PHA-767491 hydrochloride

Cat. No.: HY-13461A

CAS No.: 942425-68-5

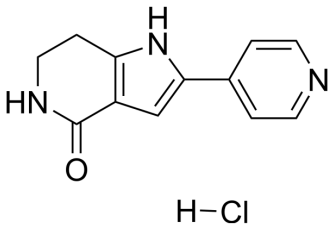
Molecular Formula: $C_{12}H_{12}ClN_3O$

Molecular Weight: 249.7

Target: CDK; Apoptosis

Pathway: Cell Cycle/DNA Damage; Apoptosis

Storage: 4°C, sealed storage, away from moisture
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro

H_2O : 50 mg/mL (200.24 mM; Need ultrasonic)
DMSO : 17.33 mg/mL (69.40 mM; Need ultrasonic and warming)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	4.0048 mL	20.0240 mL	40.0481 mL
5 mM	0.8010 mL	4.0048 mL	8.0096 mL
10 mM	0.4005 mL	2.0024 mL	4.0048 mL

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

In Vivo

1. Add each solvent one by one: PBS
   Solubility: 50 mg/mL (200.24 mM); Clear solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 1 mg/mL (4.00 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 1 mg/mL (4.00 mM); Clear solution

BIOLOGICAL ACTIVITY

Description PHA-767491 hydrochloride is a dual Cdc7/Cdk9 inhibitor, with IC_{50}s of 10 nM and 34 nM, respectively.

IC_{50} & Target

CDK9 34 nM (IC_{50})	CDK2 240 nM (IC_{50})	CDK1 250 nM (IC_{50})	CDK5 460 nM (IC_{50})
GSK3-β 220 nM (IC_{50})	Mk2 470 nM (IC_{50})	Plk1 980 nM (IC_{50})	Chk2 1100 nM (IC_{50})

In Vitro	PHA-767491 inhibits proliferation in both cell lines with an IC_{50} of 0.64 μM in HCC1954 cells and 1.3 μM in Colo-205 cells. PHA-767491 is effective DDK inhibitors in vitro, with IC_{50} values of 18.6 nM. PHA-767491 (2 μM) completely abolishes Mcm2 phosphorylation by 24 hours in HCC1954 cells[1]. PHA-767491 in combination with 5-FU exhibits much stronger cytotoxicity and induces significant apoptosis manifested by remarkably increased caspase 3 activation and poly(ADP-Ribose) polymerase fragmentation in HCC cells. PHA-767491 directly counteracts the 5-FU-induced phosphorylation of Chk1 and decreases the expression of the anti-apoptotic protein myeloid leukemia cell 1ine[2]. PHA-767491 (0-10 μM) decreases glioblastoma cell viability in a time- and dose-dependent fashion, with IC_{50} of approximately 2.5 μM for U87-MG and U251-MG cells. PHA-767491 hydrochloride induces apoptosis in glioblastoma cells, suppresses glioblastoma cell proliferation, cell migration and cell invasion[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	PHA-767491 decreases Chk1 phosphorylation and increases in situ cell apoptosis in tumor tissues sectioned from nude mice HCC xenografts[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	20 ng of purified human DDK is pre-incubated with increasing concentrations of each DDK inhibitor for 5 min. Then 10 μCi (γ)-^{32}P ATP and 1.5 μM cold ATP are added in a buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 1 mM DTT and incubated for 30 min at 30°C. The proteins are denatured in 1X Laemmli buffer at 100°C followed by SDS-PAGE and autoradiography on HyBlot CL film. Auto-phosphorylation of DDK is used as an indicator of its kinase activity. ^{32}P-labeled bands are quantified using ImageJ and the IC_{50} values are calculated using GraphPad. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	For assays in 96 well plates 2500 cells are plated per well. After 24 hours, cells are treated with small molecule inhibitors and incubated for 72 hours at 37°C. Subsequently the cells are lysed and the ATP content is measured as an indicator of metabolically active cells using the CellTiter-Glo assay. IC_{50} values are calculated using the GraphPad software. For assays in six well plates, 100,000 cells are plated per well. After 24 hours, cells are treated with small molecule inhibitors and incubated for varying time points. Cells are trypsinized and a suspension is made in 5 mL of phosphate buffered saline. 30 μL of this suspension is mixed with 30 μL of CellTiter-Glo reagent followed by a 10-minute incubation at room temperature. Luminescence is measured using EnVision 2104 Multilabel Reader and BioTek Synergy Neo Microplate Reader. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Autophagy. 2021 Dec 10;1-19.
- Acta Pharmacol Sin. 2021 Jun 29.
- Sci Rep. 2021 Mar 8;11(1):5374.

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REFERENCES

[1]. Montagnoli A, et al. A Cdc7 kinase inhibitor restricts initiation of DNA replication and has antitumor activity. Nat Chem Biol. 2008 Jun;4(6):357-65.

[2]. Sasi NK, et al. The potent Cdc7-Dbf4 (DDK) kinase inhibitor XL413 has limited activity in many cancer cell lines and discovery of potential new DDK inhibitor scaffolds. PLoS One. 2014 Nov 20;9(11):e113300.

[3]. Li W, et al. Dual Inhibition of Cdc7 and Cdk9 by PHA-767491 Suppresses Hepatocarcinoma Synergistically with 5-Fluorouracil. Curr Cancer Drug Targets. 2015;15(3):196-204.

[4]. Erbayraktar Z, et al. Cell division cycle 7-kinase inhibitor PHA-767491 hydrochloride suppresses glioblastoma growth and invasiveness. Cancer Cell Int. 2016 Nov 18;16:88.

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