

Product Data Sheet

Product Name: PF-477736

Cat. No.: GC14234

Chemical Properties

Cas. No. 952021-60-2

Chemical Name (R)-2-amino-2-cyclohexyl-N-(2-(1-methyl-1H-pyrazol-4-yl)-6-oxo-5,6-dihydro-4H-[1,2]diazepino[4,5,6-cd]indol-8-yl)acetamide

SMILES O=C(C(C1([H])C([H])([H])C([H])([H])C([H])([H])C([H])([H])C1([H])([H])([H])N([H])([H])N([H])C2=C([H])C3=C4C(N=C(C5=C([H])N(C([H])([H])([H])N=C5[H])C4=C([H])N([H])N([H])C3=O)=C2[H])

Formula C₂₂H₂₅N₇O₂

M.Wt 419.48

Solubility ≥ 5.2mg/mL in DMSO

Storage Store at -20°C

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

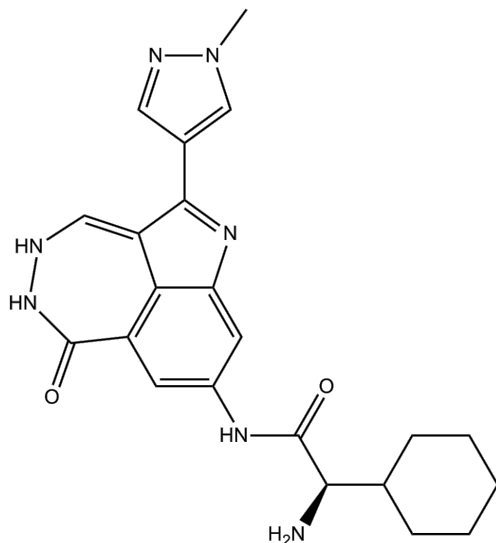
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Kinase experiment [1]:

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Kinase assays

CHK1 activity was measured by a pyruvate kinase-lactate dehydrogenase coupled, continuous spectrophotometric assay method where the phosphorylation of a CHK1 peptide substrate (Syntide-2, PLARTLSVAGLPGKK) was coupled to the oxidation of NADH and the corresponding change in absorbance intensity was measured at 340 nm. The assay was performed in a 96-well plate for 20 minutes at 30°C 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 were initiated with 1nM of CHK1 kinase domain. The inhibition of CHK1 activity was determined by measuring initial velocities in the presence of varying concentrations of PF-477736.

Cell experiment [2]:

Cell lines

HT29 cell lines

Preparation method

The solubility of this compound in DMSO is >5.2mg/mL. General tips for obtaining a higher concentration: Please warm the tube at 37°C for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Reacting condition

69 nM, 24h

Applications

The gemcitabine and PF-477736 combination induced significant potentiation of the cytotoxic activity of gemcitabine, indicating that the combination treatment caused a permanent cellular damage that cannot be overcome even after drug removal. The cell kill induced by the combination treatment is time and dose dependent.

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Animal experiment [2]:

Animal models	Colo205 Xenograft Models in Athymic Nude Mice
Dosage form	i.p., 4-60 mg/kg, q3d × 4
Application	In xenografts, PF-477736 enhanced the antitumor activity of gemcitabine in a dose-dependent manner.
Other notes	Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.

References:

[1] Blasina A1, Hallin J, Chen E, Arango ME, Kraynov E, Register J, Grant S, Ninkovic S, Chen P, Nichols T, O'Connor P, Anderes K. Breaching the DNA damage checkpoint via PF-00477736, a novel small-molecule inhibitor of checkpoint kinase 1. Mol Cancer Ther. 2008 Aug;7(8):2394-404.

Background

PF-477736 is a potent, selective ATP-competitive and small-molecule inhibitor of Chk1 with a K_i of 0.49 ± 0.29 nM for the in vitro kinase activity of Chk1 [1].

PF-477736 has shown a selective inhibition of Chk1 with the IC_{50} values of 0.49 nM, 9.9 μ M and 47 nM for Chk1, CDK1 and Chk2, respectively. In a dot-blot assay, PF-477736 has been reported to inhibit camptothecin-induced G2 arrest with the EC_{50} values of 45 nM, 38 nM and 42 nM in P53-mutated human lymphoma CA46 cells, HeLa cells and HT29 cells. Apart from these, PF-477736 has been found to selectively target p53-defective cancer cells while having minimal cytotoxic effects on normal (p53-competent) cells. In addition, PF-00477736 has been revealed to dose-dependently enhance the antitumor activity of a MTD of gemcitabine with no apparent exacerbation of systemic toxicity as assessed by monitoring body weight in the Colo205 xenograft [1].

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