Supplemental Information for JPET-AR-2022-001393

Initial pharmacological characterization of a major hydroxymetabolite of PF-5190457: inverse agonist/antagonist activity of PF-6870961 at the ghrelin receptor

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Supplementary Appendix S1. Inclusion and Exclusion Criteria

Inclusion criteria:

- Males or females 21-65 years old (inclusive)
- Heavy drinking (least 21 drinks per week for men or at least 14 drinks for women on average via the 90-day timeline follow-back done at screening)
- Negative urine drug screen and good health as confirmed by medical history, physical examination, electrocardiogram (EKG), blood/urine lab tests
- Female subjects must be of non-childbearing potential as defined by at least one of the following criteria:
    1. Females 45 – 65 years old, who are menopausal, defined as follows:

       Females who are between 45 – 55 years old will be considered menopausal if they satisfy all the following three requirements during screening:

        - In amenorrhea, defined as absence of menstruation for the previous 12 months
        - Negative urine pregnancy test
        - Serum follicle-stimulating hormone concentration within the laboratory's reference range for postmenopausal females

Females who are between 56 – 65 years old are considered menopausal if they are in amenorrhea, defined as absence of menstruation for the previous 12 months before screening

OR

2. Females 21-65 years old, who have a documented hysterectomy and/or bilateral oophorectomy

All other female subjects (including females with tubal ligations and females that do not have a documented hysterectomy) are considered to be of childbearing potential

- Male subjects must use one of the following methods of contraception from the first dose of study medication and until 28 days after dosing:
    a. Abstinence
    b. A condom AND one of the following:
        i. Vasectomy for more than 6 months
        ii. Female partner who meets one of the following conditions
        a. Has had a tubal ligation, hysterectomy, or bilateral oophorectomy
        b. Is post-menopausal
        c. Uses one of the following forms of contraception
            - Copper or hormonal containing intrauterine device
            - Spermicidal foam/gel/film/cream/suppository
            - Diaphragm with spermicide
            - Oral contraceptive
            - Injectable progesterone
            - Subdermal implant

Exclusion criteria:

- Interest in receiving treatment for heavy drinking

- Current Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) diagnosis (based on structured clinical interview for DSM-IV) of substance dependence (other than alcohol and/or nicotine);
- DSM-IV Axis I criteria for a lifetime diagnosis of schizophrenia, bipolar disorder, or other psychoses
- Active illness within the past 6 months of the screening visit that meet the DSM-IV criteria for a diagnosis of major depressive disorder or anxiety disorder; any history of attempted suicide. Clinically significant medical abnormalities (e.g., unstable hypertension, clinically significant EKG abnormalities, Creatinine ≥ 2 mg/dL, liver cirrhosis, aspartate aminotransferase or alanine transaminase > 3x the upper normal limit, hemoglobin <10.5 g/dl)
- Heart rate >100 at screening on two separate measurements
- Body Mass Index (BMI) ≤ 18.5 or anorexia
- BMI ≥ 35 kg/m^2
- Exclusionary Medications:
- Naltrexone, acamprosate, alcohol dehydrogenase inhibitors, topiramate, gabapentin, ondansetron, benzodiazepines, beta-blockers, H2-blockers, and alpha-1 blockers, baclofen and barbiturates as well as hormone replacement therapy; medications and dietary/herbal supplements (e.g. St. John's wort) that interact with Cytochrome P450 3A4. All medications in the previous sentence are exclusionary if taken within 2 weeks of study medication administration.
- PF-5190457 is a substrate for P-glycoproteins (P-gp or encoded by ABCB1 gene) based on information from in vitro or animal models. Patients that are required to take the following inhibitors and inducers of P-gp are excluded unless the subject stops taking these agents for 2 weeks for P-gp inhibitors or 6 weeks for P-gp inducers before study medication administration:

i. *Inhibitors*: Amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir and ritonavir, quercetin, quinidine, ranolazine, verapamil

ii. *Inducers*: Avasimibe, carbamazepine, phenytoin, rifampin, St John's wort, tipranavir/ritonavir

[From Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers, table 12, from http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#substrates]

- History of epilepsy or alcohol-related seizures
- Patients who have diabetes and/or are treated with any drug with glucose lowering properties such as sulfonylurea, insulin, metformin, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, or glucagon-like peptide-1 agonists (due to the glucose-lowering properties of PF-5190457 observed in healthy volunteers)
- History of alcohol-induced flushing reactions
- Clinically significant alcohol withdrawal symptoms, as assessed by a Clinical Institute Withdrawal Assessment of Alcohol Scale Revised score > 8 at screening
- Any other reason or clinical condition for which the principal investigator or the medical advisory investigator consider unsafe for a possible participant to participate in this study

Supplementary Table 1: % Inhibition of Off-Target Binding and Enzymatic Activity of PF-6870961

