl-4-phenylpiperidine hydrochloride

Molecular Formula: C₂₈H₃₇ClF₂N₂O₂

Physical Description: White to pale-coloured solid

Drug Substance Storage Condition: Store at temperatures between 2 and 8°C.

Oral Solution

A known quantity of PF-00446687-01 drug substance is dissolved in an appropriate volume of a suitable aqueous diluent to provide an extemporaneously prepared oral solution. Appropriate volumes of this oral solution are dispensed into a suitable vessel for oral administration. The PF-00446687-01 oral solution should be stored at temperatures between 2 and 8°C.

5. NONCLINICAL STUDIES

5.1. Nonclinical Pharmacology

5.1.1. In Vitro Pharmacology Studies

PF-00446687 is a selective MC4 receptor agonist that increases cAMP accumulation in CHO cells, which stably express the human recombinant MC4 receptor, with an EC_{50} of 11.6 nM and behaves as a full agonist (percent E_{max} = 95.6). Furthermore, PF-00446687 displays 112-, 78-, and 163-fold functional selectivity over human recombinant MC1, MC3, and MC5 receptors, respectively (Table 1). In receptor binding assays using [125 I]-melanotan II, PF-00446687 has a high-affinity binding at the MC4 receptor subtype (K_i = 26.2 nM). The functional potency of PF-00446687 at a native MC4 receptor in rats has been examined in hemisected rat neonate spinal cord in vitro. In this preparation, PF-00446687 inhibited the fast monosynaptic component of the dorsal root-ventral root reflex with an EC_{50} of 1.1 nM. The increased potency at the native receptor in rats is likely to reflect differences in receptor expression levels and/or post-receptor amplification mechanisms between the native and the recombinant test systems.

Table 1. Key In Vitro Pharmacologic Activities of PF-00446687

Activity	Endpoint
Functional stimulation of cAMP accumulation in human recombinant cell lines ^a	Geometric mean EC ₅₀ (95% CI) (n)
MC4	EC ₅₀ = 11.6 nM (9.3-14.6) (n = 22) % Emax = 95.6 (93.1-98.0)
MC1	EC ₅₀ = 1.3 µM (0.3-2.3) (n = 6) % Emax = 99.3 (81.6-117)%
MC3	EC ₅₀ = 0.9 µM (0.6-1.2) (n = 10) % Emax = 106.0 (99.7-113.0)
MC5	EC ₅₀ = 1.9 µM (1.3-2.3) (n = 8) % Emax = 84.7 (84.7-107.0)
In vitro binding (membrane preparation), human recombinant receptors ^b	
MC4	K _i = 26.2 nM (22.6-30.4) (n = 5)
Functional activation of rat native MC4 receptors in hemisectioned spinal cord	EC ₅₀ = 1.1 nM (0.2-5.5) (n = 3)

EC₅₀ = concentration required to produce half-maximal effect; n = number of separate experiments; MC = melanocortin receptor; % response = response expressed as a percentage of the effect to melanotan II.

^a PF00446687/DI/016/04; ^b PF00446687/DI/015/04; ^c PF00446687/DI/021/05

5.1.2. In Vivo Pharmacology Studies

In vivo efficacy studies of PF-00446687 consisted of 2 conscious rat models of sexual function and one anaesthetised model. Efficacy in a female model of sexual function was determined in a, sexual proceptivity (non-contact) test. The models used to determine efficacy in males were, penile erectile function in conscious telemetered rats and changes in intracavernosal pressure following dorsal penile nerve stimulation in anaesthetised rats. In addition, studies were conducted in conscious telemetered rats to understand the cardiovascular effects of PF-00446687, and also in conscious ferrets to understand the potential for emesis. These studies have been performed to develop confidence in the rationale that a centrally acting selective MC4 agonist will be effective in the treatment of female and male sexual dysfunction without the dose-limiting cardiovascular and emesis side effects associated with a non-selective MC agonist such as PT-141 (Hedlund, 2004).

Table 2. Key In Vivo Pharmacologic Activities of PF-00446687

Activity	Treatment	End-point
Conscious Rat Proceptive female sexual behaviour^a	(µg/kg SC)	% time investigating male minus female ± SEM (n)
	Vehicle	-15±5 (n = 14)
	PF-00446687 (1.0)	26±8 (n = 8)*
	PF-00446687 (1.0) + SHU-9119 (30)	-9±7 (n = 8)
	SHU-9119 (30) alone	-3±9 (n=4)
	Estradiol (15 µg)/Progesterone (1000 µg)	41±7 (n = 9)*
Conscious Rat Male sexual function, pro-erectile activity^b	PF-00446687 (µg/kg SC)	Number of erectile responses in 2h period post-dose ±SEM (n)
	Vehicle	12.0±1.6 (n = 12)
	0.1	11.4±2.5 (n = 12)
	1	20.4±2.9 (n = 12)*
	3	20.9±1.3 (n = 12)*
	10	20.6±2.6 (n = 12)*
	100	21.4±2.0 (n = 12)*
Anaesthetised Rat Male Sexual Function Dorsal penile nerve stimulation^c	PF-00446687 (3 mg/kg IV)	Area under the curve of 2Hz stimulation-induced increases in ICP/blood pressure ± SEM (n)
	Vehicle	22.59±1.54 (n = 7)
	PF-00446687	32.99±2.57 (n = 8)*
Conscious Ferret Emesis^d	PF-00446687	Incidence of retching and vomiting
	5, 10 mg/kg SC and 5 mg/kg IV	None detected (n = 6 at 10 mg/kg SC and n = 3 at 5 mg/kg SC and IV)
Conscious Rat Cardiovascular	PF-00446687	Incidence of haemodynamic effects
	30, 100, and 300 µg/kg SC	No biologically relevant effects

Statistical analysis was performed using ANOVA: *p<0.01 when compared with vehicle.

SEM = standard error of the mean for n separate experiments; MED = minimum effective dose.

^a PF00446687/DI/014/04; ^b PF00446687/DI/007/04; ^c PF00446687/DI/014/06; ^d Study No. 04137

5.1.2.1. Effect of PF-00446687 on Female Proceptive Sexual Behaviour

PF-00446687 has been studied in a conscious female rat model of proceptive sexual behaviour (DI/014/04). The proceptive score is calculated as the percentage of the time that an ovariectomised female, primed with a low dose of oestradiol, spends actively investigating a vigorous, sexually active male rat minus the time spent actively investigating an oestrous female rat during a 10-minute period. The test female is separate from the stimulus male and female rat by a perforate grill, so that copulatory activities cannot occur. The positive control (mimicking oestrous) is an ovariectomised rat primed with high-dose oestradiol (15 µg)/progesterone (1000 µg). PF-00446687, when administered subcutaneously to sexually-inexperienced ovariectomised female rats pre-treated with a minimal oestradiol prime (1 µg/rat), increased the time spent actively exploring a sexually-experienced male rat compared with a sexually-experienced female rat (sexual proceptivity). PF-00446687 increased sexual proceptivity when compared with vehicle-control animals, at 1 µg/kg SC (p<0.01). Furthermore, PF-00446687 at 1 µg/kg SC failed to cause an increase in female proceptivity in the presence of the MC3-MC4 receptor antagonist, SHU-9119 (30 µg/kg).

5.1.2.2. Effect of PF-00446687 in a Conscious Rat Telemetry Model of Erection

In male rats, PF-00446687, when administered subcutaneously in a blinded, randomised, crossover study design, increased the number of spontaneous penile erections observed. The number of penile erections showed a dose-dependant increase over the given range (0.1 to 100 µg/kg), with the maximum effective dose at 1 µg/kg SC. Statistical analysis demonstrated that the following doses of PF-00446687 caused a significant increase at the 1% level in the number of penile erections: 1, 3, 10, and 100 µg/kg; the NOEL was 0.1 µg/kg.

5.1.2.3. Effect of PF-00446687 in an Anaesthetised Rat Model of Penile Erection

In this model male rats are first anaesthetised and centrally mediated inhibitory inputs removed. Following compound administration, the dorsal penile nerve is electrically stimulated (1 msec, 6 V at 1, 2 or 5 Hz for 20 seconds) to produce a rise in intracavernosal pressure in the penis. The increase in the AUC is then rectified to the systemic BP to investigate drug effects compared with vehicle. PF-00446687 (3 mg/kg, IV) resulted in a 40 to 50% increase in the AUC/BP for all 3 stimulation frequencies. This increase was statistically significant following the 2 and 5 Hz stimulation ($p < 0.01$ compared with vehicle), but not the 1 Hz ($p = 0.11$). The mean free plasma concentration of PF-00446687 in samples taken immediately after the last stimulation was 28.3 ± 2.1 nM.

5.1.2.4. Effect of PF-00446687 in a Ferret Model of Emesis (Study No. 04137; Table 28)

The aim of this study was to assess the emetic potential of PF-00446687 administered by the SC route (5 and 10 mg/kg) and IV route (5 mg/kg) in ferrets (3 to 6 males/group) using a modified-crossover design. A control groups of ferrets received saline, either SC or IV. Other clinical signs (sneezing, licking and yawning) were also specifically monitored.

PF-00446687 did not induce emesis or retching, although the compound produced yawning and licking when given by either route.

Pharmacokinetic measurements of PF-00446687 in plasma and CSF were performed on Day 7. At 10 mg/kg SC, ferrets were exposed to plasma drug concentrations that decreased slowly with time with residual levels detected 24 hours after dosing. At 5 mg/kg IV ferrets were exposed to plasma PF-00446687 concentrations that decreased rapidly with time. Thirty minutes after dosing, the CSF drug levels were about 2-fold higher in the animal dosed at 5 mg/kg IV when compared with the animal dosed at 10 mg/kg SC.

Based upon parallel pharmacokinetic studies, the maximum plasma exposure achieved in this study was 178 nM.

In conclusion, PF-00446687 had no emetic effect at the doses tested.

5.1.2.5. Effect of PF-00446687 on Cardiovascular Parameters in Conscious Rats

The effects of PF-00446687 at doses of 30, 100, and 300 µg/kg SC were investigated in telemetered, male rats. No effects on blood pressure were observed, with the exception of a

statistically significant increase in mean arterial pressure 0 to 2 hours after administration of 100- μ g/kg dose of PF-00446687 (increase of 6 ± 3 mm/Hg compared with vehicle animals, ($p < 0.05$). However, since this increase was small and since neither 30- nor 300- μ g/kg had any effect on arterial pressure, this effect was considered not to be of biological relevance. There were no effects on heart rate at all dose levels.

5.1.3. Safety Pharmacology

The wider pharmacological properties of PF-00446687 were assessed to evaluate the effects, if any, on the major physiological systems. For in vitro experiments, the highest concentration tested was up to 30 μ M (14.1 mg/mL). For in vivo studies in rodents, the dose levels selected (0.01, 0.1, 1.0, and 10 mg/kg IV) achieved $C_{max,u}$ levels ranging from 0.08 nM (0.039 ng/mL) at 0.01 mg/kg IV up to $C_{max,u}$ levels of 145.6 nM (68.3 ng/mL) at 10 mg/kg IV; the latter dose showed no adverse events in the previously conducted symptomatology study in rats. For the in vivo study in dogs, PF-00446687 was administered via loading doses (50, 150, and 500 μ g/kg) given intravenously over 10 minutes, followed by an intravenous 20-minute maintenance dose (0.7, 2.4, and 7.0 μ g/kg/min, respectively) that achieved free plasma concentrations of 1.1 nM (0.5 ng/mL), 4.5 nM (2.1 ng/mL), and 15.5 nM (7.3 ng/mL), respectively.

5.1.3.1. In Vitro Safety Pharmacology Studies

5.1.3.1.1. Radioligand Binding, Ion Channel and Enzyme Assay

The profiles of PF-00446687 in radioligand binding, ion channel, and enzyme assays ($n = 2$) and in vitro safety pharmacology studies, are summarized in Table 3.

Table 3. Summary of the In Vitro General and Safety Pharmacology Studies with PF-00446687

Receptors		Ion Channels	Enzymes / Uptake Transporters
PF-00446687 had $IC_{50} > 10 \mu M$ (4.7 mg/mL) for the following targets:			
Adenosine	A_1, A_2, A_3 (human)	[3H]-dofetilide binding to HEK293 cells expressing human recombinant hERG (I_{Kr}) See section 5.1.3.1.2	Phosphodiesterase (human 2,3,4,5,6, and 11)
Adrenergic	α_1 (non-selective, rat cerebral cortex), $\alpha_{2A}, \alpha_{2B}, \beta_1, \beta_2, \beta_3$ (human)		
Angiotensin-II	AT_1, AT_2 (human)		Acetylcholinesterase (human)
Benzodiazepine	BDZ (rat cerebral cortex)		Choline transporter
Bradykinin	B_2 (human)		Monoamine Oxidase –A (human)
Cannabinoid	CB_1, CB_2 (human)		Monoamine Oxidase –B (rat brain)
Calcitonin gene-related peptide	CGRP (human)		Tyrosine hydroxylase (rat striatum)
Dopamine	$D_1, D_{2s}, D_3, D_{4.4}$ (human)		Na^+/K^+ ATPase (dog kidney)
Endothelin	ET_B (human)		
GABA	$GABA_A, GABA_B$		

n = 2, unless otherwise stated.

NMDA = N-methyl-D-aspartate; Na^+/K^+ = sodium/potassium ion ratio; ATPase = adenosine triphosphatase;

NE = norepinephrine; DA = dopamine; 5-HT = serotonin;

Table 3. Summary of the In Vitro General and Safety Pharmacology Studies with PF-00446687 (Continued)

Receptors		Ion Channels	Enzymes / Uptake Transporters
PF-00446687 had $IC_{50} > 10 \mu M$ (4.7 mg/mL) for the following targets:			
Glutamate	NMDA (rat cerebral cortex)	Action potentials recorded from dog isolated Purkinje fibres See section 5.1.3.1.2.	NE transporter (human)
Glycine	Strychnine-insensitive		DA transporter (human)
Histamine	H ₁ (guinea-pig cerebellum), H ₂ , H ₃		GABA transporter
L1	Peripheral		Choline transporter
Leukotriene	LTD4 (human)		5-HT transporter (human)
Ghrelin	Human		Guanylate cyclase
Melanocortin	MC ₁ , MC ₄ (human)		GABA transaminase
ML	ML ₁ (chicken), ML ₂ (hamster)		Catechol-O-methyl transferase
Muscarinic	M ₁ , M ₂ , M ₃ (human)		NO synthase (iNOS)
Neurokinin	NK ₁		Metalloproteinase (1,2,3,7 and 9 human)
N	(neuronal) (alpha -BGTX-insensitive)		Neutral endopeptidase (human)
Opiate	kappa, mu, $\delta 2$ (human)		Angiotensin Converting Enzyme (human)
Nicotinic	Neuronal, non-selective (rat cerebral cortex)		HIV-1 protease
Nociceptin	ORL1 (human)		Tryptase (human)
Oxytocin	OT (human)		Elastase
Phencyclidine	PCP (rat)		Phosphatase 1B (human)
Purinoreceptors	P2X (rat)		ZAP70 kinase
Serotonin	5-HT _{1A} (human), 5-HT _{1B} (rat cerebral cortex), 5-HT _{1D} (bovine), 5-HT _{2A} , 5-HT _{2B} , 5-HT _{2C} , 5-HT ₃ , 5-HT _{4e} , 5-HT ₆ , 5-HT ₇ (human)		
Sigma	Non-selective		P55fyn kinase

n = 2, unless otherwise stated.

NMDA = N-methyl-D-aspartate; Na^+/K^+ = sodium/potassium ion ratio; ATPase = adenosine triphosphatase; NE = norepinephrine; DA = dopamine; 5-HT = serotonin.

Table 3. Summary of the In Vitro General and Safety Pharmacology Studies with PF-00446687(Continued)

Receptors	Ion Channels	Enzymes / Uptake Transporters
PF-00446687 had IC ₅₀ >10 µM (4.7 mg/mL) for the following targets:		
Somatostatin	Sst4, sst5	P56lyn kinase
Glucocorticoid	GR (human)	Abl kinase
Estrogen	ERalpha	MAP kinase
Tyrosine hydroxylase	TH	Calcium modulated kinase
Urotensin	UT-II	
Vasoactive intestinal peptide	VPAC1 (human)	
Vasopressin	V1a (human)	
Ryanodine	RY3	
Chemokines	CCR1 (human)	

Summary of binding IC₅₀ values (µM) for PF-00446687:

Receptor	IC ₅₀ µM
Histamine	H ₂
Melanocortin	MC4
Muscarinic	M1
Muscarinic	M2
Opioid	Kappa
Opioid	Mu
Neurokinin	NK1
Sigma	Sigma (non-selective)
Amine transporter	Noradrenaline transporter
Amine transporter	5-HT transporter
Ca ²⁺ channels	L-type, verapamil site
Na ⁺ channels	Site 2

n = 2, unless otherwise stated.

NMDA = N-methyl-D-aspartate; Na⁺/K⁺ = sodium/potassium ion ratio; ATPase = adenosine triphosphatase; NE = norepinephrine; DA = dopamine; 5-HT = serotonin; DHP = dihydropyridine; K⁺ = potassium ion; ATP = adenosine triphosphate; Ca²⁺ = Calcium ion; Na⁺ = sodium ion; Cl⁻ = chloride ion ; nH = Hill slope.

5.1.3.1.2. Action Potential Duration in Isolated Dog Purkinje Fibres

PF-00446687 was tested in vitro at concentrations of 0.1, 0.3, 1, and 10 µM (0.047, 0.141, 0.47, 4.7 µg/mL, n = 3) for effects on cardiac action potentials evoked in isolated dog Purkinje fibers. At 0.1 µM, PF-00446687 had no effects on resting membrane potential, action potential amplitude APD₅₀, APD₉₀, or maximum rate of depolarization. At 0.3 to 10 µM, PF-00446687 induced a significant (p <0.05) shortening of APD₅₀ and APD₉₀ (0.3 µM: APD₅₀ -17.3±2.8%, APD₉₀ -10.6±1.2%). At 10 µM, a reduction in V_{max} was also observed (-12.4±5.5%).

