Crizotinib

Cat. No.: HY-50878

CAS No.: 877399-52-5

Molecular Formula: $C_{21}H_{22}Cl_2FN_5O$

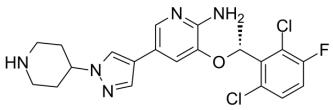
Molecular Weight: 450.34

Target: Anaplastic lymphoma kinase (ALK); c-Met/HGFR; ROS Kinase; Autophagy

Pathway: Protein Tyrosine Kinase/RTK; Autophagy

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 : 20 mg/mL (44.41 mM; ultrasonic and warming and heat to 60°C)

H_2O : < 0.1 mg/mL (ultrasonic) (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2205 mL	11.1027 mL	22.2054 mL
5 mM	0.4441 mL	2.2205 mL	4.4411 mL
10 mM	0.2221 mL	1.1103 mL	2.2205 mL

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

In Vivo

1. Add each solvent one by one: 50% PEG300 >> 50% saline
   Solubility: 20 mg/mL (44.41 mM); Suspended solution; Need ultrasonic and warming and heat to 40°C
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 1.25 mg/mL (2.78 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 1 mg/mL (2.22 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 1 mg/mL (2.22 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Crizotinib (PF-02341066) is an orally bioavailable, ATP-competitive ALK and c-Met inhibitor with IC_{50}s of 20 and 8 nM, respectively. Crizotinib inhibits tyrosine phosphorylation of NPM-ALK and tyrosine phosphorylation of c-Met with IC_{50}s of 24 and 11 nM in cell-based assays, respectively. Crizotinib is also a ROS1 inhibitor. Crizotinib has effective tumor growth inhibition[1][2][3].