Target	Radioligand	% Inhibition at 100 nM	% Inhibition at 10000 nM
Binding			
A_1 (h)	Antagonist	-8.5	-5.3
A_{2A} (h)	Agonist	1.3	5.8
A_{2B} (h)	Antagonist	-1.3	-30.2
A_3 (h)	Agonist	-7.6	-5.6
α_1 (Non-selective)	Antagonist	1.9	7.9
α_2 (Non-selective)	Antagonist	-5.2	1.8
β_1 (h)	Agonist	7.2	5.7
AT_1 (h)	Antagonist	-4.5	0.7
AT_2 (h)	Agonist	2.4	1.7
BZD	Agonist	-19.6	-17.6
B_2	Agonist	-2.9	1.6
CB_1	Agonist	-6.4	-2.8
CB_2	Agonist	2.9	0.7
CCK_1	Agonist	9.5	13.7
CCK_2	Agonist	-0.6	-2.0
CRF_1	Agonist	-1.1	-3.3
$D_{4.4}$	Antagonist	13.1	43.5
ET_A	Agonist	-6.6	-4.0
ET_B	Agonist	-11.0	-26.0
$GABA_{A1}$	Agonist	-7.6	6.9
$GABA_{B(1b)}$	Antagonist	-13.9	-1.1
GAL_1	Agonist	-3.2	-0.4
GAL_2	Agonist	-0.7	-2.1
AMPA	Agonist	-8.1	-11.4
kainate	Agonist	3.1	37.9
NMDA	Antagonist	2.2	1.1
mGluR1	Agonist	-11.6	7.3
mGluR5	Agonist	-1.6	-8.0
Glycine (strychnine-sensitive)	Antagonist	15.9	1.6
Glycine (strychnine-insensitive)	Antagonist	-2.9	-6.7
H_1	Antagonist	3.4	1.5
H_2	Antagonist	-1.5	10.7
H_3	Agonist	-11.4	9.1
$CysLT_1$ (LTD_4)	Agonist	-1.5	2.5
M (non-selective)	Antagonist	8.4	39.8
M_1	Antagonist	6.4	19.2
M_2	Antagonist	-18.1	9.1
M_3	Antagonist	-0.4	4.8
NK_1	Agonist	-14.6	8.1
NK_2	Agonist	-7.4	14.5
NK_3	Antagonist	7.6	13.3
N neuronal α4β2	Antagonist	1.6	0.2
N neuronal α7	Antagonist	0.5	30.4
N muscle type	Antagonist	-8.0	3.8
δ (DOP)	Agonist	10.0	10.7
κ (KOP)	Agonist	-9.9	8.5
μ (MOP)	Agonist	1.7	12.8
Oxytocin	Agonist	-0.5	4.3

NOP	Agonist	5.8	0.8
PAF	Agonist	1.1	6.6
PCP	Antagonist	3.8	1.5
GR	Agonist	7.3	-9.8
ER (non-selective)	Agonist	4.9	-3.6
AR	Agonist	11.3	-4.1
TRH_1	Agonist	-2.9	-8.4
$VPAC_2$	Agonist	-6.3	-12.1
$VPAC_1$	Agonist	-8.1	-10.4
V_{1a}	Agonist	7.8	5.0
V_{1b}	Agonist	-1.7	10.9
Ca+2 channel (L, dihydropyridine site)	Antagonist	-31.5	-8.6
Ca +2 channel (L, diltiazem site)	Antagonist	-1.5	-8.5
Ca^{2+} channel (N)	Antagonist	2.2	2.8
K_{ATP} channel	Antagonist	6.6	-1.3
SK_{ca} channel	Antagonist	-8.8	-6.5
Na^+ channel (site 2)	Antagonist	11.6	8.2
Enzyme			
Choline Acetyl Transferase		-4.0	46.4
TXA_2 Synthetase		-1.1	15.2
Constitutive NOS (endothelial)		-17.7	30.7
Acetylcholinesterase (h)		5.8	36.3
MAO-A		-0.1	2.9
MAO-B		-24.0	-14.6

Supplementary Table 2: %Inhibition of Dopamine, Serotonin, and Opioid Receptors and Related Transporters