5.1.3.1.3. Effect on hERG Current Stably Expressed in HEK-293 Cells

PF-00446687 was evaluated for its effect on the hERG potassium channel stably expressed in HEK-293 cells. PF-00446687 at concentrations of 0.3 µM (0.14 µg/mL) to 30 µM

(14.12 µg/mL) reduced peak hERG potassium current amplitude in a concentration dependent-manner with an IC₅₀ value of 3.98 µM (1.87 µg/mL).

5.1.4. In Vivo Safety Pharmacology studies

Results of the in vivo safety pharmacology studies with PF-00446687 are summarized in Table 4.

Table 4. Summary of Exposures Obtained in In Vivo Safety Pharmacology Studies of PF-00446687

Species	Dose (IV)	Mean Maximum Unbound Drug Concentration in Plasma	
		nM	ng/mL
Rodent	0.01 mg/kg	0.084	0.039
	0.1 mg/kg	1.6	0.75
	1.0 mg/kg	13.5	6.4
	10 mg/kg	145.6	68.3
Dog	50 µg/kg followed by	1.1	0.5
	0.7 µg/kg/min		
	150 µg/kg followed by	4.5	2.1
	2.4 µg/kg/min		
	500 µg/kg followed by	15.5	7.3
	7.0 µg/kg/min		

In rats, drug exposure in plasma was obtained 30 minutes after dosing. In dogs, drug levels were determined at the end of each maintenance infusion. The unbound drug values in plasma were calculated using fraction unbound plasma protein binding values of 0.07 (rats) and 0.15 (dogs)

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Table 5. Summary of In Vivo Safety Pharmacology Studies with PF-00446687

Experiment	Doses	Standard	Result/Observation
Maximum tolerated dose rat, male, Sprague Dawley (n = 5)	3, 10, and 30 mg/kg (IV)	None	3 mg/kg – 2 rats had slightly decreased activity, 1 hour after dosing. Diarrhoea occurred episodically in 2 rats. One rat had "wet dog shakes" approximately 5 minutes after dosing. Body weight and temperature remained unchanged. 10 mg/kg – irregular respiration in 3 rats during the first 15 minutes after dosing. Four rats had slightly increased reactivity to acoustic stimuli between 15 and 30 minutes. One rat had slightly increased home cage activity 15 minutes after dosing. Chromodacryorrhoea occurred in 3 rats between 15 and 30 minutes. Two rats were chewing between 0 and 30 minutes and were stretching 1 hour after dosing. One rat had "wet dog shakes" 1 hour after dosing. Body weight and temperature remained unchanged. 30 mg/kg – the first rat of the group died immediately after administration of PF-00446687 and consequently other rats were not treated. The maximum tolerated dose was 10 mg/kg IV corresponding to a C_{max} of 119 nM (56 ng/mL).
Effects on spontaneous locomotor activities rat, male, Sprague Dawley (n = 6)	0.01, 0.1, 1.0, and 10 mg/kg (IV)	Diazepam	No effect on any measured behaviour at 0.01, 0.1, and 1.0 mg/kg over 5 hours after dosing. PF-00446687 at 10 mg/kg significantly reduced rearing behaviour for the duration of the assessment period ($p < 0.05$) and also reduced exploratory locomotor activity 20 minutes after dosing.
Respiratory and Renal system rat, male, Sprague-Dawley (n = 10)	0.01, 0.1, 1.0, and 10 mg/kg (IV)	None	Respiratory parameters: No effect at 0.01 and 0.1 mg/kg on either respiratory rate or tidal volume up to 5 hours after dosing. A statistically significant increase in respiratory rate was observed at 1.0 and 10 mg/kg ($p = 0.01$ and $p < 0.001$, respectively); the change at 10 mg/kg corresponded to a mean increase of 12 breaths per minute (breaths / minute: vehicle, 107 ± 4 (n = 7) compared with 10 mg/kg, 119 ± 3 (n = 10)) over the course of the experiment. A significant ($p < 0.05$) decrease in tidal volume occurred at 10 mg/kg (mean 0.07 mL compared with the vehicle group, when averaged over time. Minor effects observed on respiratory rate at 1 mg/kg were unlikely to be of biological relevance. Renal parameters: Over 0 to 2 hours, doses of 0.01 and 0.1 mg/kg had no effect on the urine volume, pH or ion excretion. The statistical significance ($p < 0.05$) assigned to effects on urine volume and Cl^- ion concentration at 0.01 mg/kg are unlikely to be biologically relevant. At 1 and 10 mg/kg, PF-00446687 significantly increased the quantity of urine ($p < 0.01$) and Na^+ , K^+ and Cl^- ($p < 0.01$ and $p < 0.05$) excretion in a dose-dependent manner. Over the 2- to 5-hour period, there was a significant increase in urine volume, Na^+ and Cl^- ion excretion at 10 mg/kg. A statistically significant increase in Na^+ ion concentration was also produced at 1 mg/kg over the 2- to 5-hour period, although this is unlikely to be biologically relevant.
Haemodynamic and electrocardiogram (ECG) parameters ^c conscious dog, male, Beagle (n = 4)	50 µg/kg loading dose followed by 0.7 µg/kg/min maintenance infusion, 150 µg/kg followed by 2.4 µg/kg/min, 500 µg/kg followed by 7.0 µg/kg/min (IV)	None	After the first maintenance infusion of 0.7 µg/kg/min, there were no biologically relevant effects on any of the parameters measured (systolic BP, diastolic BP, mean BP, LV end diastolic pressure, LV systolic pressure, LV+dP/dt, HR, T wave amplitude, QRS interval, PR interval, QT interval, or QTc ⁶⁰). After the second infusion of 2.4 µg/kg/min, there were small significant falls in LVSP (-6 mmHg $p < 0.05$), SBP (-8 mmHg $p < 0.05$) and a small increase in myocardial contractility (approximately 170 mmHg/sec). After the highest dose of 7.0 µg/kg/min, there were small significant falls in LVSP (-6 mmHg $p < 0.05$), SBP (-8 mmHg $p < 0.05$), MBP (-4 mmHg $p < 0.05$), PR interval (-9 msec, $p < 0.05$), a small increase in HR (12 bpm, $p < 0.05$) and a small increase in myocardial contractility (approximately 300 mmHg/sec).

The unbound drug values in plasma from rats and dogs were calculated using fu values of 0.07 and 0.15, respectively.

CO₂ = carbon dioxide; K⁺ = potassium ion; Cl⁻ = chloride ion; Na⁺ = sodium ion; LV = left ventricular; dP/dt = change in ventricular pressure over the change in time; HR = heart rate; QTc⁶⁰ = QT interval corrected for heart rate and normalized to 60 beats/minute.

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5.1.4.1. Effects on Central and Peripheral Nervous System

The maximum tolerated dose for IV administration of PF-00446687 in rats was defined as 10 mg/kg. At 30 mg/kg, the first rat dosed died immediately after PF-00446687 administration. There were no effects on locomotor activity at 0.01, 0.1, and 1.0 mg/kg IV; however, a significant ($p < 0.05$) reduction in rearing behaviour and a lesser reduction in exploratory locomotor activity were seen at 10 mg/kg IV. Given that the behavioural effects were observed at 10 mg/kg IV doses ($C_{\max}$ 976 ng/mL, $C_{\max,u}$ 145.6 nM) they are considered unlikely to be clinically relevant.

5.1.4.2. Effects on the Respiratory System

PF-00446687 at 0.01 and 0.1 mg/kg in rats had no discernable effect on either respiratory rate or tidal volume up to 5 hours after dosing. The top 2 doses of PF-00446687 produced significant ($p = 0.01$ and < 0.001 , respectively) increases in respiratory rate over the vehicle group with the treatment effect increasing with dose. This change was equivalent to a mean of 12 breaths per minute over the course of the experiment following a 10-mg/kg dose. Also, the top dose of PF-00446687 showed a statistically significant ($p < 0.05$) decrease in tidal volume (mean 0.07 mL following the 10 mg/kg) over the vehicle group, when averaged over time. The effects observed on respiratory rate at 1 mg/kg were minor and unlikely to be clinically relevant.

5.1.4.3. Effects on Renal System

At the lower doses of 0.01 and 0.1 mg/kg, PF-00446687 in rats had little effect on the urine volume, pH, and ions excreted when compared with the vehicle-treated animals. The significant effects ($p < 0.05$) obtained at 0.01 mg/kg for urine volume and chloride ion concentration are unlikely to be biologically relevant. At 1 and 10 mg/kg, PF-00446687 significantly ($p < 0.05$ and $p < 0.01$, respectively) increased the quantity of urine and the ion concentrations excreted over the 0 to 2 hour measurement period in a dose-dependent manner. Over the 2 to 5 hour collection period, PF-00446687 at 10 mg/kg produced statistically significant ($p < 0.05$ and $p < 0.01$, respectively) increases in urine volume and the sodium and chloride ion concentration. A statistically significant increase in sodium ion concentration was also induced at 1 mg/kg over the 2 to 5 hour collection period, although this is unlikely to be biologically relevant. Overall, at 1 mg/kg IV, the increased urine volume and excretion of Na^+ , K^+ , and Cl^- occurred at clinically relevant levels. However, urinary creatinine, osmolality and electrolytes (sodium, potassium, and chloride) were monitored in the first-in-human study and there were no dose-related changes of note.

5.1.4.4. Effects on the Cardiovascular System

The effect of intravenously administered PF-00446687 on haemodynamic and ECG parameters was studied in conscious telemetered dogs. After the first maintenance infusion of $0.7 \mu\text{g/kg/min}$, there were no biologically relevant effects on any of the parameters measured (systolic BP, diastolic BP, mean BP, LV end diastolic pressure, LV systolic pressure, LV+dP/dt, HR, T wave amplitude, QRS interval, PR interval, QT interval, or QTc⁶⁰). After the second and third infusions, there were dose-related reductions in blood pressure and small reflex dose-related increases in myocardial contractility. These data indicate that PF-00446687 may have a

mild vasodilator action at unbound plasma concentrations of 15.5 nM (7.3 ng/mL). The reduction in the PR interval seen during the third infusion of 7.0 µg/kg/min was consistent with the small increase in heart rate, given the reciprocal relationship between these parameters. There were no significant changes in QTc at any dose level. The lack of effect of PF-00446687 on QTc is consistent with the observations at similar dose concentration ranges in the in vitro I_{Kr} assay. Collectively, these in vitro and in vivo data support the cardiovascular safety of PF-00446687 in humans at therapeutic dose levels.

5.1.4.5. Safety and General Pharmacology Summary

In conclusion, studies examining the general pharmacological properties of PF-00446687 demonstrated that the compound was well tolerated and had little interaction with physiologically important receptors, binding sites, enzymes, or ion channels at relevant concentrations (up to 10 to 100 µM). In the conscious dog, PF-00446687 has a modest vasodilator action at 15.5 nM (7.3 ng/mL) resulting in small reflex changes in heart rate and myocardial contractility. PF-00446687 at doses of 30 to 300 µg/kg SC also produced no dose-related change in heart rate or blood pressure in conscious telemetered rats. Although there was a concentration dependent block of hERG potassium current in vitro there were no significant changes in QTc at any dose level in vivo, which is consistent with observations at similar concentration ranges in the in vitro I_{Kr} assay. In ferrets, PF-00446687 was devoid of emesis up to a C_{max,u} of 178 nM. In rats, a dose of 1 mg/kg (C_{max,u} of 13.5 nM, 6.4 ng/mL) produced minor changes in respiratory parameters and significant increases in urine volume and electrolyte (Na⁺, K⁺, and Cl⁻) secretion. However, urinary creatinine, osmolality, and electrolytes (sodium, potassium, and chloride) were monitored in the first-in-human study and there were no dose-related changes of note. PF-00446687 was well tolerated centrally and there were no effects on locomotor activity up to doses of 1.0 mg/kg IV (C_{max,u} of 13.5 nM, 6.4 ng/mL); CNS effects seen at doses of 10 mg/kg IV (C_{max,u} of 145.6 nM, 68.3 ng/mL) are unlikely to be of clinical relevance. Overall, the non-clinical data generated in rats and dogs indicate that PF-00446687 should be well tolerated in humans at therapeutic doses levels.

Reference

Hedlund P. PT-141 Palatin. Curr Opin Investig Drugs 2004;5(4):456-62.

5.2. Pharmacokinetics and Product Metabolism in Animals

Oral, intravenous, and sublingual pharmacokinetics of PF-00446687 have been investigated in rats and dogs using specific chromatographic methods (HPLC and mass spectrometry) for the analysis of drug and metabolites in biological fluids. Strains of animals used (Sprague-Dawley rats and Beagle dogs) correspond to those used in toxicology studies.

5.2.1. Absorption and Bioavailability

PF-00446687 is a lipophilic base (LogD_{7.4} octanol 2.8 and pKa 9.0) with a molecular weight of 470.59.

Following sublingual administration of PF-00446687 to male and female dogs (2, 10, and 30 mg), absorption of PF-00446687 was rapid with t_{max} occurring in the first hour. The sublingual bioavailability values for PF-00446687 were between 20% and 35%.

Following oral administration of 1 and 0.5 mg/kg of PF-00446687 to rats and dogs, respectively, the bioavailability of PF-00446687 was 20% and $\leq 1\%$, respectively, limited by hepatic first-pass metabolism. Maximum plasma concentrations of PF-00446687 were reached between 1 and 2 hours after dosing.

5.2.2. Single-Dose Pharmacokinetics

Pharmacokinetic parameters following single IV, PO and SL doses to male rats (jugular vein cannulated) and male and female dogs are summarised in Table 6.

Single-dose intravenous pharmacokinetic studies were conducted in jugular vein-cannulated male rats at a dose level of 1 mg/kg ($n = 3$). A mean plasma elimination half-life of 2.4 hours was observed due to a plasma clearance of 89 mL/min/kg combined with a volume of distribution of 19 L/kg.

In male dogs, single-dose intravenous pharmacokinetic studies were conducted at a dose level of 0.5 mg/kg ($n = 2$). PF-00446687 exhibited an elimination half-life of 4.2 hours due to a plasma clearance of 46 mL/min/kg and a volume of distribution of 17 L/kg.

The sublingual pharmacokinetics of PF-00446687 were determined in male and female dogs following a single sublingual dose of 2, 10, or 30 mg. PF-00446687 was rapidly absorbed with maximum plasma concentrations being reached within the first hour after dosing.

Following oral administration to rats and dogs (1 and 0.5, 100, and 300 mg/kg, respectively), PF-00446687 was rapidly absorbed with unbound C_{max} values of 0.91 nM (rat), 0.23 and 1.88 μ M (dog; 100 and 300 mg/kg, respectively) with T_{max} occurring at 1.5 and 0.5 hours (rat, and dog, respectively).

Summary pharmacokinetic data are shown in Table 6.

Table 6. Single-Dose Pharmacokinetics of PF-00446687 in Non-clinical Species

Species	Dose (mg/kg)	Route	N Gender	CL (mL/min/kg)	Vd (L/kg)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·h/mL)	F (%)
Rat	1	IV	3/M	89	19	2.4	-	-	191	-
	1	PO	3/M	-	-	-	1.5	6.1	38	20
Dog	0.5	IV	2/M	46	17	4.2	-	-	183	-
	2	SL	2/M&2/F	-	-	3.7	0.65	2.6	17	35
	10	SL	2/M&2/F	-	-	3.6	0.31	15	64	26
	30	SL	2/M&2/F	-	-	4.0	0.31	41	146	20
	0.5	PO	2/M	-	-	-	-	ND	ND	<1
	100	PO	1/M	-	-	-	0.5	709	1810	-
(06AM142)	300	PO	1/M	-	-	-	0.5	5890	32500	-

ND not done

5.2.3. Toxicokinetics

Toxicokinetic data have been obtained from Sprague-Dawley rats (3 rats/gender/dose) and Beagle dogs (3 dogs/gender/dose) in 1-month intravenous toxicology studies and a 4-Day IVT oral exploratory study in Sprague-Dawley rats (3 rats/dose). The results are summarised in Table 6, along with human pharmacokinetics for comparison. (Study Nos. 05157 and 06AM037 (rat) 05158 (dog), A8361001 (human)). No apparent gender differences were seen in dogs; therefore, the data were combined.

Following repeated daily intravenous bolus administration of PF-00446687 at doses of 1, 3, and 9 mg/kg/day to male and female rats, the mean C_{max} and $AUC_{(5min-24h)}$ values of PF-00446687 on Days 1 and 27 were determined. Mean values for male and female rats are summarised in Table 7.

Table 7. Summary of Toxicokinetic Data for PF-00446687 in Non-clinical Species with Comparative Pharmacokinetic Data in Human

Species	Dose (mg/kg/day)	Route	N/ Gender	C_{max} (ng/mL) (free nM in brackets) ^a	C_{max} (ng/mL) (free nM in brackets) ^a	$AUC_{5min-24h}$ (ng.h/mL) (free nM.h in brackets) ^a	$AUC_{5min-24h}$ (ng.h/mL) (free nM.h in brackets) ^a
				Day 1	Day 27	Day 1	Day 27
Rat (Study 05157)	1	IV	3M	109 (16.2)	118 (17.6)	219 (32.6)	233 (34.7)
	1	IV	3F	78.7 (11.7)	91.6 (13.6)	269 (40.0)	318 (47.3)
	3	IV	3M	NR	382 (56.8)	NR	787 (117)
	3	IV	3F	NR	274 (40.8)	NR	826 (123)
	9	IV	3M	1020 (152)	NR	1890 (281)	NR
	9	IV	3F	917 (136)	768 (114)	3130 (466)	3090 (460)
	100	PO	3M	1020 (152)	NA	15400 (2290) ^c	NA
	300	PO	3M	7000 (1041)	NA	18700 (2782) ^c	NA
	1000	PO	3M	9550 (1421)	NA	76200 (11335) ^c	NA
Dog (Study 05158)	1	IV	3M/3F	114 (36.3)	127 (40.5)	341 (109)	356 (113)
	3	IV	3M/3F	377 (120)	439 (140)	844 (269)	1100 (351)
	10	IV	3M/3F	1370 (437)	1580 (504)	3350 (1068)	3990 (1272)
Human	195 mg ^b	PO	4M	30.0 (8.29) ^c	NA	523 (144) ^{cd}	NA

^a Calculated using the molecular weight of 470.59 and the free fraction in rat (0.07), dog (0.15) and human (0.13)

^b 195 mg total dose Study A8361001

^c geometric mean

^d AUC_{∞}

^e AUC_{0-24}

NR no result

NA not applicable

PF-00446687 plasma concentrations were similar in male and female rats. Exposure appeared to increase in a dose-proportional manner over the dose range studied and was similar on Days 1 and 27.

Following repeated daily intravenous bolus administration of doses of 1, 3, or 10 mg/kg/day of PF-00446687 to male and female dogs, the mean maximum C_{max} and $AUC_{(5min-24h)}$ values of PF-00446687 on Days 1 and 27 were determined. There were no obvious differences in the plasma concentrations observed in male and female ($n = 3$ per gender) dogs; therefore, the observed plasma concentration data for the two genders were combined and the mean values are shown in Table 6. Over the dose range examined in dogs, exposure appeared to increase in a dose-proportional manner and plasma concentrations were similar on Days 1 and 27.

Plasma concentrations were similar in male and female rats. Exposure appeared to increase in a dose-proportional manner over the dose range studied and was similar on Days 1 and 27.

Following repeated daily intravenous bolus administration of doses of 1, 3, or 10 mg/kg/day of PF-00446687 to male and female dogs, the mean C_{max} and $AUC_{(5min-24h)}$ values of PF-00446687 on Days 1 and 27 were determined. There were no obvious differences in the plasma concentrations observed in male and female ($n = 3$ per gender) dogs; thus, the observed plasma concentration data for the two genders were combined and the mean values are shown in Table 7.

5.2.4. Distribution

5.2.4.1. Plasma-Protein Binding

Preliminary estimates of plasma-protein binding of PF-00446687 have been determined by equilibrium dialysis in control plasma from rats, dogs, and humans. Mean values for percent bound were 93%, 85%, and 87%, respectively.

5.2.4.2. In Vitro Blood:Plasma Concentration Ratio.

The in vitro blood:plasma concentration ratio of PF-00446687 has been determined in fresh blood from rats, dogs, and humans. Mean values were 1.1, 1.1, and 1.1, respectively. Therefore, PF-00446687 distributes evenly between the plasma and red-cell fractions.

5.2.4.3. Distribution into the CNS

The CNS penetration of PF-00446687 was assessed in rats following intravenous infusion to steady state. The ratio of the concentration of PF-00446687 in the CSF over the unbound concentration in plasma was 0.9 ± 0.3 , indicating that PF-00446687 can freely penetrate the blood-brain barrier.

5.2.5. Excretion and Metabolism of PF-00446687

5.2.5.1. In Vitro Metabolism Studies

The rate of metabolism of PF-00446687 has been determined in hepatic microsomal fractions from rats, dogs, and humans. All incubations were carried out under standard conditions of 1 μ M substrate and 0.5 μ M P450 concentrations. PF-00446687 was metabolised extensively in rat, dog, and human liver microsomes with mean apparent intrinsic clearance values of 60, 242, and 45 μ L/min/mg protein, respectively. In rat, dog and human hepatocytes, PF-00446687 was

metabolised extensively with intrinsic clearance values of 67, 105, and 9 $\mu\text{L}/\text{min}/\text{million cells}$ respectively.

These data are in keeping with the high clearance of this compound in non-clinical species.

Following incubation with human hepatocytes several metabolites of PF-00446687 were detected, including a mono-oxidised metabolite consistent with oxidation of the t-butyl, and an N-desalkyl metabolite consistent with the loss of the t-butyl. A similar metabolite profile was seen following incubation with hepatocytes of rats and dogs. No glucuronide or sulphate conjugates were detected in any of the hepatocyte extracts.

5.2.5.2. In vivo Metabolism Studies

In an isolated perfused liver preparation in the rat, PF-00446687 demonstrated almost complete extraction as judged by a clearance value of 13.2 mL/min (15 mL/min flow rate), which is in keeping with the high in vivo clearance of this compound. Less than 1% of the parent compound was eliminated unchanged in the bile.

Analysis of urine excreted by rats following intravenous administration of PF-00446687, indicated that <3% of the dose was excreted renally as parent compound.

These data suggest that metabolism is likely to be the major route of clearance and that renal and biliary elimination of unchanged PF-00446687 are minimal.

5.2.5.3. Potential for Drug Interactions

In human liver microsomes, PF-00446687 was shown to be a weak to moderate inhibitor of CYP2C19 ($\text{IC}_{50} = 19 \mu\text{M}$) and CYP3A4 ($\text{IC}_{50} \geq 30 \mu\text{M}$) metabolism. Inhibition of the other major CYPs (CYP1A2, CYP2C9, and CYP2D6, $\text{IC}_{50} > 30 \mu\text{M}$) was not observed. In addition, a time-dependent inhibition was seen for the CYP3A4 substrate midazolam. Following incubation for 30 minutes prior to addition of midazolam, the IC_{50} of PF-00446687 decreased from 30 μM to 6.9 μM . This observed time dependency may be an indication of mechanism-based inhibition. In silico predictions (using SimCYP®) of the magnitude of the DDI with midazolam as a result of time dependant inhibition of CYP3A4, indicate a potential 1.5-fold increase in AUC of midazolam when coadministered with 100 mg daily dose of PF-00446687. The predicted free concentration of PF-00446687 was approximately 10 nM on Day 4.

Characterisation of the CYP enzymes involved in the metabolism of PF-00446687 in vitro suggests that CYP3A4 is solely responsible for the metabolism of this compound, with almost complete inhibition of PF-00446687 metabolism (92%) observed in the presence of ketoconazole (1 μM). Simulations using SimCYP® software suggest that PF-00446687 plasma concentrations (AUC) may increase approximately 20-fold when co-administered with ketoconazole. A clinical ketoconazole interaction study is ongoing to establish the extent of this interaction and the likelihood of clinical significance.

5.2.6. Summary for Humans

In keeping with its physicochemistry, PF-00446687 combines significant solubility and membrane permeability, which results in complete CNS penetration and adequate exposure following oral dosing in humans.

5.3. Toxicology

PF-00446687 was assessed in a series of non-clinical in vivo and in vitro studies outlined in Table 8.

For in vivo studies, the intravenous route was generally used in order to maximize exposure, since the original intended clinical route (sublingual) is not practical in laboratory animals. Exceptions were the irritation studies in rats (oral route) and hamsters (topical application). An oral route toxicology program is being developed to accommodate the change in the intended clinical route to oral administration. A 7-day oral irritancy study in rats and 4-day oral exploratory studies in rats and dogs were performed to support oral administration in human subjects.

Table 8. Non-clinical Toxicity Studies

Study	
In Vivo Assays:	Dose (mg/kg) *
Acute Intravenous Toxicity Study in Mice (Study No. 05160)	20 and 40
Acute Intravenous Toxicity Study in Rats (Study No. 05159)	10 and 20
4-Day oral exploratory study in male rats (Study No. 06AM037)	100, 300, 1000
7-day oral gavage irritation study in rats (Study No. 05156)	10 and 20
4-day Intravenous Range-Finding Study in Rats (Study No. 05125)	7.5, 15 and 25→20
1-Month Intravenous Toxicity Study in Rats and In Vivo Micronucleus Assay (Study No. 05157)	1, 9→3 and 18→15→9
Oral escalating dose toxicity study in dogs (Study No. 06AM142)	100, 200, 300
Intravenous Escalating Dose Toxicity Study in Dogs (Study No. 05052)	3, 10, 12 and 16
1-Month Intravenous Toxicity Study in Dogs (Study No. 05158)	1, 3 and 10
6-Day Cheek Pouch Irritation Study in Hamsters (Study No. 04332)	1 and 1.6 mg/animal
In Vitro Assays:	Concentration
Bacterial Mutagenicity Assay (Study No. 05-2840-02)	0.01 to 5.0 mg/plate
Chromosome Aberration Assay in Human Lymphocytes (Study No. 05-2840-03)	To assay limits
Evaluation of the Phospholipidosis Potential in the In Vitro Human Lymphocyte Assay (Study No. 04351)	0.01, 0.10 and 1 µM

* if not stated otherwise

The respiratory and renal safety pharmacology assessment was performed using PF-00446687 formulated as a 10 mg/mL solution in 0.15 M acetate buffer, pH 4.5 to 5.0 in 5% w/v glucose (dextrose) solution in purified water and diluted as appropriate in this vehicle. The haemodynamic study was performed using PF-00446687-01 formulated in sterile water for injection B.P. The appearance and behavior assessment was performed using PF-00446687-01 formulated with 0.02 M acetic acid in 5% (w/v) glucose.

The acute and 1-month intravenous, and the oral irritation studies as well as the oral escalating dose study in dogs, and the in vitro genetic toxicology studies used PF-00446687 as a base. The

other studies used the compound as a hydrochloride salt. The vehicle employed in acute and 1-month toxicology studies was acetate buffer made isotonic with 5% glucose at a pH of 4.5 to 5. Details of other vehicles used are provided in the study summaries. For oral studies PF-00446687 was prepared as a suspension in the following vehicle: methylcellulose as a 0.5% (w/v) aqueous solution containing 0.1% (w/v) Tween 80.

Pivotal repeat-dose studies included assessments of clinical observations, ophthalmology, haematology and clinical chemistry parameters, urinalysis, plasma drug concentrations, full necropsy, and histopathology. In dogs, food consumption and cardiovascular parameters (blood pressure and electrocardiography) were also measured.

The key findings of these studies are summarized in the following paragraphs and presented in the accompanying tables.

5.3.1. Single-Dose Toxicity Studies

5.3.1.1. Acute Intravenous Toxicity Study in Mice (Study No. 05160; Table 17)

PF-00446687 was administered by IV injection to CD1 mice as single doses of 20 or 40 mg/kg. The animals (1 to 3/gender) were observed for up to 14 days following treatment.

At 40 mg/kg, one female died within 5 minutes of dosing and showed clinical signs of decreased activity, dyspnoea, inability to rise, and convulsions. Decreased activity and tremors were recorded in the male within 15 minutes after administration. At 20 mg/kg, there were no effects of treatment.

In conclusion, the highest non-lethal intravenous dose in mice was 20 mg/kg.

5.3.1.2. Acute Intravenous Toxicity Study in Rats (Study No. 05159; Table 18)

PF-00446687 was administered by IV injection to Sprague-Dawley rats as single doses of 10 or 20 mg/kg. The animals (1 to 3/gender) were observed for up to 14 days following treatment.

At 20 mg/kg, one male died within 5 minutes of dosing. Clinical signs were tremor and inability to rise (in both animals), and convulsions (within 5 minutes of dosing) and decreased activity (until 1 hour after administration) in females. At 10 mg/kg, there were no treatment effects.

In conclusion, the highest non-lethal intravenous dose in rats was 10 mg/kg.

5.3.2. Repeat-Dose Toxicity Studies

5.3.2.1. 4-Day Oral Exploratory Study in Male Rats (Study No. 06AM037; Table 19)

PF-00446687 was orally administered to male Sprague-Dawley rats (5/dose) for 4 days at 100, 300, and 1000 mg/kg. Satellite groups of 3 rats/dose were used for toxicokinetic assessment.

Animals were exposed to plasma PF-00446687 concentrations that increased with dose on Day 1, with significant drug concentration detected 24 hours after dosing.

The treatment induced death at 1000 mg/kg (2 out of 5) on Day 1. Deaths were preceded by decreased activity, prostration and convulsions. Death observed at 300 mg/kg was limited to the toxicokinetic animals (2 out of 3) and was possibly related to anaesthesia and/or blood sampling and treatment in this group as no adverse clinical signs were noted in the main group at this dose.

Tremors, partially closed-eyes and rough fur were observed in surviving animals of the 1000 mg/kg dose level. Decreased activity and increased salivation were noted at ≥ 300 mg/kg. Mean food consumption was decreased from 100 mg/kg (up to 91 % at 1000 mg/kg, compared with control) and body weight was decreased from 300 mg/kg (up to 21% at 1000 mg/kg, compared with control). Increase in liver weight without microscopic findings was noted from 100 mg/kg and was considered to be adaptative. Reticulocyte count was decreased from 100 mg/kg (up to 74% at 1000 mg/kg, compared with control). Variations in plasma chemistry parameters (increase in bilirubin and glucose, decrease in phosphate) and increases in red blood cell count, haemoglobin and haematocrit were observed from 300 mg/kg, possibly indicative of haemoconcentration. The white blood cell count decreased at 1000 mg/kg and may be the result of the combination of stress and the reduced food intake.

5.3.2.2. 4-Day Intravenous Range-Finding Study in Rats (Study No. 05125; Table 20)

PF-00446687 was administered by IV injection to Sprague-Dawley rats (5/gender/dose) at daily doses of 0, 7.5, 15, and 20 mg/kg for 4 days. Two high-dose animals received 25 mg/kg on Day 1, but as this was not well tolerated, the dose was reduced to 20 mg/kg. Satellite groups of 6/gender/dose were used for pharmacokinetic measurements of PF-00446687 on Days 1 and 4. Blood samples were taken 0.08, 0.33, 1, 3, 7, and 24 hours after dosing.

The rats were exposed to PF-00446687 concentrations in plasma that increased proportionally with dose, were slightly higher in females, and decreased slightly over the 4-day treatment period.

The treatment induced death 5 minutes after dosing on Day 1 in 1 of the 10 rats at the 15-mg/kg dose. In addition, 2 of 12 PK satellite animals died 5 minutes after administration of the 20-mg/kg dose on Days 1 and 2; blood sampling possibly contributed to the death of one of them. The causes of death were unknown.

The incidence and severity of clinical signs were dependent on dose, with minor clinical signs (stretching, yawning, urogenital licking, and sporadic excessive grooming) observed at all doses and marked clinical signs (tachypnoea, tremor, decreased activity, and prostration) from the 15-mg/kg dose. All clinical signs were transient and disappeared 30 minutes after dosing. At 25 mg/kg, severe clinical signs (prostration, tremor, and dyspnoea) were observed on Day 1 and necessitated reducing the high dose.

In conclusion, the intravenous NOAEL was 7.5 mg/kg in the rat given the death and marked clinical signs observed at 15 mg/kg.

5.3.2.3. 1-Month Intravenous Toxicity Study in Rats and In Vivo Micronucleus Assay (Study No. 05157; Table 21)

PF-00446687 was administered by IV injection to Sprague-Dawley rats (10/gender/dose). The initial daily doses were 1, 9, and 18 mg/kg. The initial high dose was reduced from 18 to 15 mg/kg on Day 2 due to death in the PK satellite group. Further dose adjustment occurred to the mid- and high-dose groups on Day 8 when doses were reduced to 3 and 9 mg/kg, respectively, due to poor toleration at the injection site. Satellite groups of 3 rats/gender/dose were used for pharmacokinetic measurements of PF-00446687 on Days 1 and 27. Blood samples were taken 5 minutes, and 1, 3, 7, and 24 hours after dosing.

Animals were exposed to PF-00446687 concentrations in plasma that increased with dose and were of the same order of magnitude in males and females. Exposure remained stable over the 27-day treatment period at 1 mg/kg in both genders and in females at 9 mg/kg.

Two rats treated with 15 mg/kg died on Days 3 and 4, 1 of which was associated with convulsions. In the PK satellite group, 2 rats treated with 18 mg/kg also died. One rat, treated with 9 mg/kg, exhibited convulsions and pedaling movements shortly after dosing on Day 24.

Treatment at the mid and high doses produced severe irritant effects at the injection site, which prevented continued daily treatment, particularly in high-dose males. As a result, 1 mid- and 1 high-dose female and 8 high-dose males were euthanised. Histopathological analysis indicated thrombosis and peri-vascular inflammation at the mid and high doses, and vascular necrosis and erosion/ulcer at the high dose. These findings correlated with clinical signs and necropsy findings (discoloration of the skin, scabs, haematoma, oedema, wound, and foci of necrosis on the tail). In the control and 3-mg/kg group, the clinical and histopathological changes relating to irritation at the injection site were similar and considered mild. The initial doses of 1, 9, and 15 mg/kg were injected as concentrations of 0.5, 4.5, and 7.5 mg/mL; after dose adjustment, the doses of 1, 3, and 9 mg/kg were injected at concentrations of 0.25, 0.75, and 2.25 mg/mL. The dose of 1 mg/kg (with injected concentrations of 0.25 and 0.5 mg/mL) produced no irritation at the injection site.

Lymphoid hyperplasia, congestion, plasmacytosis, and/or haemosiderosis occurred in the inguinal lymph nodes of some animals in the mid- and high-dose groups. Myeloid hyperplasia was present in the bone marrow of a few high-dose animals. Overall, these changes were mild and considered to be secondary to inflammation at the injection site.

In the bone marrow, there was no evidence of increased numbers of micronucleated polychromatic erythrocytes.

Because of the deaths that occurred at 15 mg/kg and the convulsions in a single animal at 9 mg/kg, the NOAEL is considered to be 3 mg/kg. PF-00446687 was irritant at the injection site at high-injection concentrations; the dose of 1 mg/kg produced no irritation with injection concentrations of 0.25 and 0.5 mg/mL.

