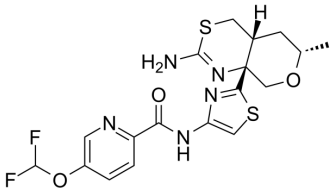
PF-06751979

Cat. No.:	HY-112157
CAS No.:	1818339-66-0
Molecular Formula:	$C_{18}H_{19}F_2N_5O_3S_2$
Molecular Weight:	455.5
Target:	Beta-secretase
Pathway:	Neuronal Signaling
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 : 150 mg/mL (329.31 mM; Need ultrasonic)
Ethanol : 50 mg/mL (109.77 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1954 mL	10.9769 mL	21.9539 mL
	5 mM	0.4391 mL	2.1954 mL	4.3908 mL
	10 mM	0.2195 mL	1.0977 mL	2.1954 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: ≥ 2.75 mg/mL (6.04 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.75 mg/mL (6.04 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

PF-06751979 is a potent, brain penetrant, β-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitor with an IC_{50} of 7.3 nM in BACE1 binding assay.

IC_{50} & Target

IC50: 7.3 nM (BACE1), 194 nM (BACE2)[1]

In Vitro

PF-06751979 shows improved selectivity over BACE2 (IC_{50}=194 nM) in binding (27-fold) relative to the literature examples and across multiple chemical series in BACE1 program. PF-06751979 also inhibits BACE1 and BACE2 in a fluorescent polarization (FP) assay with IC_{50}s of 26.9 nM and 238 nM, respectively. PF-06751979 has excellent potency at BACE1 in binding or FP assay formats along with cellular activity looking at production of sAPPβ in H4 cells with an IC_{50} of 5 nM[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

PF-06751979 displays excellent brain penetration, potent in vivo efficacy, and broad selectivity over related aspartyl proteases including BACE2. Acute administration of PF-06751979 yields a robust dose-responsive and time-dependent reduction of cerebral spinal fluid (CSF) Aβx-40 with peak inhibition at 3 h of >77%. To determine if the reduction in brain and CSF Aβ is maintained during sustained exposure to PF-06751979, a 5 day subchronic study is executed, dosing once daily by subcutaneous (SC) administration (10 or 50 mg/kg/day). Brain and CSF samples are collected on day 5, following the last dose. PF-06751979 produces a dose-responsive and time-dependent inhibition of Aβ42 in mouse brain. At the 50 mg/kg/day dose, maximal brain lowering is 63% at 7 to 9 h. Administration of PF-06751979 (10 or 50 mg/kg/day for 5 days) produces a dose-responsive and time-dependent inhibition of Aβx-40 in mouse CSF resulting in 77% inhibition of CSF at 3 h post-final 50 mg/kg dose[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]

Both BACE1 and BACE2 enzymatic activity is measured with the aid of an optimized synthetic peptide substrate biotin-GLTNIKTEEISEISYEVEFR-C[Oregon green]KK-OH. Upon cleavage of the peptide substrate, a decrease in fluorescence polarization is measured. Compounds are diluted by half log in 100% DMSO 11 times with a top concentration of 10mM in a 384-well polypropylene plate. The 100% DMSO dose response curve is then added to a 384-well black assay plate as 0.150 μ L per well. The final working top concentration is 0.1 mM, and the DMSO concentration is 1%. A volume of 7.5 μL of BACE substrate is then added in assay buffer (100 mM sodium acetate, pH to 4.5 with glacial acetic acid, 0.001% Tween 20). The background wells in column 1 of the 384-well assay plate receive 7.5 μL of assay buffer. The reaction is started with the addition of 7.5 μL of BACE1 or BACE2 enzyme in assay buffer to all wells except the background wells in column 1. The final concentration of peptide substrate is 150 nM, and the final concentration of BACE1 and BACE2 enzyme is 0.15 and 2.5 nM, respectively. The assay plate is sealed and incubated at 37°C for 3 or 1 h (BACE1 or BACE2, respectively). After incubation, 15 μL of stop solution (1.5 μM streptavidin in Dulbecco's PBS) is added to all wells, and the plate is read. Percent effect values for each concentration of compound are calculated based on fluorescence polarization (FP) readings in the 100% effect control wells containing no enzyme and the 0% effect control wells containing no compound. Curve-fitting analysis utilizing concentrations and percent effect values for a given compound is plotted, and the IC_{50} is determined using a sigmoidal four-parameter fit algorithm[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay [1]

The neuroglioma cell line H4 cells are grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 200 mM glutamax. Cells are plated overnight in tissue culture treated Falcon 384-well plates at a cell density of 4500 cells/well in 50 μL of media. The next day, media is removed, and cells are washed once with PBS, after which 25 μL of media is placed in all wells, followed by the addition of the diluted PF-06751979 dose response curve. The highest concentration tested is 30 μM with 1% DMSO. Cells serving as the background controls receive 30 μM of a proprietary compound. Compounds are allowed to incubate with cells overnight in a 37°C incubator. Concurrently, 384-well black Nunc Maxisorp plates are also incubated overnight at 4°C with 10 μL of 4 μg/mL Aβ antibody in coating buffer (0.1 M sodium bicarbonate, pH 8.8 to 9.0)[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration [1]

Mice[1]

Male 129/sve wild-type mice (20-25 g) are in a nonfasted state prior to subcutaneous dosing with vehicle or PF-06751979 using a dosing volume of 10 mL/kg. The mice are dosed subcutaneously once a day for 5 days with PF-06751979 (10 or 50 mg/kg/day) or vehicle. The mice (n=5 per group) are then sacrificed at 1, 3, 5, 7, 14, 20, and 30 h postdose. Following cardiac puncture into ethylenediaminetetraacetic acid (EDTA)-containing tubes, whole blood samples (0.5-1.0 mL) are collected, and plasma is separated by centrifugation (1500× g for 10 min at 4°C). The generated plasma is distributed into separate tubes on wet ice for exposure measurements (50 μL) and Aβ analysis (remainder). CSF samples (8-12 μL) are obtained by cisterna magna puncture using a sterile 25 gauge needle and collected with a P-20 Eppendorff pipet. CSF samples are distributed into separate tubes on dry ice for exposure measurements (3 μL) and Aβ analysis (remainder). Whole brain is removed and divided for exposure measurements (cerebellum) and Aβ analysis (left and right hemispheres), weighed, and

frozen on dry ice. Prior to the assay, all samples are stored at -80°C[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Alzheimers Dement. 2019 Sep;15(9):1183-1194.

See more customer validations on www.MedChemExpress.com

REFERENCES

[1]. O'Neill BT, et al. Design and Synthesis of Clinical Candidate PF-06751979: A Potent, Brain Penetrant, β-Site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) Inhibitor Lacking Hypopigmentation. J Med Chem. 2018 May 24;61(10):4476-4504.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA