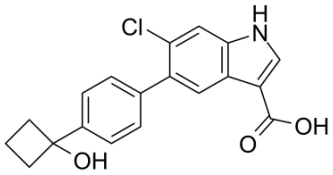
PF-06409577

Cat. No.:	HY-103683
CAS No.:	1467057-23-3
Molecular Formula:	$C_{19}H_{16}ClNO_3$
Molecular Weight:	341.79
Target:	AMPK
Pathway:	Epigenetics; PI3K/Akt/mTOR
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (292.58 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	2.9258 mL	14.6289 mL	29.2577 mL
	5 mM	0.5852 mL	2.9258 mL	5.8515 mL
	10 mM	0.2926 mL	1.4629 mL	2.9258 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: **10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline**
   Solubility: ≥ 2.5 mg/mL (7.31 mM); Clear solution
2. Add each solvent one by one: **10% DMSO >> 90% (20% SBE-β-CD in saline)**
   Solubility: ≥ 2.5 mg/mL (7.31 mM); Clear solution
3. Add each solvent one by one: **10% DMSO >> 90% corn oil**
   Solubility: ≥ 2.5 mg/mL (7.31 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

PF-06409577 is a potent and selective allosteric activator of **AMPK** α1β1γ1 isoform with an **EC_{50}** of 7 nM.

IC_{50} & Target

AMPK α1β1γ1
7 nM (EC50)

In Vitro	PF-06409577 possesses similar potency toward the human and rat α1β1γ1 isoforms. In broad panel screening against other receptors, channels, PDEs and kinases, PF-06409577 exhibits minimal off-target pharmacology. PF-06409577 shows no detectable inhibition of hERG in a patch-clamp assay (100 μM) and is not an inhibitor (IC_{50}>100 μM) of the microsomal activities of major human cytochrome P450 isoforms[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	PF-06409577 demonstrates moderate plasma clearance in rats, dogs, and monkeys, and is well distributed with steady state distribution volume. Following oral administration of crystalline PF-06409577 in 0.5% methylcellulose suspension, PF-06409577 is rapidly absorbed in rats, dogs, and monkeys. The corresponding oral bioavailability values in rats, dogs, and monkeys, are 15%, 100%, and 59%, respectively. Dose responsive increases in pAMPK relative to total AMPK (tAMPK) in whole kidney tissue are observed with a maximal 3.8-fold response at 300 mg/kg PF-06409577 treatment[1]. Oral administration of PF-06409577 (10, 30, and 100 mg/kg QD) results in dose-dependent reductions in proteinuria in the obese ZSF1 animals, with greater than 2-fold reduction in 24-hour urinary albumin loss compared to vehicle control after 60 days of treatment[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	PF-06409577 is prepared in DMSO. PF-06409577 is incubated with fully phosphorylated AMPK in assay buffer at room temperature for 15 min followed by addition of PP2a and another incubation for 60 min at room temperature. The phosphatase treatment is quenched and the kinase assay initiated with the addition of okadaic acid (50 nM final), 50 nM Cy-5 SAMS peptide and ATP equal to Km for each isoform. Reactions are incubated for an additional 60 min and the kinase reaction is quenched with the addition of 10 mM EDTA and 2 nM Eu-pACC antibody in detection Buffer. Kinase activity is monitored by excitation at 320 nM and measuring emission at 665 and 615 nM, respectively [1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Rats: Daily administration of 0.5% methylcellulose (p.o.), PF-06409577 at 10, 30, or 100 mg/kg (p.o.), PF- 249 at 3, 10, or 30 mg/kg (p.o.), or ramipril in drinking water (1 mg/kg/day) is initiated and continued for 68 days. Urine is collected for 24-hours and volume recorded from all lean and obese rats after 14, 28, 42, and 60 days of dosing. On Day 63 all rats are administered a final dose after 16-hour overnight fasting. One hour following the final dose, blood glucose is measured by glucometer and a 100 μL tail vein blood sample collected and processed for determination of insulin levels and total protein. Each rat is then anesthetized with isoflurane. The right kidney is collected and immediately freeze-clamped and transferred to liquid nitrogen storage; the left kidney is fixed in 10% formalin. Rats are then euthanized by exsanguination from the vena cava[1] MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Cameron KO, et al. Discovery and Preclinical Characterization of 6-Chloro-5-[4-(1-hydroxycyclobutyl)phenyl]-1H-indole-3-carboxylic Acid (PF-06409577), a Direct Activator of Adenosine Monophosphate-activated Protein Kinase (AMPK), for the Potential Treatment of Diabetic Nephropathy. J Med Chem. 2016 Sep 8;59(17):8068-81.

[2]. Salatto CT, et al. Selective Activation of AMPK β1-Containing Isoforms Improves Kidney Function in a Rat Model of Diabetic Nephropathy. J Pharmacol Exp Ther. 2017 May;361(2):303-311.

Caution: Product has not been fully validated for medical applications. For research use only.

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