5.3.2.4. Oral Escalating Dose Toxicity Study in Dogs (Study No. 06AM142; Table 22)

PF-00446687 was administered orally by gavage to female Beagle dogs at single escalating doses of 100 and 300 mg/kg (1 animal) or at 200 mg/kg for 2 days (1 animal).

The dogs were exposed to plasma drug concentrations that increased in a supra-proportional manner with increased dose.

Convulsions and other CNS signs such as titubation, inability to rise and tachypnoea were observed after a single dose at 300 mg/kg. These signs were not observed after 2 daily administrations at 200 mg/kg. At doses from 100 mg/kg emesis, arched back with extended limbs, and body weight decrease were observed. From doses of 200 mg/kg, there was an increased number of white blood cells, neutrophils, monocytes, fibrinogen and globulin that may indicate an inflammatory process. Minimal increase in transaminases was observed from 200 mg/kg and ALP from 300 mg/kg.

5.3.2.5. Intravenous Escalating-Dose Toxicity Study in Dogs (Study No. 05052; Table 23)

PF-00446687 was administered by IV injection to Beagle dogs. One male received 3 and 16 mg/kg on Days 1 and 3, and 1 female received 10 mg/kg on Day 2. Two additional dogs (1/gender) received 12 mg/kg on Days 8 and 9. The vehicle was an acidified 5% glucose solution. Pharmacokinetic measurements of PF-00446687 were performed on Days 1, 2, 3, and 8. Blood samples were taken 0.08, 0.25, 0.5, 1, 4, and 24 hours after dosing.

The dogs were exposed to drug concentrations in plasma that increased with dose, though not in direct proportion.

Single doses of PF-00446687 produced emesis from 10 mg/kg, increased salivation from 12 mg/kg and tremor, tachypnoea, and titubation at 16 mg/kg. Treatment for 2 days with 12 mg/kg indicates that more severe signs appear on the second day (whining, rigid extended limbs, pedalling movements, and inability to rise).

5.3.2.6. 1-Month Intravenous Toxicity Study in Dogs (Study No. 05158; Table 24)

PF-00446687 was administered by IV injection to Beagle dogs (3/gender/group) at daily doses of 0, 1, 3, and 10 mg/kg for 1 month (corresponding to injection concentrations of 0.5, 1.5, and 5 mg/mL, respectively). Pharmacokinetic measurements of PF-00446687 were performed on Days 1 and 27. Blood samples were taken 0.08, 0.25, 0.5, 1, 4, and 24 hours after dosing.

The dogs were exposed to drug concentrations in plasma that increased proportionally with dose, were similar in males and females, and remained stable over the 27-day treatment period.

PF-00446687 produced a dose-related increase in activity at all doses with clinical signs, comprising rolling over the ground, pedaling movements, stretching down, extended tail, and whining. These signs were continuous and appeared involuntary in nature for 15 minutes following administration of 10 mg/kg, becoming occasional thereafter for up to 60 minutes. At 1 and 3 mg/kg, these signs were less frequent and were generally no longer seen after 30 minutes. Individually, these behaviours are occasionally seen in untreated dogs, but at 10 mg/kg, their

combined and continuous nature indicated marked effects on the central nervous system and was considered adverse.

At 3 and 10 mg/kg, there was a dose-related increase in emesis that may have contributed to a slight haemoconcentration at these doses, indicated by minor increases in sodium, chloride, and albumin in the plasma and an increase in urine specific gravity. At 10 mg/kg, there was a body weight loss of 0.43 and 0.20 kg in male and female dogs, respectively, compared with body weight gains of 0.44 and 0.17 kg in controls.

A dose-related increase in heart rate was recorded at all doses (up to 46 beats/min at 10 mg/kg) and an increase in blood pressure at 10 mg/kg (18 mmHg). Although the cause is unknown, the increased activity may have contributed to these changes. Redness of the skin and the conjunctiva at all doses probably reflected the changes in cardiovascular function.

At 10 mg/kg, peri-vascular inflammation and necrosis at the injection site indicated a local irritant effect at the intravenous injection sites. This dose was associated with an injection concentration of 5 mg/mL. Minimal thymic atrophy observed at 10 mg/kg was probably related to non-specific stress.

In conclusion, given the behavioural signs indicative of effects on the central nervous system, together with the body weight loss at 10 mg/kg, the NOAEL is considered to be 3 mg/kg IV. PF-00446687 produced irritant effects at the injection site at 10 mg/kg, with an injection concentration of 5 mg/mL.

5.3.3. Genotoxicity (Study Nos. 05-2840-04 and 05-2840-03; Table 25)

The genetic safety of PF-00446687 was assessed in a comprehensive series of in vitro (bacterial mutation, chromosomal aberrations in human lymphocytes, and micronucleus induction in CHO cells) and in vivo (bone marrow micronucleus) tests. PF-00446687 did not induce microbial gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 or in *Escherichia coli* strain WP2uvrApKM101 when tested up to cytotoxic concentrations with and without metabolic activation. In human lymphocytes in vitro, PF-00446687 did not induce significant increases in chromosomal aberrations in 3-hour tests with (26.2 to 65.4 µg/mL) and without (47.9 to 127 µg/mL) metabolic activation or a 24-hour test without metabolic activation (17 to 65.1 µg/mL). PF-00446687 showed significant increases in polyploid cells in human lymphocyte cultures in the 3-hour test (1.33% at 91.8 µg/mL) and the 24-hour test (2.00% at 47.0 µg/mL) without metabolic activation compared with vehicle control values of 0.1 and 0.18% polyploid cells, respectively. A significant increase in polyploid cells was observed in an initial 3-hour test (1.30% at 78.0 µg/mL) with metabolic activation, but was not reproducible in a second 3-hour metabolic test. The increase in polyploidy is not considered biologically relevant because no increases in micronucleated cells were detected in either in vitro or in vivo micronucleus tests that are both sensitive in detecting clastogenic and aneugenic chromosomal damage. PF-00446687 showed no significant increases in micronucleated cells in an exploratory in vitro micronucleus assay in CHO cells when tested up to cytotoxic limit concentrations of 94, 121, and 58.5 µg/mL in 3-hour tests with and without metabolic activation and in a 24-hour direct test, respectively. In the 1-month intravenous toxicity study in rats with micronucleus, PF-00446787 was administered by intravenous injection once a day for 28 consecutive days as

described in section 5.3.1.2. This dosing regimen produced mean male and female combined total C_{max} and $AUC_{(5min-24h)}$ values of 768 ng/mL and 3090 ng•h/mL on Day 27, respectively. No chromosomal damage occurred in bone marrow cells as evidenced by the lack of induction of micronuclei in the presence of a maximum tolerated dose that exceeded the intended clinical exposure in humans. These data indicate that PF-00446687 does not pose a genetic safety risk for clinical exposure in humans (see Section 5.3.1.2).

5.3.4. Carcinogenicity

PF-00446687 has not been tested for carcinogenicity activity in a lifetime rodent bioassay.

5.3.5. Reproduction Toxicity

Fertility, embryo/foetal development, and peri-natal and post-natal development toxicity studies with PF-00446687 have not been conducted.

5.3.6. Juvenile Toxicity

Juvenile toxicity studies with PF-00446687 have not been conducted.

5.3.7. Special/Other Studies

5.3.7.1. 6-Day Cheek Pouch Irritation Study in Hamsters (Study No. 04332; Table 26)

The aim of this study was to assess the irritant potential of PF-00446687 in the buccal cavity. PF-00446687 or vehicle (sodium citrate/ethanol) was applied in the right cheek pouch of Golden hamsters (4 animals/gender/concentration) at 0, 50, or 80 mg/mL. At the end of the study, histopathological examination of the application site was performed.

PF-00446687 produced no irritation to the hamster cheek pouch or any other treatment effects.

In conclusion, 80 mg/mL was the NOEL.

5.3.7.2. Evaluation of the Phospholipidosis Potential in the In Vitro Human Lymphocyte Assay (Study No. 04351)

The aim of this study was to assess the potential of PF-00446687 to induce phospholipidosis. The compound was tested in vitro using human lymphocytes at concentrations of 0 (0.1% DMSO), 0.01, 0.1, and 1 μ M and was shown not to induce phospholipidosis, assessed either by the accumulation of multi-lamellar bodies or by the phospholipidosis ratio.

In conclusion, PF-00446687 did not show evidence of phospholipidosis potential in vitro at concentrations up to 1 μ M.

5.3.7.3. 7-Day Oral Gavage Irritation Study in Rats (Study No. 05156; Table 27)

The aim of this study was to assess the gastrointestinal irritant potential of PF-00446687 when administered orally. PF-00446687 was administered once daily, orally by gavage for 7 days to

Sprague-Dawley rats (5/gender/group) at concentrations of 1 and 2 mg/mL in acidified water (10 and 20 mg/kg, respectively). There were no treatment-related clinical signs or histopathological evidence of gastrointestinal irritation or hepatic change.

In conclusion, the oral administration of PF-00446687 produced no evidence of gastrointestinal irritation at concentrations up to 2 mg/mL.

5.3.8. Significance to Human Risk

5.3.8.1. Relationship of Findings to Pharmacokinetics

PF-00446687 exposure in rats and dogs, as defined by C_{max} and AUC, increased with dose over the dose ranges tested. In studies with repeated daily administration, there were no marked gender differences or differences when comparing exposure parameters at the start and end of the study. The threshold concentrations of PF-00446687 in plasma associated with key responses in studies in rats and dogs can be found in the following table.

Table 9. Threshold Concentrations of PF-00446687 Associated With Key Responses

Key Response(s)	Dose (mg/kg)	Mean		Mean Cmax ^a (ng/mL)	
		AUC(5min-24h) ^a (ng•h/mL)			
		Total	Free ^b	Total	Free ^b
4-Day oral exploratory in male rats					
↓ food consumption; ↓ reticulocyte; ↑ liver weight (relative to body weight)	100	15400	1078	1020	71.4
Death (TK animals only, 2/3); ↓ body weight, ↓ activity, ↑ salivation; chromodacryorrhea; ↑ red blood cell, ↑ haemoglobin, ↑ haematocrit; ↑ glucose, ↑ bilirubin, ↓ phosphate;	300	18700	1309	7000	490
Death (preceding signs were convulsion, ↓ activity, prostration, lacrimation). Surviving animals : tremors, prostration, partially closed- eyes, rough fur; ↓white blood cells; ↓K; ↑GGT	1000	76200	5334	9550	669
1-Month IV Study in Rats					
Oedema and haematoma at the injection site	1	276	19.3	105	7.35
Injection site (perivascular inflammation, thrombosis, paleness/redness of the skin)	3	810	56.7	317	22.2
Inguino-femoral lymph nodes (enlarged or discolored)					
Thymus discoloration					
Convulsions	9	3090	216	768	53.8
Injection site (necrosis, erosion/ulcer)					
Inguino-femoral lymph nodes (lymphoid hyperplasia, congestion, hemosiderosis, plasmocytosis)					
Bone marrow (myeloid hyperplasia)					
Death	15	NA	NA	NA	NA
Oral Escalating Dose Toxicity Study in Female Dogs					
Arched back with extended limbs; emesis; ↓ body weight; ↑ calcium; ↓ urea	100	1810	272	709	106
↑ white blood cell; ↑ neutrophils; ↑ monocytes; ↑ transaminases; ↑ globulin and protein	200	ND	ND	ND	ND
Convulsions, titubations, inability to rise, tachypnoea, ↑salivation; ↑ fibrinogen; ↑ ALP	300	32500	4875	5890	884
1-Month IV Study in Dogs					
↑ activity (rolling over, pedalling, stretching down, extended tail, whining), ↑ salivation, redness of skin and conjunctiva, ↑ heart rate	1	356	53.4	127	19.1
Inability to rise, stamping, emesis	3	1100	165	439	65.9
↑ plasma Na ⁺ , Cl ⁻ , albumin, protein and albumin/protein ratio					
↑ urine specific gravity					
Tremor, titubation, body weight loss, ↑ blood pressure, ↓ plasma K ⁺	10	3990	599	1580	237
Injection site (perivascular inflammation and necrosis), minimal thymic atrophy					

NA= Not applicable ND = no data

^a male and female combined

^b free fraction: 0.07 in rats and 0.15 in dogs

PF-00446687 has shown evidence of efficacy at the oral dose of 200 mg in a biomarker (Rigiscan) study in healthy male volunteers. In the first-in-human study (A8361001) the mean free C_{max} and AUC at the oral dose of 195 mg were 3.90 ng/mL and 67.95 ng•h/mL.

5.3.8.2. Signs Relevant to Human Risk

Death was recorded in mice following a single IV dose at 40 mg/kg, but not at 20 mg/kg and in rats at 20 mg/kg, but not at 10 mg/kg. In repeat-dose IV studies, deaths were observed in rats from 15 mg/kg (4-day and 1-month) but not at 9 mg/kg, at a free C_{max} 14-fold greater than the human exposure at the oral dose of 195 mg (free C_{max} of 3.9 ng/mL). Following 4-day oral administration, deaths occurred in rats from 300 mg/kg (free C_{max} of 490 ng/mL), but not at 100 mg/kg (free C_{max} 71.4 ng/mL). There were no deaths in dogs at an IV dose up to 10 mg/kg (free C_{max} 237 ng/mL) and at an oral dose of 300 mg/kg (free C_{max} 884 ng/mL).

Convulsions were observed in mice, rats and dogs. In mice, convulsions were observed after a single intravenous dose of 40 mg/kg, but not at 20 mg/kg. In rats, convulsions occurred after a single IV dose of 20 mg/kg, but not at 10 mg/kg. Convulsions were also observed in one rat at 9 mg/kg IV towards the end of the 1-month study. There were no convulsions at the 3 mg/kg IV dose in rats for 1 month, (free C_{max} 22.2 ng/mL) this exposure was 6-fold the free C_{max} observed in human after an oral dose of 195 mg. Following oral administration in rats, convulsions were noted at 1000 mg/kg but no CNS signs were associated with the dose of 100 mg/kg (C_{max} 71.4 ng/mL). In dogs, convulsions were not reported in the 1-month study up to an IV dose of 10 mg/kg (free C_{max} of 237 ng/mL) at an exposure 61-fold the human exposure following oral doses of 195 mg. Single oral administration of 300 mg/kg (free C_{max} 884 ng/mL) resulted in convulsions but not at 100 mg/kg (free C_{max} 106 ng/mL). Doses used for the oral studies resulted in exposures (C_{max} and AUC) higher than those observed following IV route.

Treatment-related emesis was seen in dogs at the IV dose of 3 mg/kg, but not at the IV dose of 1 mg/kg, and following oral administration of 100 mg/kg and above. The free C_{max} at which emesis did not occur is 5-fold higher than that in humans following oral administration at 195 mg. Furthermore, studies using an emetic model in ferrets indicate no emesis at doses up to 5 mg/kg (free C_{max} 84 ng/mL) representing a multiple of over 21, compared with the C_{max} in humans at oral dose of 195 mg. Emesis has not been observed in clinical studies at doses up to 195 mg.

PF-00446687 produced behavioural changes in rats, ferrets, and dogs. In rats, there was an increase in stretching, yawning, urogenital licking, and excessive grooming at all doses in the 4-day IV study. Oral administration for 4-days resulted in decreased activity from 300 mg/kg, at C_{max} exposures much higher than those observed after IV administration. In ferrets, behavioural changes comprised of sneezing, yawning, and licking were observed. In dogs, treatment induced a dose-related increase in activity at all doses in the 1-month IV study. Although in this study, the individual activities were normal and occasionally seen in untreated dogs, the combined and continuous appearance immediately after the dose of 10 mg/kg was considered an adverse effect of treatment on the central nervous system. Similar changes were not encountered at 3 mg/kg, associated with a C_{max} about 17-fold greater than the human exposure at the oral dose of 195 mg. Melanocortin agonists are reported to induce increased activity in rats, characterized by grooming, stretching, yawning, and penile erection (Argiolas et al., 2000).

A general increase in activity in dogs may have contributed to the slight increase in heart rate that remained within historical controls, redness of the skin, and conjunctiva observed at all doses, and the slight increase in blood pressure at 10 mg/kg. In the absence of histopathological changes, these effects were not considered adverse. Increases in heart rate were also recorded in conscious telemetered dogs. Given the changes in blood pressure and heart rate in dogs, cardiovascular parameters should be carefully monitored during early clinical studies.

Irritant potential of PF-00446687 has been assessed. A hamster cheek pouch irritation, a pre-clinical model of sublingual irritation, showed no irritation at the application sites. Sublingual administration of PF-00446687 is not considered to represent a risk to humans in terms of irritation to the tongue and mouth. Oral gavage administration of PF-00446687 in rats did not produce irritation of gastrointestinal tract up to 2 mg/mL (equivalent to 20 mg/kg). Therefore sublingual or oral administration in human are not considered to represent a risk to humans in terms of irritation of the tongue, mouth and gastrointestinal tract.

In clinical trials, PF-00446687 has been administered to human via the oral route (32.5 and 195 mg). The incidence of adverse events was similar to that reported for placebo. At a dose of 195 mg, a number of potentially CNS mediated adverse events were reported (restlessness, yawning, stretching).

5.3.9. Systems Not Tested

Fertility, embryo/foetal development (including teratogenic potential assessment), and peri-natal post-natal development studies with PF-00446687 have not been conducted. Safety for women of childbearing potential cannot be implied from the existing data.

PF-00446687 has not been tested for carcinogenic activity in a lifetime rodent bioassay.

Reference

Argiolas A, Melis MR, Murgia S, et al. ACTH- and alpha-MSH-induced grooming, 5 stretching, yawning and penile erection in males rats: site of action in the brain and role of melanocortin receptors. Brain Res Bull 2000;51(5):425-31.