IC_{50} & Target	IC50: 20 nM (ALK), 8 nM (c-Met)[3]
In Vitro	Crizotinib (PF-02341066) displays similar potency against c-Met phosphorylation in mIMCD3 mouse or MDCK canine epithelial cells with IC_{50} of 5 nM and 20 nM, respectivly. PF-2341066 shows improved or similar activity against NIH3T3 cells engineered to express c-Met ATP-binding site mutants V1092I or H1094R or the P-loop mutant M1250T with IC_{50} of 19 nM, 2 nM and 15 nM, respectively, compared with NIH3T3 cells expressing wild-type receptor with IC_{50} of 13 nM. In contrast, a marked shift in potency of PF-2341066 is observed against cells engineered to express c-Met activation loop mutants Y1230C and Y1235D with IC_{50} of 127 nM and 92 nM, respectively, compared with wild-type receptor. PF-2341066 also potently prevents the phosphorylation of c-Met in NCI-H69 and HOP92 cells, with IC_{50} of 13 nM and 16 nM, respectively, which express the endogenous c-Met variants R988C and T1010I, respectively[1]. Crizotinib (PF-02341066) also potently inhibits NPM-ALK phosphorylation in Karpas299 or SU-DHL-1 ALCL cells with an IC_{50} of 24 nM. PF-2341066 potently prevents cell proliferation, which is associated with G(1)-S-phase cell cycle arrest and induction of apoptosis in ALK-positive ALCL cells with IC_{50} of 30 nM, but not ALK-negative lymphoma cells[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Crizotinib (PF-02341066) reveals the ability to cause marked regression of large established tumors (> 600 mm^3) in both the 50 mg/kg/day and 75 mg/kg/day treatment cohorts, with a 60% decrease in mean tumor volume over the 43-day administration schedule in the GTL-16 model. In an another study, PF-2341066 displays the ability to completely inhibits GTL-16 tumor growth for >3 months, with only 1 of 12 mice exhibiting a significant increase in tumor growth over the 3-month treatment schedule at 50 mg/kg/day. A significant dose-dependent reduction of CD31-positive endothelial cells is observed at 12.5 mg/kg/day, 25 mg/kg/day, and 50 mg/kg/day in GTL-16 tumors, indicating that inhibition of MVD shows a dose-dependent correlation to antitumor efficacy. PF-2341066 displays a significant dose-dependent reduction of human VEGFA and IL-8 plasma levels in both the GTL-16 and U87MG models. Marked inhibition of phosphorylated c-Met, Akt, Erk, PLCλ1, and STAT5 levels is observed in GTL-16 tumors following p.o. administration of PF-2341066[1]. Treatment of c-MET-amplified GTL-16 xenografts with 50 mg/kg PF-2341066 elicits tumor regression that is associated with a slow reduction in 18F-FDG uptake and decreases expression of the glucose transporter 1, GLUT-1[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Cells are seeded in 96-well plates in media supplemented with 10% fetal bovine serum (FBS) and transferred to serum-free media [with 0.04% bovine serum albumin (BSA)] after 24 h. In experiments investigating ligand-dependent RTK phosphorylation, corresponding growth factors are added for up to 20 min. After incubation of cells with PF-2341066 for 1 h and/or appropriate ligands for the designated times, cells are washed once with HBSS supplemented with 1 mM Na_3VO_4, and protein lysates are generated from cells. Subsequently, phosphorylation of selected protein kinases is assessed by a sandwich ELISA method using specific capture antibodies used to coat 96-well plates and a detection antibody specific for phosphorylated tyrosine residues. Antibody-coated plates are (a) incubated in the presence of protein lysates at 4°C overnight; (b) washed seven times in 1% Tween 20 in PBS; (c) incubated in a horseradish peroxidase-conjugated anti-total-phosphotyrosine (PY-20) antibody (1:500) for 30 min; (d) washed seven times again; (e) incubated in 3,3′,5,5′-tetramethyl benzidine peroxidase substrate to initiate a colorimetric reaction that is stopped by adding 0.09 N H_2SO_4; and (f) measured for absorbance in 450 nm using a spectrophotometer. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Tumor cells are seeded in 96-well plates at low density in media supplemented with 10% FBS (growth media) and transferred to serum-free media (0% FBS and 0.04% BSA) after 24 h. Appropriate controls or designated concentrations of PF-2341066 are added to each well, and cells are incubated for 24 to 72 h. Human umbilical vascular endothelial cells (HUVEC) are seeded in 96-well plates in EGM2 media for 5 to 6 h at > 20,000 cells per well and transferred to serum-free media overnight. The following day, appropriate controls or designated concentrations of PF-2341066 are added to each well, and after 1 h incubation, HGF is added to designated wells at 100 ng/mL. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay is done to determine the relative tumor cell or HUVEC numbers. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration [1]	Athymic mice bearing xenografts (300-800 mm^3) are given PF-2341066 in water by oral gavage at designated dose levels. At designated times following PF-2341066 administration, mice are humanely euthanized, and tumors are resected. Tumors are snap frozen and pulverized using a liquid nitrogen-cooled cryomortar and pestle, protein lysates are generated, and protein concentrations are determined using a BSA assay. The level of total and phosphorylated protein is determined using a capture ELISA or immunoprecipitation-immunoblotting method. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Hematol Oncol. 2018 Aug 29;11(1):109.
- Cancer Discov. 2018 Mar;8(3):354-369.
- Nat Biomed Eng. 2018 Aug;2(8):578-588.
- Blood. 2021 Oct 17;blood.2020008136.
- Sci Transl Med. 1 Sep 2021.

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REFERENCES

[1]. Zou HY, et al. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. Cancer Res. 2007, 67(9), 4408-4417.

[2]. Christensen JG, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. Mol Cancer Ther. 2007, 6(12 Pt 1), 3314-3322.

[3]. Cui JJ, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem. 2011 Sep 22;54(18):6342-63.

[4]. Cullinane C, et al. Differential (18)F-FDG and 3'-deoxy-3'-(18)F-fluorothymidine PET responses to pharmacologic inhibition of the c-MET receptor in preclinical tumor models. J Nucl Med. 2011 Aug;52(8):1261-7

[5]. Shen A, et al. c-Myc alterations confer therapeutic response and acquired resistance to c-Met inhibitors in MET-addicted cancers. Cancer Res. 2015 Nov 1;75(21):4548-59.

[6]. Umapathy G, et al. The kinase ALK stimulates the kinase ERK5 to promote the expression of the oncogene MYCN in neuroblastoma. Sci Signal. 2014 Oct 28;7(349):ra102.

[7]. Tucker ER, et al. Immunoassays for the quantification of ALK and phosphorylated ALK support the evaluation of on-target ALK inhibitors in neuroblastoma. Mol Oncol. 2017 Aug;11(8):996-1006.

[8]. Liu H, et al. Identifying and Targeting Sporadic Oncogenic GeneticLiu H, et al. Identifying and Targeting Sporadic Oncogenic Genetic Aberrations in Mouse Models of Triple Negative Breast Cancer. Cancer Discov. 2018 Mar;8(3):354-369.

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