Product Data Sheet

Product Name: PHA-767491
Cat. No.: GC11324

Chemical Properties

Cas. No.	845714-00-3		
Chemical Name	2-pyridin-4-yl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one		
SMILES	C1CNC(=O)C2=C1NC(=C2)C3=CC=NC=C3		
Formula	$C_{12}H_{11}N_3O$	M.Wt	213.24
Solubility	≥ 10.65 mg/mL in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	PHA-767491		

Protocol

Kinase experiment:

20 ng of purified human DDK is pre-incubated with increasing concentrations of each DDK inhibitor for 5 min. Then 10 µCi (γ)-32P ATP and 1.5 µM cold ATP are added in a buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 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. 32P-labeled bands are quantified using ImageJ and the IC50 values are calculated using GraphPad.

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Cell experiment: 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. IC50 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.

References:

[1]. 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.
[2]. Li W, et al. Dual Inhibition of Cdc7 and Cdk9 by PHA-767491

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Suppresses Hepatocarcinoma Synergistically with 5-Fluorouracil. Curr Cancer Drug Targets. 2015;15(3):196-204.
[3]. 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.
[4]. 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.

Background

PHA-767491 is a small-molecule inhibitor of Cdc7 kinase with IC50 value of 10 nM [1]. In the process of DNA synthesis, Cdc7 kinase is critical to the activation of replication

origins. It phosphorylates several subunits of mini chromosome maintenance proteins (MCMs), especially the Mcm2 at Ser40 and Ser53 sites, which constitute the replicative DNA helicase. Cdc7 is overexpressed in tumor cells and tumor specimens, therefore the Cdc7 inhibitors are developed as anticancer agents. As a small-molecule Cdc7 inhibitor, PHA-767491 exerted antitumor activity through a different mechanism than other drugs target DNA replication. PHA-767491 competed with ATP for binding to Cdc7 and showed an IC50 value of 10 nM in the presence of 1.5 μM ATP [1].

PHA-767491 is a selective inhibitor. It showed no inhibitory effect on 15 kinases when tested against a panel of 38 serine/theronine and tyrosine kinases. However, it inhibited cdk1, cdk2 and GSK-3β with 20-fold less potency. Besides that, PHA-767491 was found to have inhibitory effect on Cdk9 with IC50 value of 34 nM, which made it be regarded as a dual inhibitor of Cdk7/9 kinase. When treated to a panel of 61 human cell lines, PHA-767491 caused cell proliferation inhibition with an average IC50 value of 3.17μM. It killed cells both p53-positive and p53-negative. Among the exceptions, NHDF and MCF7 cells were resistant to PHA-767491. K562 cells were less sensitive to PHA-767491 [1].

In mice bearing implanted tumors derived from HL60 cell lines, administration of PHA-767491 at dose of 20 and 30 mg/kg twice a day reduced tumor volume dose-dependently. PHA-767491 administration also resulted in 50% tumor growth inhibition in xenograft models of A2780 ovary carcinoma, HCT-116 colon carcinoma or Mx-1 mammary adenocarcinoma [1].

Reference:

[1] Montagnoli A, Valsasina B, Croci V, et al. A Cdc7 kinase inhibitor restricts initiation of DNA replication and has antitumor activity. Nature chemical biology, 2008, 4(6): 357-365.

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