6. EFFECTS IN HUMANS

Two clinical studies with PF-00446687 have been completed, and a third study is ongoing. A total of 64 male subjects have received PF-00446687 to date, of which 52 have received 195 or 200 mg. Table 10 provides an overview of the studies and details on each protocol follow the table.

Table 10. Overview of Completed Clinical Studies

Completion Status	Design	Daily Doses (form)	Number of Subjects Entered/ Completed	Duration of Treatment
Protocol A8361001 (Europe)				
Completed	A double-blind (3 rd party open), randomized, placebo-controlled, dose-escalating, crossover study to investigate the safety, toleration and pharmacokinetics of single doses of PF-00446687 in healthy male subjects.	0.3, 1, 3, 32.5, 65, 130 and 195 mg (sublingual solution); 32.5 and 195 mg (oral solution), placebo	16/16	Single dose
Protocol A8361002 (Europe)				
Completed	A study to assess the pharmacodynamic effect of a single dose of PF-00446687 on penile erections in healthy male subjects (randomized, cross-over, single dose, active- and/or placebo controlled)	3, 20, 60, 200 mg (oral solution), placebo	48/48	Single dose

Table 11. Overview of Ongoing Clinical Studies

Protocol A8361005 (Europe)				
Ongoing	An open, placebo-controlled, cross-over study to investigate the effect of ketoconazole on the pharmacokinetics of PF-00446687 in healthy male subjects.	5 mg (oral solution), placebo	12/NA	Single dose

Protocol A8361001 was the first-in-human study of PF-00446687. The objectives of this study were to determine the safety and toleration of single doses of PF-00446687 and to determine the pharmacokinetics and pharmacodynamics of PF-00446687 following single-dose administration.

The objective of Protocol A8361002 was to assess the effect of PF-00446687 on penile erections by utilizing the penile plethysmography (Rigiscan® Plus) technique in healthy male subjects. This study consisted of two cohorts. In cohort 1, 200 mg PF-00446687, 10 mg vardenafil, 3 mg apomorphine, and placebo were administered in a cross-over design. In cohort 2, a range of doses of PF-00446687 (3, 20, 60, and 200 mg) and placebo were studied. Subjects were exposed to neutral video and VSS for up to 5 hours after drug administration.

The objectives of protocol A8361005 were to investigate the effect of steady state ketoconazole on the pharmacokinetics of a single dose of PF-00446687, and to investigate the safety and toleration of PF-00446687 in the presence of steady state ketoconazole in healthy subjects. Subjects received either 400 mg ketoconazole or placebo for 10 days, and a single oral dose of 5 mg PF-00446687 on day 4; they crossed over to the alternate treatment after a wash-out period. This study is ongoing.

6.1. Pharmacokinetics and Product Metabolism in Humans

PK Summary: Following sublingual dosing, peak plasma concentrations were achieved within 2 to 8 hours, exposure increased supra-proportionally with increasing dose (<2-fold relative to

dose), and terminal half-life was approximately 12 hours across the entire dose range. Following oral dosing, exposure was similar to that achieved for sublingual dosing at comparable dose levels, however, the small sample size ($n = 4$) needs to be considered. Urinary excretion was negligible with $<2.5\%$ dose excreted as parent over 48 hours after dosing. There were no discernible differences between the 195 mg dose of PF-00446687 and placebo for any of the pharmacodynamic markers evaluated (TSH, LH, FSH, GH and prolactin).

In protocol A8361001, the single-dose pharmacokinetics of PF-00446687 were investigated after sublingual and oral dosing (Table 12). Following sublingual dosing, PF-00446687 achieved peak plasma concentrations between 1 and 4 hours after dosing at the lower doses, and up to 8 hours (median) after dosing at the higher doses. After oral dosing, peak plasma concentrations were observed at 4 hours for every subject. Thereafter, plasma concentrations declined in a monophasic manner, for all doses and both routes of administration. Following sublingual dosing, systemic exposure increased supra-proportionally with increasing dose. Considering mean values, at the low end of the dose range (3 to 32.5 mg), C_{max} increased 16-fold for an 11-fold increase in dose. At the higher end of the dose range (32.5 to 195 mg), C_{max} and AUC increased 10-fold and 16-fold, respectively, for a 6-fold increase in dose. Across the entire dose range (3 to 195 mg), C_{max} increased 162-fold for a 65-fold increase in dose. Following oral dosing with PF-00446687, systemic exposure also increased supra-proportionally with increasing dose. Considering mean values, C_{max} , AUC_{last} and AUC_{inf} increased 13-fold, 17-fold, and 16-fold, respectively, for a 6-fold increase in dose. Peak concentration at 195 mg (oral) was 30.0 ng/mL total which is equivalent to 3.9 ng/mL or 8.3 nM free. Mean terminal half-life was approximately 12 hours at all reported dose levels, and for both routes of administration. When comparing the oral versus sublingual routes of administration, the overall profiles were similar at comparable dose levels. The percentage of dose (195 mg) excreted as intact drug in urine was low ($<2.5\%$ dose) and similar for both routes of administration. Five analytes (TSH, FSH, GH, LH and prolactin) were measured in the pharmacodynamic samples collected after sublingual administration of 195 mg dose of PF-00446687 or placebo. There were no apparent differences between the placebo and 195 mg dose of PF-00446687 treatment groups for any of the analytes.

Table 12. Summary Pharmacokinetic Parameters After Single-Dose Administration of PF-00446687 (Protocol A8361001)^d

Dose of PF-00446687	N	AUC _{last} ^a (ng.h/mL)	AUC _{inf} ^a (ng.h/mL)	C _{max} ^a (pg/mL)	T _{max} ^b (h)	t _{1/2} ^c (h)	V _z /F _c (L)	CL/F _c (L/h)
Cohort 1								
0.3 mg	6	NC	NC	NC	NC	NC	NC	NC
1 mg	5	NC	NC	NC	NC	NC	NC	NC
3 mg	6	1.604 (1.204)	NC	197.6 (102.03)	3.0 (1.0 – 4.0)	NC	NC	NC
32.5 mg	6	38.96 (19.19)	40.26 (20.59)	3166.4 (1736.4)	2.0 (2.0 – 4.0)	10.02 (2.01)	12212 (4923.2)	876.6 (382.7)
Cohort 2								
65 mg	8	91.14 (31.79)	92.27 (31.77)	5519.8 (2161.7)	4.0 (2.0 – 8.0)	12.05 (2.39)	12712 (4693.5)	757.2 (353.4)
130 mg	8	329.9 (141.5)	331.0 (141.5)	18067 (7572.0)	6.0 (2.0 – 8.0)	12.75 (2.36)	7850.8 (3753.8)	434.3 (238.6)
195 mg	8	642.0 (227.5)	644.0 (227.2)	31919 (14435)	8.0 (2.0 – 8.0)	12.62 (2.80)	5826.0 (2422.5)	329.1 (166.4)
32.5 mg oral	4	31.50 (15.80)	32.59 (15.54)	2274.0 (1045.6)	4.0 (4.0 – 4.0)	11.16 (2.15)	16954 (7020.4)	1050 (345.0)
195 mg oral	4	521.4 (115.8)	522.7 (116.0)	30033 (6639.0)	4.0 (4.0 – 4.0)	12.85 (4.01)	6950.8 (2107.0)	382.1 (102.5)

Source Table 13.5.1

^a geometric mean (SD)

^b median (range)

^c arithmetic mean (SD)

^d dosing is via the sublingual route, except where indicated

N=Number of subjects assessed; NC= not calculated as the majority of the samples were BLQ; SD = standard

6.2. Safety and Efficacy

6.2.1. Phase 1 Studies

Safety Summary: Safety data are available from two protocols. A total of 64 subjects have received PF-00446687 (of which 52 have received doses of 195 or 200 mg) and 63 subjects have received placebo in the two completed studies. The highest incidence of adverse events was due to low toleration locally for sublingual administration; reports included application site anaesthesia/irritation and hypoesthesia. The sublingual route is no longer being pursued. Across both studies, the most common treatment-related systemic adverse events were restlessness (6.3%), increased libido (3.1%), somnolence (3.1%), dizziness (3.1%); both nausea (6.3%) and abdominal pain (3.1%) were mostly reported with sublingual dosing. There have been no clinically meaningful changes in laboratory tests, vital signs or ECG parameters that could be attributed to study drug. There have been no discontinuations due to adverse events nor any serious adverse events in the PF-00446687 program.

6.2.1.1. Adverse Events

Treatment-emergent adverse events are described by protocol in the following sections. Comprehensive lists of all-causality and treatment-related AEs reported in PF-00446687-treated subjects across all Phase 1 protocols are provided in 8.2.1.

6.2.1.1.1. Protocol A8361001

All-Causality, Treatment-Emergent Adverse Events

There was a dose-related increase in the number of local adverse events reported for the sublingual treatment groups only; these events included application site anaesthesia, irritation and hypoesthesia) and only occurred in the PF-00446687 sublingual treatment groups. The most commonly reported systemic adverse events included yawning, somnolence and restlessness which were reported predominantly at 195 mg. Treatment-emergent, all causality AEs reported in $\geq 20\%$ PF-00446687-treated subject per dose group are shown in Table 13.

Treatment-Related, Treatment-Emergent Adverse Events

There was a dose-related increase in the number of local adverse events reported for the sublingual treatment groups only; these events included application site anaesthesia, irritation and hypoesthesia and only occurred in the PF-00446687 sublingual treatment groups. The most commonly reported systemic adverse events included restlessness and nausea. Treatment-related, treatment-emergent AEs reported in $\geq 20\%$ PF-00446687-treated subject per dose group are shown in Table 14.

PF-00446687

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Table 13. All-Causality Treatment-Emergent Adverse Events Reported at Incidences $\geq 20\%$ in PF-00446687-Treated Subject in Protocol A8361001

	Cohort 1					Cohort 2					
	PF-00446687 Doses					PF-00446687 Doses					
	0.3 mg SL No (%)	1 mg SL No (%)	3 mg SL No (%)	32.5 mg SL No (%)	Pbo No (%)	65 mg SL No (%)	130 mg SL No (%)	195 mg SL No (%)	32.5 mg Orally No (%)	195 mg Orally No (%)	Pbo No (%)
Evaluable	6	5	6	6	8	8	8	8	4	4	7
Gastrointestinal disorders	-	1 (20.0)	-	2 (33.3)	-	2 (25.0)	2 (25.0)	5 (62.5)	1 (25.0)	-	-
Abdominal pain	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Aphthous stomatitis	-	1 (20.0)	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	1 (25.0)	-	-
Hypoaesthesia oral	-	-	-	2 (33.3)	-	-	2 (25.0)	2 (25.0)	-	-	-
Nausea	-	-	-	-	-	-	-	3 (37.5)	-	-	-
General disorders and administration site conditions	-	-	-	-	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Application site anaesthesia	-	-	-	-	-	-	2 (25.0)	-	-	-	-
Application site irritation	-	-	-	-	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Infections and infestations	-	1 (20.0)	-	-	-	-	-	-	1 (25.0)	-	-
Otitis externa	-	1 (20.0)	-	-	-	-	-	-	-	-	-
Tonsillitis	-	-	-	-	-	-	-	-	1 (25.0)	-	-
Musculoskeletal and connective tissue disorders	-	1 (20.0)	-	-	-	-	-	-	-	-	-
Back pain	-	1 (20.0)	-	-	-	-	-	-	-	-	-
Nervous system disorders	-	-	-	-	-	-	-	3 (37.5)	1 (25.0)	2 (50.0)	2 (28.6)
Dizziness	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Headache	-	-	-	-	-	-	-	-	1 (25.0)	-	2 (28.6)
Paraesthesia	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Somnolence	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Psychiatric disorders	-	-	-	-	-	-	-	4 (50.0)	-	1 (25.0)	-
Restlessness	-	-	-	-	-	-	-	4 (50.0)	-	1 (25.0)	-
Respiratory, thoracic and mediastinal disorders	-	-	-	-	-	-	2 (25.0)	2 (25.0)	-	2 (50.0)	-
Pharyngolaryngeal pain	-	-	-	-	-	-	2 (25.0)	-	-	1 (25.0)	-
Yawning	-	-	-	-	-	-	-	2 (25.0)	-	2 (50.0)	-

Reported in MedDRA version 9.1

Pbo = Placebo

SL - Sublingual

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Table 14. Treatment-Related, Treatment-Emergent Adverse Events Reported at Incidences $\geq$ 20% PF-00446687-Treated Subject per Dose Group in Protocol A8361001

Evaluable	Cohort 1					Cohort 2					
	PF-00446687 Doses					PF-00446687 Doses					
	0.3 mg SL	1 mg SL	3 mg SL	32.5 mg SL	Pbo	65 mg SL	130 mg SL	195 mg SL	32.5 mg Orally	195 mg Orally	Pbo
	No (%) 6	No (%) 5	No (%) 6	No (%) 6	No (%) 8	No (%) 8	No (%) 8	No (%) 8	No (%) 4	No (%) 4	No (%) 7
Gastrointestinal Disorders	-	-	-	2 (33.3)	-	2 (25.0)	2 (25.0)	5 (62.5)	1 (25.0)	-	-
Abdominal pain	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	1 (25.0)	-	-
Hypoaesthesia oral	-	-	-	2 (33.3)	-	-	2 (25.0)	2 (25.0)	-	-	-
Nausea	-	-	-	-	-	-	-	3 (37.5)	-	-	-
General Disorders and Administration Site Conditions	-	-	-	-	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Application site anaesthesia	-	-	-	-	-	-	2 (25.0)	-	-	-	-
Application site irritation	-	-	-	-	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Nervous system disorders	-	-	-	-	-	-	-	3 (37.5)	-	2 (50.0)	-
Dizziness	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Paraesthesia	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Somnolence	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Psychiatric disorders	-	-	-	-	-	-	-	4 (50.0)	-	1 (25.0)	-
Restlessness	-	-	-	-	-	-	-	4 (50.0)	-	1 (25.0)	-
Respiratory, thoracic and mediastinal disorders	-	-	-	-	-	-	2 (25.0)	2 (25.0)	-	2 (50.0)	-
Dysphonia	-	-	-	-	-	-	-	-	-	-	-
Yawning	-	-	-	-	-	-	-	1 (12.5)	-	1 (25.0)	-
Vascular disorders	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Hot flush	-	-	-	-	-	-	-	-	-	1 (25.0)	-

Reported in MedDRA version 9.1

Pbo = Placebo

SL - Sublingual

6.2.1.1.2. Protocol A8361002

All-Causality, Treatment-Emergent Adverse Events

The incidence of adverse events was similar to placebo for PF-00446687 treatment groups. Of interest, there were two reports of increased libido (at doses of 200 mg, 20 mg) and one report of somnolence (3 mg). For 10 mg vardenafil, and 3 mg apomorphine, there was 37.5% and 18.8% incidence of headache, respectively. All-causality, treatment-emergent AEs reported in more than one PF-00446687-treated subject per dose group are shown in Table 15.

Table 15. All-Causality Treatment-Emergent Adverse Events Occurring in Protocol A8361002

	PF-00446687 Doses				Vard	Apomor	Pbo
	3 mg No (%)	20 mg No (%)	60 mg No (%)	200 mg No (%)	10 mg No (%)	3 g No (%)	No (%)
Evaluable With Adverse events	24 3 (12.5)	24 3 (12.5)	24 5 (20.8)	40 7 (17.5)	16 6 (37.5)	16 3 (18.8)	48 5 (10.4)
Eye Disorders	-	-	1 (4.2)	-	-	-	1 (2.1)
Eye irritation	-	-	1 (4.2)	-	-	-	1 (2.1)
Gastrointestinal Disorders	1 (4.2)	-	1 (4.2)	1 (2.5)	-	-	-
Diarrhoea	1 (4.2)	-	1 (4.2)	-	-	-	-
Nausea	-	-	-	1 (2.5)	-	-	-
General Disorders and Administration Site conditions	-	-	-	1 (2.5)	-	2 (12.5)	1 (2.1)
Fatigue	-	-	-	1 (2.5)	-	2 (12.5)	1 (2.1)
Infections and infestations	1 (4.2)	1 (4.2)	3 (12.5)	2 (5.0)	-	-	3 (6.3)
Nasopharyngitis	1 (4.2)	1 (4.2)	2 (8.3)	2 (5.0)	-	-	2 (4.2)
Rhinitis	-	-	1 (4.2)	-	-	-	1 (2.1)
Musculoskeletal and connective tissue disorders	-	1 (4.2)	1 (4.2)	-	-	-	-
Back pain	-	1 (4.2)	1 (4.2)	-	-	-	-
Nervous system disorders	1 (4.2)	-	1 (4.2)	-	6 (37.5)	3 (18.8)	1 (2.1)
Headache	-	-	1 (4.2)	-	6 (37.5)	3 (18.8)	1 (2.1)
Somnolence	1 (4.2)	-	-	-	-	-	-
Psychiatric disorders	-	1 (4.2)	-	1 (2.5)	-	-	-
Libido increased	-	1 (4.2)	-	1 (2.5)	-	-	-
Respiratory, thoracic and mediastinal disorders	-	-	-	1 (2.5)	-	-	1 (2.1)
Cough	-	-	-	1 (2.5)	-	-	1 (2.1)
Skin and subcutaneous tissue disorders	-	-	-	1 (2.5)	-	-	-
Erythema	-	-	-	1 (2.5)	-	-	-

Reported in MedDRA version 9.1

Vard= Vardenafil

Apomor = Apomorphine

Pbo = Placebo

Treatment-Related, Treatment-Emergent Adverse Events

The only adverse event that was reported in more than one subject per dose level was fatigue (apomorphine 3 mg). Of interest, there were two reports of increased libido (at doses of 200 mg, 20 mg) and one report of somnolence (3 mg). Treatment-related, treatment-emergent AEs reported in more than one PF-00446687-treated subject per dose group are shown in Table 16.

