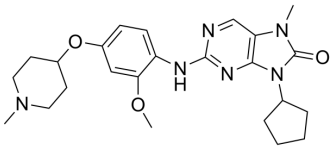
AZ3146

Cat. No.:	HY-14710
CAS No.:	1124329-14-1
Molecular Formula:	$C_{24}H_{32}N_6O_3$
Molecular Weight:	453
Target:	Mps1
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (220.75 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2075 mL	11.0375 mL	22.0751 mL
5 mM	0.4415 mL	2.2075 mL	4.4150 mL
10 mM	0.2208 mL	1.1038 mL	2.2075 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: ≥ 2.5 mg/mL (5.52 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.52 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

AZ3146 is a reasonably potent Mps1 and TTK inhibitor, with IC_{50} of 35 nM for $Mps1^{Cat}$.

IC_{50} & Target

$Mps1^{Cat}$
35 nM (IC_{50})

In Vitro

In in vitro kinase assays, AZ3146 inhibits human $Mps1^{Cat}$ with IC_{50} of ~35 nM. AZ3146 also efficiently inhibits autophosphorylation of full-length Mps1 immunoprecipitated from human cells[1]. TTK specific kinase inhibitor AZ3146 can decrease HCC cell growth. In vitro cell cytotoxicity assays are performed on SMMC-7721 and BEL-7404 cells. IC_{50}s are calculated as being 7.13 μM (BEL-7404) and 28.62 μM (SMMC-7721). Both cells are further treated under the concentration of IC50 for 4 days. Significant inhibitions of cell proliferation are observed[2]. HCT116 cells are cultured for 10 days in 0.8 μM

(the GI_{50}) of AZ3146 , then 2 μM AZ3146 for 3 weeks. Sixteen clones are isolated and cell lines generated, named AzR1-16, all of which are resistant to AZ3146-induced cell death in cell viability assays; AzR3 and 4 have a GI_{50} of approximately 3 μM (4-fold resistance), while the remaining clones have a GI_{50} of approximately 9 μM (11-fold resistance). When analyzing mitosis by time-lapse microscopy, while 2 μM AZ3146 causes the parental cell line to rapidly exited mitosis in 10 minutes[3].

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

PROTOCOL

Kinase Assay [1]

His-tagged human $Mps1^{Cat}$ encoding amino acids 510-857 is generated. For kinase assays, 500 ng is added to buffer (25 mM Tris-HCl, pH 7.4, 100 mM NaCl, 50 μg/mL BSA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 10 mM $MgCl_2$, and 0.5 μg/mL myelin basic protein), AZ3146, and 100 μM γ-[^{32}P]ATP (2 μCi/assay). Reactions are incubated at 30°C for 20 min, spotted onto P81 paper, washed in 0.5% phosphoric acid, and immersed in acetone. Phosphate incorporation is determined by scintillation counting. For immunoprecipitation kinase assays, HeLa cells are treated with nocodazole for 14 h, mitotic cells isolated, washed in PBS, and lysed for 30 min in 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.5% NP-40, 5 mM EDTA, 5 mM EGTA, 40 mM β-glycerophosphate, 0.2 mM PMSF, 1 mM DTT, 1 mM sodium orthovanadate, 20 mM sodium fluoride, 1 μM okadaic acid, and complete EDTA-free protease inhibitor cocktail. Full-length Mps1 is immunoprecipitated. Purified complexes are washed with lysis buffer containing 100 mM NaCl and assayed as described for the recombinant protein. To quantify ^{32}P incorporation, reactions are stopped with SDS sample buffer and separated by SDS-PAGE followed by phosphorimaging. The plate is analyzed using a phosphorimager using AIDA software. To assess the specificity of AZ3146, a single-point screen is carried using kinase profiling service. 50 kinases are selected and assayed with 1 μM AZ3146[1].

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

Cell Assay [2]

The TTK inhibitor AZ3146 is disolved in DMSO at a concentration in 100 mM and diluted into 100 μM, 10 μM, 1 μM and 0.1 μM sequentially with DMEM containing 10% FBS before use. In vitrocytotoxicity assays are performed. HCC cells are plated into 96-well plates at the density of 3×10^3 per well. AZ3146 is added in the indicated concentrations the next day. The inhibitor treated cells are cultured and tested at a 24-hour intervals for 3-4 days using CCK-8[2].

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

CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- J Biol Chem. 2019 Feb 8;294(6):2021-2035.
- bioRxiv. September 11, 2018.

See more customer validations on www.MedChemExpress.com

REFERENCES

[1]. Hewitt L, et al. Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. J Cell Biol. 2010 Jul 12;190(1):25-34.

[2]. Liu X, et al. TTK activates Akt and promotes proliferation and migration of hepatocellular carcinoma cells. Oncotarget. 2015 Oct 27;6(33):34309-20.

[3]. Gurden MD, et al. Naturally Occurring Mutations in the MPS1 Gene Predispose Cells to Kinase Inhibitor Drug Resistance. Cancer Res. 2015 Aug 15;75(16):3340-54.

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

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