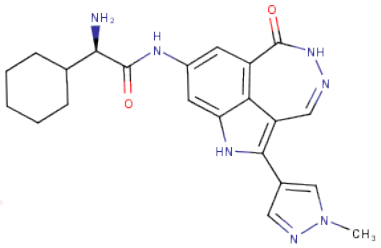
Data Sheet (Cat.No.T6028)

TargetMol
A DRUG SCREENING EXPERT

PF 477736

Chemical Properties



CAS No. : 952021-60-2

Formula: C22H25N7O2

Molecular Weight: 419.48

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	PF 477736 (PF-736,PF-00477736) is a specifc, effective and ATP-competitive Chk1 inhibitor (Ki: 0.49 nM) and also inhibits FGFR3, Aurora-A, VEGFR2, Flt3, Fms (CSF1R), Ret and Yes.
Targets(IC50)	c-Fms,VEGFR,FGFR,FLT,c-RET,Chk,CDK,Src,Aurora Kinase
In vitro	PF-477736 (128 nM) abrogates the camptothecin-induced DNA damage checkpoint in a dose-dependent manner in CA46 and HeLa cells. PF-477736 effectively abrogates the gemcitabine-induced S-phase arrest with a corresponding increase in apoptotic cell populations in HT29 cells. PF-477736 (540 nM) enhances gemcitabine-induced cytotoxicity in a time- and dose-dependent manner in HT29 cells. PF-477736 potentiates the growth-inhibitory activity of a panel of chemotherapeutic agents across a broad spectrum of p53-deficient human cancer cell lines in the MTT assay. Addition of PF-477736 (360 nM) to gemcitabine-arrested cells induces a dramatic increase in the intensity of H2AX phosphorylation, reflecting a greater number of γ-H2AX molecules near sites of DNA damage. [1] PF-477736 (0.5 nM) selectively blocks p73 and P53 phosphorylation in presence of curcumin in HL-60 cells. [2] PF-477736 (360 nM) suppresses docetaxel-induced phosphorylation of histone H3 (Ser10) and Cdc25C (Ser216) and potentiates apoptosis in COLO205 cells. [3] PF-477736 (250 nM) combined with MK-1775 has marked synergistic cytotoxic activity in OVCAR-5 cells. PF-477736 (250 nM) combined with MK-1775 causes accumulation of cells with a DNA content between 2N and 4N in OVCAR-5 cells. PF-477736 (250 nM) combined with MK-1775 causes premature mitosis before the end of DNA replication, with damaged DNA leading to apoptotic cell death in OVCAR-5 cells. [4]
In vivo	PF-477736 (4 mg/kg i.v.) results in terminal half-life (T1/2) of 2.9 hours, AUC of 5.72 μg×hr/mL and CLp of 11.8 mL/min/kg in rats. PF-477736 dose-dependently enhances the antitumor activity of a maximum tolerated dose of gemcitabine in the Colo205 xenograft mouse model. PF-477736 (12 mg/kg) induces an increase in the phosphorylation of histone H3 (Ser10) and of phospho-histone H2AX in the Colo205 xenograft mouse model. [1] PF-477736 (15 mg/kg i.p.) enhances docetaxel induced tumor growth inhibition and tumor growth delay in COLO205 and MDA-MB-231 xenograft models. [3] PF 477736 (10 mg/kg once daily i.p.) combined with MK-1775 (30 mg/kg twice a day oral) leads to greater tumor growth inhibition in mice bearing OVCAR-5 xenografts. [4]

Kinase Assay	Binding assay: The assay is performed in a 96-well plate for 20 minutes at 30℃ in 0.1 mL of assay buffer containing 50 mM TRIS pH 7.5, 0.4 M NaCl, 4 mM PEP, 0.15 mM NADH, 28 units of lactate dehydrogenase/mL, 16 units of pyruvate kinase/mL, 3 mM DTT, 0.125 mM Syntide-2, 0.15 mM ATP and 25 mM magnesium chloride. Assays are initiated with 1 nM of CHK1 kinase domain. The inhibition of CHK1 activity is determined by measuring initial velocities in the presence of varying concentrations of PF-477736. The data is analyzed using Enzyme Kinetic and Excel software and fit to a kinetic model for competitive inhibition to obtain a Ki value. The kinase selectivity of PF-477736 is evaluated by screening the compound at 1 μM or 10 μM against a panel 2 of about 100 protein kinases.
Cell Research	The IC50 assay measures the antiproliferative effects of PF-477736 on p53-defective human cancer cell lines. Cells in each line are seeded in complete medium at an exponentially growing density in 96-well assay plate and allowed to attach for 16 hours. Serial dilutions of PF-477736 are then done, and appropriate controls are added to each plate. Cells are incubated with drug for 96 hours. After incubation, MTT working stock diluted in complete medium is added to each well, and cells are incubated for 4 hours. After centrifugation and supernatant removal, DMSO is added to each well and plates are read on SpectraMax plate reader at 540 nm. (Only for Reference)

Solubility Information

Solubility	DMSO: 31.5 mg/mL (75 mM), (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.3839 mL	11.9195 mL	23.839 mL
5 mM	0.4768 mL	2.3839 mL	4.7678 mL
10 mM	0.2384 mL	1.192 mL	2.3839 mL
50 mM	0.0477 mL	0.2384 mL	0.4768 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

Blasina A, et al. Mol Cancer Ther, 2008, 7(8), 2394-2404.
Chakraborty J, et al. J Biol Chem, 2010, 285(43), 33104-33112.