Table 16. Treatment-Related, Treatment-Emergent Adverse Events in Protocol A8361002

	PF-00446687 Doses				Vard	Apomor	Placebo
	3 mg No (%)	20 mg No (%)	60 mg No (%)	200 mg No (%)	10 mg No (%)	3 g No (%)	No (%)
Evaluable	24	24	24	40	16	16	48
With Adverse events	2 (8.3)	1 (4.2)	1 (4.2)	4 (10.0)	-	2 (12.5)	2 (2.1)
Gastrointestinal Disorders	1 (4.2)	-	1 (4.2)	1 (2.5)	-	-	-
Diarrhoea	1 (4.2)	-	1 (4.2)	-	-	-	-
Nausea	-	-	-	1 (2.5)	-	-	-
General Disorders and Administration Site conditions	-	-	-	1 (2.5)	-	2 (12.5)	1 (2.1)
Fatigue	-	-	-	1 (2.5)	-	2 (12.5)	1 (2.1)
Nervous System Disorders	1 (4.2)	-	-	-	-	-	-
Somnolence	1 (4.2)	-	-	-	-	-	-
Psychiatric Disorders	-	1 (4.2)	-	1 (2.5)	-	-	-
Libido increased	-	1 (4.2)	-	1 (2.5)	-	-	-
Skin and subcutaneous tissue disorders	-	-	-	1 (2.5)	-	-	-
Erythema	-	-	-	1 (2.5)	-	-	-

Reported in MedDRA version 9.1

Vard= Vardenafil

Apomor = Apomorphine

Pbo = Placebo

6.2.2. Laboratory Test Abnormalities

There were no clinically significant findings that could be attributed to study drug for any of the laboratory safety data collected.

One subject (A8361001) temporarily discontinued treatment due to elevated serum enzymes; in the opinion of the investigator, this was caused by excessive exercise.

6.2.3. ECG Results

In A8361001, there did not appear to be any clinically relevant treatment effect on any of the ECG parameters across the dose range studied. Protocol A8361002 monitored ECG for on-study safety.

6.2.4. Discontinuations Due to Adverse Events

No subject has discontinued due to adverse events in the PF-00446687 clinical program.

6.2.5. Serious Adverse Events

There have been no serious adverse events reported in the PF-00446687 clinical program.

6.2.6. Efficacy

Efficacy Summary: In a healthy volunteer Rigiscan study, PF-00446687 200 mg demonstrated improvement over placebo during visual sexual stimulation. A second cohort demonstrated no evidence of a dose response (down to 3 mg); however, the activity of 200 mg was confirmed.

6.2.6.1. Phase 1 Studies

In protocol A8361002, the pro-erectile effects of PF-00446687 were monitored in healthy volunteers using the Rigiscan technique. In cohort 1, subjects received (in a randomized, cross-over design) placebo, 10 mg vardenafil, 3 mg apomorphine and 200 mg PF-00446687. During the visual sexual stimulation (erotic video, all sessions, base of penis), PF-00446687 demonstrated approximately 4.5-fold improvement over placebo for time above 60% rigidity and approximately 2.1-fold improvement over placebo for the AUEC. Vardenafil performed as expected (7-fold improvement for rigidity, 2.7-fold improvement for AUEC), whereas apomorphine was not distinguishable from placebo. Cohort 2 explored PF-00446687 at doses of 3, 20, 60, and 200 mg and placebo. There was no evidence of dose response for any of the endpoints measured; however, the activity of 200 mg was confirmed.

7. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

PF-00446687 is a selective melanocortin MC4 receptor agonist that is being investigated for the oral treatment of the symptoms of FSD and MED. The initial study explored sublingual delivery, but due to low local toleration, oral dosing is now the preferred route.

PF-00446687 is presented as a powder for oral solution. The powder is dissolved in an appropriate aqueous diluent to provide the extemporaneously prepared solution.

Animal models support the use of a selective MC4 agonist both in terms of efficacy and potential for improved therapeutic window in comparison with non-selective MC agonists. To date, a total of 64 male subjects have received single doses of PF-00446687 up to a dose of 200 mg. Overall, PF-00446687 has been well-tolerated with the majority of adverse events (including nausea) being ascribed to the sublingual route of administration.

Dose Rationale

Non-clinical models of sexual behaviour provided the rationale for the initial development of specific MC4 agonists. The dose range explored (0.3 to 200 mg) in the clinic has been well-tolerated. The biomarker study of erectile activity has demonstrated activity at 200 mg in healthy volunteers. Additionally, adverse events that are believed to be pharmacologically-driven (restlessness, yawning, stretching) have been reported at 195 mg. Hence, 200 mg will be used as the initial dose for confirmation of activity in patient populations and the activity of lower doses will subsequently be explored.

Non-Clinical Safety Assessment

PF-00446687 has been investigated in a range of in vitro and non-clinical studies designed to evaluate its safety pharmacology and its toxicity. The majority of studies were conducted in rats and dogs, with mice, ferrets, and hamsters being used for specific investigations. Additionally, genetic safety studies have been conducted in vivo (micronucleus assessment in rats) and in vitro (bacterial mutagenicity assay and human lymphocyte assay).

PF-00446687 behaves as a full agonist at the MC4 receptor. It is highly selective for the MC4 receptor with >70-fold functional selectivity over human recombinant MC1, MC3, and MC5 receptors. A greater MC4 agonist potency (EC_{50} of 1.1 nM) was demonstrated using a rat functional assay (hemisected rat neonate spinal cord); this finding may reflect differences in the native versus recombinant systems.

Convulsions were observed in mice, rats and dogs at high exposures. The lowest exposure at which convulsions occurred was in the 1-month rat study where a single animal was affected (9 mg/kg) towards the end of the study. No convulsions were observed in rats at 3 mg/kg (C_{max} 317 ng/mL total, 22.2 ng/mL free). The highest dose studied in the clinic is 200 mg which has an associated C_{max} of approximately 31 ng/mL total (~4 ng/mL free). At 9 mg/kg, there is approximately a 10-fold margin over the C_{max} (free) at the highest dose being investigated in the clinic (200 mg). A total of 52 subjects have received ~200 mg single-dose to date and there have been no safety issues of concern. At steady state, exposure may increase 1.2- to 3-fold relative to single dose, dependent on the effect of PF-00446687 on drug metabolizing enzymes. Doses and associated exposures upon multiple dosing will be closely monitored to ensure safe dose escalation in the multiple dose study. Future toxicology studies are aimed at refining this upper limit, but currently, the clinical program is designed such that unbound C_{max} does not exceed approximately 10 nM.

Cardiovascular assessment was performed both in the conscious rat and dog models. The data indicate that PF-00446687 may have had a mild vasodilator action in dogs at unbound plasma concentrations at and above 15.5 nM (7.3 ng/mL), resulting in dose-related reductions in blood pressure (<10 mmHg), small reflex increases in myocardial contractility, increase in heart rate (up to 12 bpm) and reduction in PR interval; no QTc effects were noted. In the 1-month toxicity study in dogs, an increase in heart rate was recorded at all doses (up to 46 beats/min at 10 mg/kg, C_{max} 1580 ng/mL total, 237 ng/mL free) and an increase in blood pressure was observed at 10 mg/kg (18 mmHg); these changes may have been due to the overall increased general activity. The concentrations previously explored in the clinic are below those at which the cardiovascular effects were observed; multiple dosing may result in similar concentrations. Hence, cardiovascular monitoring (telemetry, ECGs) will be conducted in the early studies, particularly the multiple dose studies.

The effects of PF-00446687 on urine and electrolyte excretion were investigated in a rat model at doses up to 10 mg/kg IV. At 1 mg/kg ($C_{max,u}$ of 13.5 nM, 6.4 ng/mL), significant increases in urine volume and electrolyte (Na^+ , K^+ , and Cl^-) secretion were observed; this effect was further amplified at 10 mg/kg IV. In the 1-month IV study, haemoconcentration was observed in dogs from the IV dose of 3 mg/kg. Urinary creatinine, osmolality, and electrolytes (sodium, potassium, and chloride) were monitored in the first-in-human study and there were no dose-related changes of note.

The minor increases in respiratory rate and tidal volume observed in rats at 10 mg/kg ($C_{\max,u}$ of 145.6 nM, 68.3 ng/mL) are not expected to have a clinical impact given the concentrations at which they were observed. There were no adverse events suggesting a respiratory effect in clinical studies to date.

PF-00446687 is expected to be a centrally acting agent, and melanocortin agonists are reported to induce an increased activity in rats, characterized by grooming, stretching, yawning, and penile erection (Argiolas et al., 2000). In the toxicology studies, PF-00446687 induced behavioural changes (including yawning, stretching, licking, excessive grooming, rolling over, and whining) in all 3 species studied. In rats and dogs, these effects generally occurred only up to 30 to 60 minutes after IV dosing, suggesting that they are concentration related. In the first-in-human study (195 mg), a number of potentially centrally mediated adverse events were reported including restlessness, yawning and stretching. Subjects will continue to be monitored for such signs.

Non-selective melanocortin agonists, such as PT-141, have nausea and vomiting as dose-limiting side effects. PF-00446687 did not induce retching or vomiting in the ferret model of emesis up to 10 mg/kg SC and 5 mg/kg IV (maximum $C_{\max,u}$ estimated as 178 nM). However, in dogs, emesis was observed at high concentrations (3 mg/kg IV: $C_{\max}$ of 439 ng/mL total, 65.9 ng/mL free). In the two clinical studies conducted to date, there have been reports of nausea although the incidence is low (6.3%) and was generally associated with sublingual administration.

Local irritancy studies have been conducted to support the dosing concentrations to be used clinically for various routes. Concentrations up to 80 mg/mL may be used for sublingual dosing of PF-00446687, as supported by the lack of irritation in the hamster cheek pouch model. However, the sublingual route is unlikely to be continued in the clinic, given its poor toleration at high doses and the superior toleration with similar exposure profile afforded by the oral route. Concentrations up to 2 mg/mL may be used for oral administration of PF-00446687, as supported by the lack of gastrointestinal irritation in rats. However, for IV dosing, only dose concentrations up to 0.5 mg/mL are supported based on the injection site irritation seen in rats at higher concentrations. The oral toxicology studies, in conjunction with the IV 1-month studies, support short term oral dosing in the clinic.

Other findings of note in the toxicity studies were body weight loss in dogs (0.4 to 0.8 kg lower than controls) over the month of the study at 10 mg/kg, and redness of the skin and conjunctiva, also in dogs. The impact of PF-00446687 on food consumption is being assessed as part of the multiple dose study in healthy volunteers.

Death was recorded in mice at 40 mg/kg IV and at doses ≥ 15 mg/kg IV in rats (4-day and 1-month studies). There were no deaths at 9 mg/kg in rats ($C_{\max}$ 738 ng/mL total, 53.8 ng/mL free).

The genetic safety of PF-00446687 was assessed in a comprehensive series of in vitro (bacterial mutation, chromosomal aberrations in human lymphocytes) and in vivo (rat bone marrow) tests. PF-00446687 displayed no mutagenic or clastogenic effects in these studies.

The predominant clearance pathway for PF-00446687 is expected to be metabolism by cytochrome P450 3A4; in vitro data also indicate a potential inhibition of cytochrome P450s 3A4 and 2C19 by PF-00446687 at higher concentrations. These data suggest a high potential for drug-drug interactions; wherein PF-00446687 could act as the victim (where CYP 3A4 is inhibited by another agent and exposures to PF-00446687 are increased) or as the perpetrator (where PF-00446687 could itself alter CYP3A4 activity and therefore the exposure of other substrates). The impact of these interactions will need to be assessed within the context of the therapeutic index. An assessment of the impact of CYP 3A4 inhibition (by ketoconazole) on the pharmacokinetics of PF-00446687 is currently ongoing. Insight into the ability of PF-00446687 to itself inhibit CYP3A4 will be gained from the multiple dose safety study (wherein a higher than expected accumulation ratio would indicate this phenomenon) and may also be assessed definitively later in the programme.

Clinical Safety Assessment

Two single-dose studies with PF-00446687 in healthy volunteers have been completed in a total of 64 subjects. The pharmacokinetic profile indicates a greater than proportional (<2-fold) increase in exposure with increasing dose, however, the terminal half-life is constant across a wide dose range (12 hours) supporting once daily dosing. The peak concentration occurs between 2 and 8 hours after dosing, although current data indicate that for oral dosing, the peak occurs at approximately 4 hours after dosing. As sublingual and oral (albeit with fewer subjects) dosing result in similar exposure and profiles, oral dosing is the preferred route moving forward.

The most frequently reported adverse events (not considered related to sublingual dosing) were restlessness, increased libido, somnolence and dizziness. The incidence of adverse events increased with dose, however, even at 200 mg, PF-00446687 is well-tolerated. There were no serious adverse events and no discontinuations during the studies.

There have been no changes in vital signs, ECG parameters or laboratory safety assessments of clinical concern.

Urinary creatinine, osmolality, and electrolytes (sodium, potassium, and chloride) and were monitored in the first-in-human study and there were no dose-related changes of note.

The potential for drug-drug interactions is being investigated early in the program in order to determine the potential exposure increases. A study to determine the impact of CYP3A4 inhibition by ketoconazole on the exposure of PF-00446687 is ongoing.

Rigiscan methodology has demonstrated pro-erectile effects of PF-00446687 at 200 mg in healthy volunteers.

Risks, Side Effects, Precautions, Special Monitoring

If toxicity or severe intolerance develops in an individual, further dosing must be stopped and the event discussed with Pfizer immediately.

In the case of overdose, standard supportive treatment is recommended; antidote studies have not been performed.

Nonspecific melanocortin receptor agonists have had transient side effects of nausea (38% incidence, dose-related and in some instances severe), stretching, yawning, and decreased appetite; some patients reported increased sexual desire at the higher doses and one patient noted skin tanning (Melanotan II; Wessells et al., 2000). Side effects reported with PT-141 include flushing, nausea, feeling hot, and taste disturbance in healthy volunteers, and flushing and nausea in MED patients; all AEs were mild or moderate and resolved spontaneously (Diamond et al., 2004). Non-selective melanotropin analogues have been developed to promote skin pigmentation, some of these effects have been observed for PF-00446687 in healthy subjects upon single dose (yawning, stretching, increased libido). It is not known yet what the profile will be upon multiple dosing, but the adverse event profile will be further characterised in the early studies.

Due to the central mode of action of PF-00446687 and its potential impact on the dopamine pathway, its use will initially exclude those populations in whom use of non-selective dopamine agonist drugs is cautioned (ie, patients with arrhythmias, underlying cardiovascular disease, and major psychotic disorders).

Fertility and teratology studies with PF-00446687 have not been conducted. Safety for women of childbearing capacity cannot be implied from the existing data.

Until more information is available, PF-00446687 should not be administered to the following: children, females of childbearing potential, patients with cardiac arrhythmias or severe cardiovascular disease, patients with major psychotic disorders and those receiving antipsychotic medication.

Haemodynamic parameters (pulse rate and blood pressure) will continue to be monitored in early studies. During early clinical studies, there should be routine monitoring of liver and kidney function, and haematological parameters. Until more information is available, caution is advised regarding the study of subjects with compromised liver function.

Ongoing and future studies will assess potential risks of and precautions in the use of PF-00446687 in humans.

References

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8. APPENDIX

8.1. Toxicology Tables

Table 17. Single-Dose Toxicity Study in Mice

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Mouse/CD1	Intravenous injection, 5 mL/kg, 10 mL/kg (isotonic solution with 5% (w/v) glucose + 0.075M acetate buffer at pH 4.5-5.0/Solution)	20 40	3M, 3F 1M, 1F	20	40	40 : Death (F). ↓ activity, tremor. Dyspnoea, convulsions, inability to rise (F).	05160

Table 18. Single-Dose Toxicity Study in Rats

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Rat/Sprague-Dawley	Intravenous injection, 5 mL/kg (isotonic solution with 5% (w/v) glucose + 0.075 M acetate buffer at pH 4.5-5.0/Solution)	10 20	3M, 3F 1M, 1F	10	20	20 : Death (M). Tremor, inability to rise. Dyspnoea (M), Convulsions, ↓ activity (F)	05159

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Table 19. Nonpivotal Studies Repeat-Dose Toxicity Studies in Rats (Study No: 06AM037)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses ^a (mg/kg/day)	Number/ Sex/Group	NOAEL (mg/kg/day)	Noteworthy Findings	Study Number
Rat/Sprague- Dawley	Oral gavage, once daily 10 mL/kg (Methylcellulose 0.5% [w/v] aqueous solution + 0.1% [w/v] Tween 80 /Suspension)	4 days	0,	5M		≥100: ↓food consumption. ↓RETI, ↓erythropoiesis, ↑liver weight (relative to body weight)	06AM037
			100,			≥300: ↑salivation, ↓activity, chromodacryorrhea, ↓body weight. ↑RBC, ↑HGB, ↑HCT, ↑GLUC, ↓PHOS, ↑BILI	
			300, 1000			1000: Death (2M) following ↓activity, convulsions (1M), prostration with lacrimation (1M). Tremors, prostration, partially closed-eyes, rough fur. Mild to moderate ↑absolute liver weight, ↓MCV, ↓WBC, ↓LYMP, ↓EOSI, ↓K, ↑GGT	
Toxicokinetic Parameters (M):							
Dose (mg/kg/day)			100	300	1000		
Day 1							
C _{max} (µg/mL)			1.02	7.00	9.55		
AUC ₍₀₋₂₄₎ (µg.h/mL)			15.4	18.7	76.2		

NOAEL = No-observed-adverse-effect-level; M = Male; C_{max} = Maximum (peak) observed drug concentration after single-dose administration; AUC₍₀₋₂₄₎ = Area under concentration-time curve from time 0 to 24 hours after dosing.

^a All doses are expressed as mg of active moiety per kg of body weight per day.

