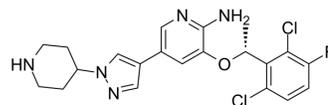


Crizotinib

Cat. No.:	HY-50878		
CAS No.:	877399-52-5		
Molecular Formula:	C ₂₁ H ₂₂ Cl ₂ FN ₅ O		
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	1 year
		-20°C	6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : 20 mg/mL (44.41 mM); ultrasonic and warming and heat to 60°C
 H₂O : < 0.1 mg/mL (ultrasonic) (insoluble)

	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	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

- 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
- Add each solvent one by one: 10% DMSO >> 90% corn oil
 Solubility: ≥ 1.25 mg/mL (2.78 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
 Solubility: ≥ 1 mg/mL (2.22 mM); Clear solution
- 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 IC₅₀s of 20 and 8 nM, respectively. Crizotinib inhibits tyrosine phosphorylation of NPM-ALK and tyrosine phosphorylation of c-Met with IC₅₀s 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]}.

IC₅₀ & Target	IC ₅₀ : 20 nM (ALK), 8 nM (c-Met) ^[3]
In Vitro	<p>Crizotinib (PF-02341066) displays similar potency against c-Met phosphorylation in mIMCD3 mouse or MDCK canine epithelial cells with IC₅₀ of 5 nM and 20 nM, respectively. 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 IC₅₀ of 19 nM, 2 nM and 15 nM, respectively, compared with NIH3T3 cells expressing wild-type receptor with IC₅₀ 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 IC₅₀ 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 IC₅₀ of 13 nM and 16 nM, respectively, which express the endogenous c-Met variants R988C and T1010I, respectively^[1].</p> <p>Crizotinib (PF-02341066) also potently inhibits NPM-ALK phosphorylation in Karpas299 or SU-DHL-1 ALCL cells with an IC₅₀ 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 IC₅₀ of 30 nM, but not ALK-negative lymphoma cells^[2].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
In Vivo	<p>Crizotinib (PF-02341066) reveals the ability to cause marked regression of large established tumors (> 600 mm³) 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].</p> <p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

PROTOCOL

Kinase Assay ^[1]	<p>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 Na₃VO₄, 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 H₂SO₄; and (f) measured for absorbance in 450 nm using a spectrophotometer.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Cell Assay ^[1]	<p>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.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

Animal Administration ^[1]

Athymic mice bearing xenografts (300-800 mm³) 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

- Signal Transduct Target Ther. 2024 Mar 9;9(1):65.
- J Hematol Oncol. 2018 Aug 29;11(1):109.
- Cancer Discov. 2023 Mar 1;13(3):598-615.
- Cancer Discov. 2018 Mar;8(3):354-369.
- Nat Biomed Eng. 2018 Aug;2(8):578-588.

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- [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.
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- [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 Genetic Aberrations in Mouse Models of Triple Negative Breast Cancer. *Cancer Discov.* 2018 Mar;8(3):354-369.

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