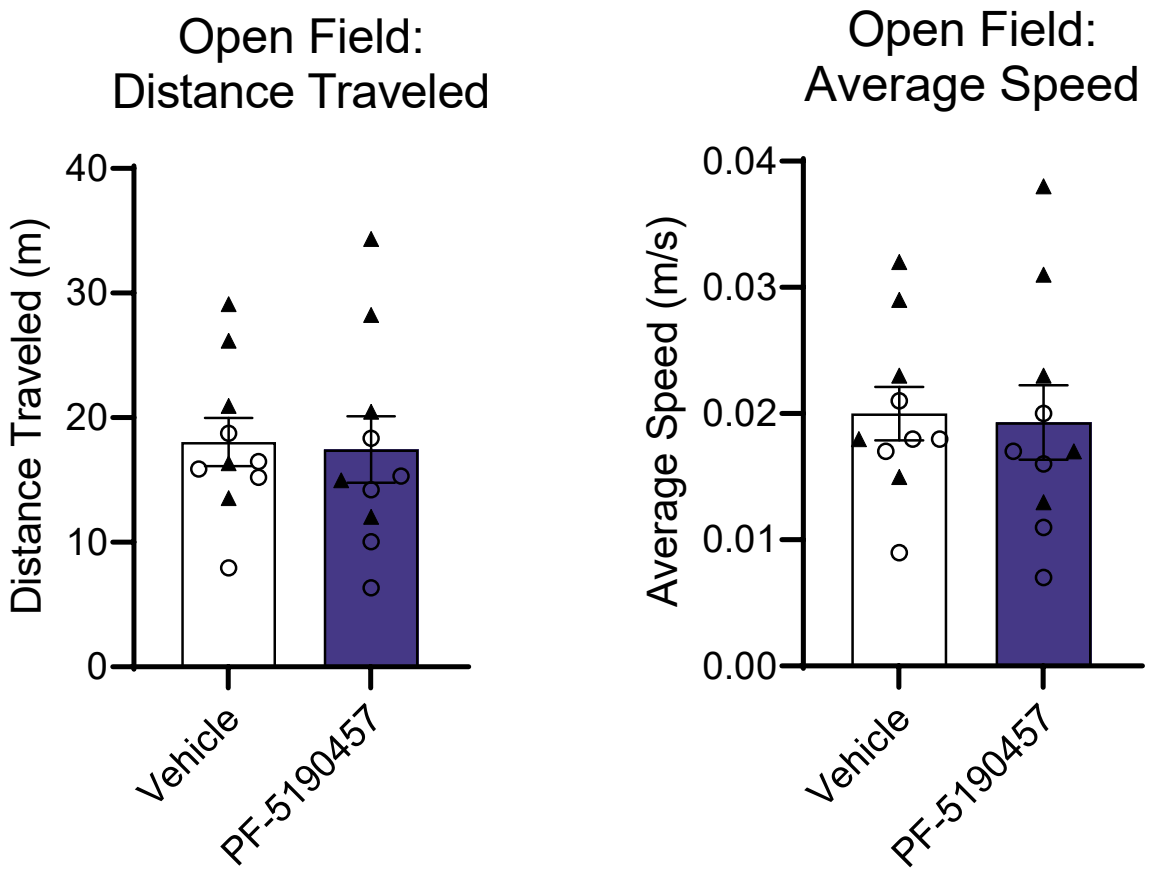
Target	Radioligand	% Inhibition at 100 nM	% Inhibition at 10 µM
5HT1A	[^{3}H]8OH-DPAT	0.85 ± 4.01%	14.71 ± 0.99%
5HT2A	[^{3}H]5-HT	14 ± 14%	0.50 ± 16.79%
5HT2C	[^{3}H]5-HT	3.9 ± 5.6%	18.16 ± 0.09%
DA D1	[^{3}H]SCH 23390	-4.3 ± 5.1%	-7.0 ± 6.6%
DA D2	[^{3}H]YM-09151-2	0.89 ± 6.15%	9.58 ± 0.31%
DA D3	[^{3}H]YM-09151-2	-3.0 ± 5.4%	10.57 ± 0.44%
DA D4.4	[^{3}H]YM-09151-2	-2.2 ± 6.2%	22.3 ± 1.5%
δ (DOP)	[^{3}H]DPDPE	-2.7 ± 1.5%	3.36 ± 0.24%
κ (KOP)	[^{3}H]U69,593	5.7 ± 3.2%	11.8 ± 4.1%
µ (MOP)	[^{3}H]DAMGO	11.5 ± 4.3%	26.1 ± 3.7%
DAT	[^{125}I]RTI-55	16.1 ± 1.7%	17.5 ± 3.0%
SERT	[^{125}I]RTI-55	5.07 ± 0.91%	7.9 ± 2.4%
NET	[^{125}I]RTI-55	14.22 ± 0.58%	23.8 ± 1.5%

Data are expressed as the % inhibition of specific control binding. Numbers represent the means ± range from two independent experiments, each conducted with at least triplicate determinations. Negative inhibition values indicate that more specific binding was measured in the presence of PF-6870961than under control conditions

Supplementary Table 3: Mean Plasma Concentration of PF-5190457 and PF-6870961

	50 mg BID PF-5190457		100 mg BID PF-6870961	
	PF-5190457 (ng/mL)	**PF-6870961 (ng/mL)**	**PF-5190457 (ng/mL)**	**PF-6870961 (ng/mL)**
Day 1				
Pre-Dose	-	2.06	-	2.34
Post-Dose	105.52	26.40	218.07	48.17
Day 2				
Pre-Dose	21.26	10.94	41.01	19.35
Post-Dose	106.57	33.92	260.34	74.13

BID = twice daily



Supplementary Figure S1. PF-51904575 (30 mg/kg) had no effect on spontaneous locomotion in rats as measured by distance traveled (m) (**left**) and average speed (m/s) (**right**) in an open field test. Data are shown as mean ± S.E.M and analyzed using a two-tailed, paired t test. *n* = 10, ○ = male rats, ▲ = female rats.