RETI: Reticulocyte count; RBC: Red blood cell count; HGB: Haemoglobin; HCT: Haematocrit; GLUC: Glucose; PHOS: Phosphate; K: Potassium; BILI: Bilirubin; MCV: Mean corpuscular volume; WBC: White blood cell count; LYMP: Lymphocytes; EOSI: Eosinophils; GGT: Gamma glutamyl transferase.

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Table 20. Nonpivotal Repeat-Dose Toxicity Studies in Rats

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg/day)	Duration of Dosing	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Rat/Sprague- Dawley	Intravenous injection, once daily 5 mL/kg (0.02M acetic acid in a 5%[w/v]glucose/Solution)	0, 7.5, 15, 20 ^b	4 days	5M, 5F	7.5	≥ 7.5: Dyspnea, stretching, yawning, urogenital licking and excessive grooming. 15: Death 1M. ≥ 15: Prostration, ↓ activity, tremor, tachypnoea.	05125
		7.5, 15, 20		6M, 6F		<u>Pharmacokinetic data:</u> 20: Death (2M). Plasma drug concentration ↑ with dose, slightly higher in females, and ↓ slightly over the 4-day treatment period.	

^a 0.02 M acetic acid in a 5% (w/v) glucose solution^b 1M and 1F received 25 mg/kg/day on Day 1, due to severe clinical signs observed, the high dose was adjusted to 20 mg/kg/day for the remaining animals.

Table 21. 1-Month Intravenous Toxicity Study in Rats

Species/Strain: Rat/Sprague-Dawley Study Number: 05157
Initial Age: 7 weeks
Date of First Dose: 27 Jul 2005 Lot Number: E010002696
Duration of Dosing: 1 Month
Duration of Postdose: None GLP Compliance: Yes
Method of Administration: Intravenous injection, once daily, 2 mL/kg from Day 1 to 5 (high dose) or from Day 1 to 6 (control, low and mid dose), then 4 mL/kg for all groups.
Vehicle/Formulation: Aqueous solution of acetate buffer (pH 4.5-5) + glucose 50 g/L (w/v).
Special Features: Cyclophosphamide was administered by oral gavage for 2 days (Days 27 and 28) in positive control groups for the micronucleus test (5 animals/group/sex).
Ophthalmology was performed on all animals on prior to study and on Day 23 or 24 of the study.
Plasma drug concentrations were measured on Days 1 and 27. Blood samples were taken at 5 minutes, 1, 3, 7, and 24 hours after dosing.
Dose adjustment: The high dose, 18 mg/kg was reduced to 15 mg/kg on Day 1, after death in the Toxicokinetic group, and to 9 mg/kg on Day 8 after irritation at the injection site. The mid dose was reduced from 9 mg/kg to 3 mg/kg on Day 8, also due to irritation at the injection site.
No-Observed-AdverseEffect Level: 3 mg/kg/day

Dose ^a (mg/kg/day)	0 (Control)		1		9 → 3		18 → 15 → 9	
Gender (M/F)	M	F	M	F	M	F	M	F
Number of Animals								
Toxicity	10	10	10	10	10	10	10	10
Toxicokinetics	3	3	3	3	3	3	3	3
Toxicokinetics:								
Mean Total Cmax (ng/mL)								
Day 1	BLQ	BLQ	109	78.7	1020	917	1450	1310
Day 27	BLQ	BLQ	118	91.6	382	274	NA	768
Mean Total AUC(5min-24h) ^b (ng.h/mL)								
Day 1	BLQ	BLQ	219	269	1890	3130	4730	7510
Day 27	BLQ	BLQ	233	318	787	826	NA	3090
Noteworthy Findings								
Died or Euthanised Moribund								
Main study groups								
Euthanised ^c	0	0	0	0	2	1	8	1
Found dead ^d	0	0	0	0	0	0	1	1
Toxicokinetic groups								
Euthanised ^c	0	0	0	0	1	0	2	2
Found dead ^d	0	0	0	0	0	0	1	0
Body Weight ^e								
Day 7	297.57 g	-	+1	-	-1	-	-4	-

- = No noteworthy findings; BLQ = Below the lower limit of quantification (1 ng/mL).

NA = Not applicable (only data for females were available).

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b AUC(5min-24h): AUC terms were calculated using 5 minutes to 24 hours data.

^c Due to signs at the injection site or inability to dose.

^d High dose animals were found dead on Day 3 (male) and 4 (female) in the Main study groups and on Day 1 (male) in the Toxicokinetic groups.

^e Group means in grams are shown for controls. Percent differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.

Table 21. 1-Month Intravenous Toxicity Study in Rats (Continued)

Dose ^a (mg/kg/day)	0 (Control)		1		9 → 3		18 → 15 → 9	
Gender (M/F)	M	F	M	F	M	F	M	F
Body Weight Gain ^b								
Day 7	37.97 g	-	0	-	0	-	-40 [‡]	-
Clinical Observations								
Main study groups								
Convulsions	0	0	0	0	0	0	1 ^c	1 ^c
Pedalling movements	0	-	0	-	0	-	1	-
Diarrhea	0	-	1	-	0	-	1	-
Injection site								
Oedema	0	0	1	2	5	4	3	9
Haematoma	0	3	1	6	6	4	7	9
Lesion	0	0	0	1	5	2	10	9
Necrosis	0	-	0	-	1	-	2	-
Paleness of the skin	0	0	0	0	2	0	0	1
Redness of the skin	0	0	0	0	5	3	2	5
Clinical Observations								
Toxicokinetic groups								
Convulsions	0	0	0	0	0	0	0	1
Injection site								
Oedema	0	0	1	0	2	2	0	3
Haematoma	0	0	0	0	3	3	2	3
Lesion	0	0	0	0	1	1	2	3
Necrosis	0	0	0	0	1	0	0	1
Paleness of the skin	-	0	-	0	-	0	-	1
Redness of the skin	-	0	-	0	-	1	-	1
Ophthalmology	-	-	-	-	-	-	-	-
Hematology								
Red blood cell count (10 ⁶ /μL)	8.416	7.852	8.103	7.899	8.159	8.043	7.480 ^c	7.415*
Haemoglobin (g/dL)	15.75	14.78	15.40	15.36	15.43	15.17	14.90 ^c	14.41
Haematocrit (%)	46.83	43.89	45.78	45.08	46.13	44.60	45.20 ^c	42.58
Reticulocyte count (10 ³ /μL)	259.07	285.57	254.75	274.75	313.41	283.87	472.00 ^c	353.98
Red cell distribution width (%)	11.37	11.18	11.38	11.40	11.90	11.37	12.90 ^c	12.13 [‡]
Platelet count (10 ³ /μL)	1228.4	1361.3	1241.3	1322.0	1357.9	1442.8	1412.0 ^c	1587.2 ^s
Blood cell morphology	-	-	-	-	-	-	-	-
Clinical Chemistry								
Chloride (mmol/L)	103.4	105.2	102.7	104.0	102.6	103.6*	100.0 ^d	101.8 [‡]
Urinalysis	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology								
Number Examined	10	10	10	10	10	10	10	10
Injection site								
discoloration	0	0	0	0	1	0	7	0
abnormal surface	0	-	0	-	0	-	1	-
scar/wound/scab	0	0	0	0	1	0	5	5

Dunnett's test: * p≤0.05, † p≤0.01; Modified T test: ^s p≤0.05, [‡] p≤0.01; - = No noteworthy findings.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b Group means in grams are shown for controls. Percent differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the percent differences

^c Convulsions occurred on Day 24, when the animal received a dose of 9 mg/kg.

^d One value.

Table 21. 1-Month Intravenous Toxicity Study in Rats (Continued)

Dose ^a (mg/kg/day)	0 (Control)		1		9 → 3		18 → 15 → 9	
Gender (M/F)	M	F	M	F	M	F	M	F
Gross Pathology								
Thymus								
discoloration	0	0	0	7	2	3	0	1
Inguinofemoral lymph nodes								
discoloration	0	0	0	0	0	1	1	1
enlarged	-	0	-	0	-	0	-	4
Histopathology								
Number Examined	10	10	10	10	10	10	10	10
Injection site								
Thrombosis								
minimal	1	2	3	1	1	2	0	1
mild	1	0	1	2	5	5	4	2
moderate	1	1	0	0	2	0	6	7
Perivascular inflammation								
minimal	2	2	3	1	2	4	0	0
mild	0	5	0	5	6	5	5	3
moderate	0	1	0	0	2	1	5	7
Vascular necrosis								
minimal	0	0	0	2	2	1	1	1
mild	3	3	0	2	4	1	2	8
moderate	0	3	0	0	0	0	6	0
Erosion/Ulcer								
moderate	0	0	0	0	0	0	1	1
marked	0	0	0	0	0	0	1	0
Inguinofemoral lymph nodes								
Lymphoid hyperplasia								
mild	0	0	0	0	0	0	2	1
moderate	0	0	0	0	0	0	2	2
Congestion								
mild	0	0	0	0	0	0	1	0
Plasmocytosis								
mild	0	0	0	0	0	0	1	3
moderate	0	0	0	0	0	0	1	3
Haemosiderosis								
minimal	0	0	0	0	0	0	0	1
mild	0	0	0	0	0	0	0	3
Bone marrow								
Myeloid hyperplasia								
minimal	0	0	0	0	0	0	0	1
mild	0	0	0	0	0	0	2	0
Additional Examinations								
Micronucleus assay	-	-	-	-	-	-	-	-

- = No noteworthy findings.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

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Table 22. Oral Escalating Dose Toxicity Study in Dogs(Study No: 06AM142)

Species/Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses ^a (mg/kg/day)	Number/ Sex/Group	NOAEL (mg/kg/day)	Noteworthy Findings	Study Number
Dog/Beagle	Oral gavage, once daily, 1 mL/kg (Methylcellulose 0.5% [w/v] aqueous solution + 0.1% [w/v] Tween 80/Suspension	1 Day	100,	1F		≥100: Arched back with extended limbs, emesis, ↓body weight, ↑Ca, ↓urea.	06AM142
		2 Days	200,	1F			
		1 Day	300	1F		200: ↑PROT, ↑GLOB	
						≥200: ↓food consumption. ↑WBC, ↑NEUT, ↑MONO. ↑ASAT, ↑ALAT	
						≥300: titubation, convulsions, inability to rise, tachypnea, ↑salivation. ↑fibrinogen, ↑ALP	
Toxicokinetic Parameters (1F):							
Dose (mg/kg/day)			100	300	200		
Day			Day 1	Day 2	Day 3		
C _{max} (ng/mL)			709	5890	NI		
AUC ₍₀₋₂₄₎ (ng.h/mL)			1810	32500	NI		

NOAEL = No-observed-adverse-effect-level; F = Female; C_{max} = Maximum (peak) observed drug concentration after single-dose administration.AUC₍₀₋₂₄₎ = Area under concentration-time curve from time 0 to 24 hours after dosing.^a All doses are expressed as mg of active moiety per kg of body weight per day.

PROT: Protein; GLOB: Globulin; WBC: White blood cell count; NEUT: Neutrophils; MONO: Monocytes; ASAT: Aspartate aminotransferase; ALAT: Alanine aminotransferase; ALP: Alkaline phosphatase.

NI = Not Interpreted.

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Table 23. Nonpivotal Repeat-Dose Toxicity Studies

Species/Strain	Method of Administration (Vehicle/Formulation)	Doses (mg/kg/day)	Dosing Period	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Dog/Beagle	Intravenous injection, once daily, 2 mL/kg (0.02M acetic acid in 5% glucose solution)	3,	1 day (Day 1)	1M	10	3: None.	05052
		16,	1 day (Day 3)			≥10: Emesis.	
		10,	1 day (Day 2)	1F		12: Diarrhoea (Day 1), ↑salivation, whining. Rigid extended limbs, pedaling movements and unable to rise (M).	
		12	2 days (Day 8 to 9)	1M, 1F		Slight ↑ in white blood cell count, neutrophils, fibrinogen (M). 16: Tremor, tachypnea, titubation, ↑salivation. <u>Pharmacokinetic data:</u> Plasma drug concentrations ↑with dose, though not in direct proportion. At 12 mg/kg/day the values in the female were higher than those observed in the male.	

Table 24. 1-Month Intravenous Toxicity Study in Dogs

Species/Strain: Dog/Beagle	Study Number: 05158															
Initial Age: About 6 months																
Date of First Dose: 04 Aug 2005	Lot Number: E010002696															
Duration of Dosing: 1 month																
Duration of Postdose: None	GLP Compliance: Yes															
Method of Administration: Intravenous bolus injection, once daily, 2 mL/kg																
Vehicle/Formulation: 0.075 M acetate buffer (pH 5.5) in 5% (w/v) dextrose/Solution																
Special Features:																
Ophthalmology was performed on all animals before study start and on Day 26 of the study.																
Cardiovascular measurements (heart rate, ECG) and blood pressure were recorded on all animals before study start and on Day 22, before and at about 15 minutes after dosing.																
Drug concentrations in plasma were measured on all animals, on Days 1 and 27. Blood samples were taken at 5, 15, and 30 minutes, and 1, 4, and 24 hours after dosing.																
Additional blood samples were taken on Day 22, immediately after the cardiovascular and blood pressure recordings for plasma drug and Sodium, Potassium, Chloride, and Calcium concentrations. .																
No-Observed-Adverse-Effect Level: 3 mg/kg/Day																
Dose^a (mg/kg/day)	0 (Control)		1		3		10									
Gender (M/F)	M	F	M	F	M	F	M	F								
Number of Animals	3	3	3	3	3	3	3	3								
Toxicokinetics:	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined									
Mean Total Cmax (ng/mL)	BLQ		114		377		1370									
Day 1	BLQ		127		439		1580									
Day 27																
Mean Total Concentration (ng/mL)	BLQ		122		278		1290									
Day 22																
Mean Total AUC(5min-24h) ^b (ng.h/mL)	NA		341		844		3350									
Day 1	NA		356		1100		3990									
Day 27																
Noteworthy Findings																
Died or Euthanised Moribund	0	0	0	0	0	0	0	0								
Body Weight Gain ^c																
Day 14	+3.32	+0.91	0	-0.90	0	+1.65	-5.34*	-2.21								
Day 28	+4.87	+2.20	+0.73	+1.67	+1.11	+3.81	-4.89*	-2.60								
Body Weight ^d																
Day 28	9.47 kg	7.90 kg	-13.1	0	-4.2	3.42	-11.6	-5.1								
Ophthalmology	-	-	-	-	-	-	-	-								

Dunnett's test: * p≤0.05

NA = Not applicable; BLQ= Below Limit of Quantification (≤1 ng/mL).

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b AUC(5min-24h): AUC terms were calculated using 5 minutes to 24 hours data.

^c Percent differences from value on Day 1 are shown for controls and treated groups. Statistical significance is based on actual data and not on the percent differences.

^d Group means in kg are shown for controls. Percent differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.

Table 24. 1-Month Intravenous Toxicity Study in Dogs (Continued)

Dose ^a (mg/kg/day)	0 (Control)		1		3		10	
Gender (M/F)	M	F	M	F	M	F	M	F
Number of Animals	3	3	3	3	3	3	3	3
Clinical Observations	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
Incidence (occurrence ^b)								
Increased salivation	6 (26.5)		6 (28.3)		6 (27.3)		6 (28.3)	
Rolling over on the ground ^c	5 (6.8)		6 (16.7)		6 (21.8)		6 (24.8)	
Emesis	1 (1) ^d		1 (1)		2 (3.5)		6 (18.8)	
Whinnying	0		5 (1.4)		6 (8.2)		6 (9.7)	
Extended tail	0		3 (2)		6 (9.2)		6 (9.2)	
Stretching down ^c	0		6 (15.5)		6 (26.7)		6 (27.5)	
Tremor	0		0		0		2 (2.0)	
Titubation ^c	0		0		0		4 (3.3)	
Inability to rise	0		0		2 (1)		3 (14.7)	
Pedalling movements ^c	0		1 (2)		2 (16)		3 (8)	
Stamping ^c	0		0		1 (1)		6 (10)	
Redness of the skin	1 (1)		5 (12.6)		4 (7.3)		5 (4.0)	
Red conjunctiva	0		2 (3.5)		1 (1)		2 (21)	
Electrocardiography (predose/ 15 min after dose)								
Heart rate (beats/min)	108.3/91.3		104.2/127.8 [‡]		105.7/135.7 [‡]		101.2/147.5 [‡]	
P duration (msec)	54/56		54/52*		53/51* [#]		56/51 [§]	
P amplitude (mV)	0.35/0.34		0.40/0.41		0.42/0.42		0.42 [#] /0.50 [§]	
QT interval (msec)	203/207		203/194 [§]		203/189 [‡]		201/188 [‡]	
QTc interval (msec)	207/203		205/207		206/206		202/210 [§]	
Blood Pressure (predose/ 15 min after dose)								
Mean Blood Pressure (mm Hg)	83.8/80.0		94.7/84.8		89.3/93.7		91.5/111.2*	
Systolic Blood Pressure (mm Hg)	116.7/107.0		97.0/121.8		125.5/122.7		132.2/152.5	
Diastolic Blood Pressure (mm Hg)	50.2/51.5		65.0/58.2		59.2/69.7		64.7/73.2	
Number of Animals	3	3	3	3	3	3	3	3
Haematology								
Fibrinogen (mg/dL)								
Day -1	236.7	180.0	242.0	169.7	212.3	187.7	241.3	195.7
Day 29	250.3	195.0	277.0	192.3	250.0	235.3	270.7	253.7
Blood Cell Morphology								
Clinical Chemistry								
Potassium (mmol/L)								
Day -1	4.27	3.83	4.47	4.03	4.47	4.00	4.23	4.17
Day 22	4.30	3.93	3.87	4.03	3.97	3.80	3.40 [§]	3.50 ^{&}
Sodium (mmol/L)								
Day -1	147.0	147.7	146.0	146.3	146.0	147.7	145.7	148.3
Day 22	148.3	148.0	147.0	149.7	146.3	147.0	150.7	150.7
Day 29	148.7	147.7	147.7	146.7	151.3	148.7	150.7	149.0

Analyse of variance before dosing/after dosing: * p≤0.05; [§]p≤0.01; [‡]p≤0.001

Pairwise comparison of treated groups versus control: [#]p≤0.05, [†]p≤0.01; [‡]p≤0.001

Dunnett's test: [&]p≤0.05, [§]p≤0.01

- = No noteworthy findings.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b Mean number of days the sign occurred.

^c Associated with transient increase activity.

^d Before dosing

Table 24. 1-Month Intravenous Toxicity Study in Dogs (Continued)

Dose ^a (mg/kg/day)	0 (Control)		1		3		10	
Gender (M/F)	M	F	M	F	M	F	M	F
Number of Animals	3	3	3	3	3	3	3	3
Chloride (mmol/L)								
Day -1	108.0	110.0	108.3	108.3	109.0	109.7	108.0	110.3
Day 22	111.3	111.0	112.7	110.7	111.0	109.7	113.0	113.7
Day 29	109.7	110.3	111.0	109.7	113.3	111.0	112.3	111.0
Albumin (g/dL)								
Day -1	3.20	3.40	3.37	3.53	3.40	3.50	3.40	3.50
Day 29	3.50	3.63	3.60	3.67	3.70	3.70	4.10*	3.53
Protein (g/dL)								
Day -1	5.67	5.57	6.03*	5.70	5.77	5.87	5.83	5.67
Day 29	5.77	5.50	5.93	5.60	6.07	6.03	6.80†	5.63
Albumin/Globulin ratio								
Day -1	1.30	1.57	1.30	1.63	1.47	1.47	1.40	1.60
Day 29	1.57	1.97	1.53	1.90	1.57	1.60	1.53	1.70
Urinalysis								
Density	1.0273	-	1.0250	-	1.0383	-	1.0437 [#]	-
Bilirubin ^b	0	-	0	-	1	-	3	-
Gross Pathology								
Injection site								
Red discoloration	0	1	0	1	0	1	2	3
Histopathology								
Injection site								
Perivascular inflammation, focal/multifocal								
minimal	1	0	3	1	2	0	0	0
mild	0	0	0	1	1	1	1	0
moderate	0	0	0	0	0	1	1	3
marked	0	0	0	0	0	0	1	0
Perivascular necrosis, focal/multifocal								
minimal	0	0	0	1	1	1	0	1
mild	0	1	0	0	0	0	3	1
moderate	0	0	0	0	0	0	0	1
Perivascular hemorrhage								
minimal	0	2	1	1	0	1	0	0
mild	2	0	1	1	3	1	0	0
moderate	1	0	1	1	0	0	2	2
marked	0	1	0	0	0	1	1	1
Thymus, atrophy								
minimal	0	0	1	1	1	1	3	3

Dunnett's test: * p≤0.05, † p≤0.01; Modified T test: [#]p≤0.05

- = No noteworthy findings.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b Incidence of animals showing presence at grade 2 (moderate).

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Table 25. Genetic Toxicology Studies (Study No.'s 05-2840-04, 05-2840-03, 05158) Test Article: PF-00446687

Type of test	Lot Number	Test cells	Metabolic activation ^a	Dose/Concentration	Results
Microbial reverse mutation assay	E010002696	S. typhimurium	With	0.050 - 5.0 mg/plate	(-)
			Without	0.015 - 5.0 mg/plate	(-)
		E. coli WP2uvrApKM101	With	0.050 - 5.0 mg/plate	(-)
			Without	0.015 - 5.0 mg/plate	(-)
			With	0.050 - 5.0 mg/plate	(-)
			Without	0.015 - 5.0 mg/plate	(-)
In vitro Cytogenetics assay	E010002696	Human lymphocytes	Without	47.9 - 127 µg/mL	(-) ^b
				17.0 - 65.1 µg/mL	(-) ^b
		3 hours	With	26.2 - 65.4 µg/mL	(-)
In vitro Micronucleus assay	10964-57-6	Chinese Hamster Ovary Cells	With	Up to 94 µg/mL	(-)
			Without	Up to 121 µg/mL	(-)
			Without	Up to 58.5 µg/mL	(-)
In vivo Cytogenetics assay ^c	E010002696	Rat bone marrow cells	n/a	1, 9 → 3, 18 → 15 → 9 mg/kg	(-)

n/a = not applicable

^a Aroclor-induced rat liver S9 fraction from male SD rats.^b There was a significant increase in polyploidy frequency of 1.33% and 2.00% in the 3- and 24-hours test.^c The mean total Cmax and AUC(5min-24h) values (males and females combined) for PF-00446687 on Day 27 in rats given 10 mg/kg were 1580 ng/mL and 3990 ng•h/mL, respectively.

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Table 26. Repeat-Dose Toxicity Study in Hamsters**Nonpivotal Studies****Test Article: PF-00446687-01**

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/animal/day)	Duration of Dosing	Gender and No. per Group	NOEL (mg/animal/day)	Noteworthy Findings	Study Number
Hamster/Golden RJ:AURA	Topical application in the right cheek pouch, once daily, 0.020 mL/animal (0.15 M sodium citrate/ethanol 1:1(v/v) /Solution)	0 ^a , 0, 1 ^b , 1.6 ^c	6 days ^d	4M, 4F	1.6	None	04332

^a Non-treated control group.^{b, c} The corresponding concentrations used were 50 and 80 mg/mL, respectively.^d The duration of treatment was reduced to 6 days instead of 7 due to technical problems.**Table 27. Repeat-Dose Toxicity Study in Rats****Nonpivotal Studies****Test Article: PF-00446687-01**

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses ^a (mg/kg/day)	Duration of Dosing	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Rat/Sprague-Dawley	Oral gavage, once daily, 10 mL/kg (aqueous solution 0.01M HCl/Solution)	0, 10, 20	7 days	5M, 5F	>20	None	05156

^a Doses corresponded to respective PF-00446687-01 concentrations levels of 1 or 2 mg/mL.

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Table 28. Subcutaneous and Intravenous Emesis Study in Ferrets (Study No. 04137)

Species/Strain	Method of Administration	Doses (mg/kg)	Duration of Dosing (Study day)	Gender and Number per Group	Noteworthy Findings	Study Number
Ferret/Mustela	Group 1 Subcutaneous injection ^a , 2 mL/kg	0 (Saline)	1 day (Day 1)	3M	No retching or emesis. Yawning and body licking.	04137
	Intravenous injection ^a via catheter, 2 mL/kg	5	1 day (Day 5)		5 IV: ↑ yawning and body licking.	
	Group 2 Subcutaneous injection ^a , 2 mL/kg	5	1 day (Day 1)	3M	≥5 SC: Dose-related ↑ yawning and body licking. Red area at the injection site.	
	Subcutaneous injection ^a , 2 mL/kg	10	1 day (Day 5)			
	Group 3 Subcutaneous injection ^a , 2 mL/kg	10	1 day (Day 1)	3M		
	Intravenous injection ^a via catheter, 2 mL/kg	0 (Saline)	1 day ^b (Day 5)			
	Subcutaneous injection ^a , 2 mL/kg	0 (Saline)	1 day ^c (Day 5)			
	Group 1 Intravenous injection ^a via catheter, 2 mL/kg	5	1 day (Day 7)	3M	Pharmacokinetic data: 5 IV: Ferrets were exposed to plasma drug concentrations which ↓ rapidly with time. 10 SC: Ferrets were exposed to plasma drug concentrations which ↓ slowly with time with residual drug level detected 24 hours after dosing. CSF drug levels (30 minutes after dosing) were about 2-fold higher in the animal dosed at 5 mg/kg IV when compared to that dosed at 10 mg/kg SC.	
	Group 3 Subcutaneous injection ^a , 4 mL/kg	10	1 day (Day 7)	3M		

SC: subcutaneous injection; IV: intravenous injection; CSF: Cerebrospinal fluid. ^a in (Ultrapure water adjusted to pH 3-4/ Solution); ^b2 males; ^c1 male.

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8.2. All Causality, Treatment-Emergent Adverse Events

Table 29. All-Causality Treatment-Emergent Adverse Events Occurring in Protocol A8361001

	Cohort 1					Cohort 2					
	PF-00446687 Doses					PF-00446687 Doses					
	0.3 mg SL	1 mg SL	3 mg SL	32.5 mg SL	Pbo	65 mg SL	130 mg SL	195 mg SL	32.5 mg Orally	195 mg Orally	Pbo
Evaluable	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
With adverse events	6	5	6	6	8	8	8	8	4	4	7
Gastrointestinal Disorders	1 (16.7)	2 (40.0)	2 (33.3)	2 (33.3)	2 (25.0)	6 (75.0)	8 (100.0)	8 (100.0)	1 (25.0)	3 (75.0)	3 (42.9)
Abdominal pain	-	1 (20.0)	-	2 (33.3)	-	2 (25.0)	2 (25.0)	5 (62.5)	1 (25.0)	-	-
Aphthous stomatitis	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Diarrhoea	-	1 (20.0)	-	-	-	-	-	-	-	-	-
Glossodynia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Hypoaesthesia oral	-	-	-	2 (33.3)	-	1 (12.5)	2 (25.0)	2 (25.0)	-	-	-
Nausea	-	-	-	-	-	1 (12.5)	-	3 (37.5)	-	-	-
General Disorders and Administration Site Conditions	-	-	-	1 (16.7)	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Application site anaesthesia	-	-	-	-	-	-	2 (25.0)	-	-	-	-
Application site irritation	-	-	-	-	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Application site paraesthesia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Feeling hot	-	-	-	-	-	1 (12.5)	-	-	-	-	-
Sensation of foreign body	-	-	-	1 (16.7)	-	-	-	-	-	-	-
Infections and infestations	1 (16.7)	1 (20.0)	1 (16.7)	-	-	1 (12.5)	1 (12.5)	-	1 (25.0)	-	-
Nasopharyngitis	-	-	-	-	-	1 (12.5)	1 (12.5)	-	-	-	-
Otitis externa	1 (16.7)	1 (20.0)	1 (16.7)	-	-	-	-	-	-	-	-
Tonsillitis	-	-	-	-	-	-	-	-	1 (25.0)	-	-
Musculoskeletal and connective tissue disorders	-	1 (20.0)	2 (33.3)	-	2 (25.0)	1 (12.5)	-	1 (12.5)	-	-	1 (14.3)
Back pain	-	1 (20.0)	1 (16.7)	-	1 (12.5)	1 (12.5)	-	-	-	-	-
Myalgia	-	-	-	-	-	-	-	1 (12.5)	-	-	1 (14.3)
Pain in extremity	-	-	1 (16.7)	-	1 (12.5)	-	-	-	-	-	-
Nervous system disorders	-	-	-	-	-	1 (12.5)	1 (12.5)	3 (37.5)	1 (25.0)	2 (50.0)	2 (28.6)
Ageusia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Dizziness	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Headache	-	-	-	-	-	-	-	-	1 (25.0)	-	2 (28.6)
Paraesthesia	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Sciatica	-	-	-	-	-	1 (12.5)	1 (12.5)	-	-	-	-
Somnolence	-	-	-	-	-	-	-	1 (12.5)	-	1 (25.0)	-

Reported in MedDRA version 9.1

Pbo = Placebo

SL – Sublingual

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Table 29. All-Causality Treatment-Emergent Adverse Events Occurring in Protocol A8361001 (Continued)

	Cohort 1					Cohort 2					
	PF-00446687 Doses					PF-00446687 Doses					
	0.3 mg	1 mg	3 mg	32.5 mg	Pbo	65 mg	130 mg	195 mg	32.5 mg Orally	195 mg Orally	Pbo
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Evaluable	6	5	6	6	8	8	8	8	4	4	7
With adverse events	1 (16.7)	2 (40.0)	2 (33.3)	2 (33.3)	2 (25.0)	6 (75.0)	8 (100.0)	8 (100.0)	1 (25.0)	3 (75.0)	3 (42.9)
Psychiatric disorders	-	-	-	-	-	-	-	4 (50.0)	-	1 (25.0)	-
Restlessness	-	-	-	-	-	-	-	4 (50.0)	-	1 (25.0)	-
Respiratory, thoracic and mediastinal disorders	-	-	-	-	-	-	2 (25.0)	2 (25.0)	-	2 (50.0)	-
Dysphonia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Pharyngolaryngeal pain	-	-	-	-	-	-	2 (25.0)	-	-	1 (25.0)	-
Yawning	-	-	-	-	-	-	-	2 (25.0)	-	2 (50.0)	-
Vascular disorders	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Hot flush	-	-	-	-	-	-	-	-	-	1 (25.0)	-

Reported in MedDRA version 9.1

Pbo = Placebo

SL = Sublingual

8.2.1. All-Causality, Treatment Emergent Adverse Events Summary

The all-causality, treatment-emergent adverse events in PF-00446687-treated subjects, regardless of incidence are listed by body system below (in MedDRA version 9.1 preferred terms).

Eye disorders: eye irritation

Gastrointestinal disorders: abdominal pain, diarrhoea, glossodynia, hypoaesthesia oral, nausea

General disorders and administration site conditions: application site anaesthesia, application site irritation, application site paraesthesia, fatigue, feeling hot, sensation of foreign body

Infections and infestations: nasopharyngitis, otitis externa, rhinitis, tonsillitis

Musculoskeletal and connective tissue disorders: back pain, myalgia, pain in extremity

Nervous system disorders: ageusia, dizziness, paraesthesia, somnolence

Psychiatric disorders: libido increased, restlessness

Respiratory, thoracic and mediastinal system disorders: cough, dysphonia, pharyngolaryngeal pain, yawning

Skin and subcutaneous tissue disorders: erythema

Vascular disorders: hot flush

Includes Protocols: A8361001, A8361002

Table generated 24 Jan 07

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8.3. Treatment-Related, Treatment-Emergent Adverse Events

Table 30. Treatment-Related, Treatment-Emergent Adverse Events in Protocol A8361001

	Cohort 1					Cohort 2					
	PF-00446687 Doses					PF-00446687 Doses					
	0.3 mg SL	1 mg SL	3 mg SL	32.5 mg SL	Pbo	65 mg SL	130 mg SL	195 mg SL	32.5 mg Orally	195 mg Orally	Pbo
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Evaluable	6	5	6	6	8	8	8	8	4	4	7
With adverse events	-	-	-	2 (33.3)	-	5 (62.5)	7 (87.5)	8 (100.0)	1 (25.0)	2 (50.0)	1 (14.3)
Gastrointestinal Disorders	-	-	-	2 (33.3)	-	2 (25.0)	2 (25.0)	5 (62.5)	1 (25.0)	-	-
Abdominal pain	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	1 (25.0)	-	-
Glossodynia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Hypoaesthesia oral	-	-	-	2 (33.3)	-	1 (12.5)	2 (25.0)	2 (25.0)	-	-	-
Nausea	-	-	-	-	-	1 (12.5)	-	3 (37.5)	-	-	-
General Disorders and Administration Site Conditions	-	-	-	1 (16.7)	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Application site anaesthesia	-	-	-	-	-	-	2 (25.0)	-	-	-	-
Application site irritation	-	-	-	-	-	4 (50.0)	7 (87.5)	7 (87.5)	-	-	-
Application site paraesthesia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Feeling hot	-	-	-	-	-	1 (12.5)	-	-	-	-	-
Sensation of foreign body	-	-	-	1 (16.7)	-	-	-	-	-	-	-
Musculoskeletal and connective tissue disorders	-	-	-	-	-	1 (12.5)	-	-	-	-	1 (14.3)
Myalgia	-	-	-	-	-	-	-	-	-	-	1 (14.3)
Nervous system disorders	-	-	-	-	-	1 (12.5)	-	3 (37.5)	-	2 (50.0)	-
Ageusia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Dizziness	-	-	-	-	-	-	-	2 (25.0)	-	-	-
Paraesthesia	-	-	-	-	-	-	-	1 (12.5)	-	1 (25.0)	-
Somnolence	-	-	-	-	-	-	-	1 (12.5)	-	1 (25.0)	-
Psychiatric disorders	-	-	-	-	-	-	-	-	-	4 (50.0)	-
Restlessness	-	-	-	-	-	-	-	-	-	4 (50.0)	-
Respiratory, thoracic and mediastinal disorders	-	-	-	-	-	-	-	1 (12.5)	-	1 (25.0)	-
Dysphonia	-	-	-	-	-	-	-	1 (12.5)	-	-	-
Yawning	-	-	-	-	-	-	-	1 (12.5)	-	1 (25.0)	-
Vascular disorders	-	-	-	-	-	-	-	-	-	1 (25.0)	-
Hot flush	-	-	-	-	-	-	-	-	-	1 (25.0)	-

Reported in MedDRA version 9.1

Pbo = Placebo, SL – Sublingual

8.3.1. Treatment-Related, Treatment Emergent Adverse Events Summary

The treatment-related, treatment-emergent adverse events in PF-00446687-treated subjects, regardless of incidence are listed by body system below (in MedDRA version 9.1 preferred terms).

Gastrointestinal disorders: abdominal pain, diarrhoea, glossodynia, hypoaesthesia oral, nausea

General disorders and administration site conditions: application site anaesthesia, application site irritation, application site paraesthesia, fatigue, feeling hot, sensation of foreign body

Musculoskeletal and connective tissue disorders: myalgia

Nervous system disorders: ageusia, dizziness, paraesthesia, somnolence

Psychiatric disorders: libido increased, restlessness

Respiratory, thoracic and mediastinal disorders: dysphonia, yawning

Skin and subcutaneous tissue disorders: erythema

Vascular disorders: hot flush

Includes Protocols: A8361001,A8361002

Table generated 24 Jan 07