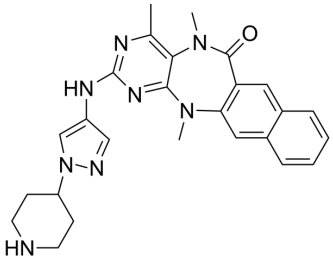
HTH-01-015

Cat. No.:	HY-12334
CAS No.:	1613724-42-7
Molecular Formula:	$C_{26}H_{28}N_8O$
Molecular Weight:	468.55
Target:	AMPK
Pathway:	Epigenetics; PI3K/Akt/mTOR
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 1 year -20°C 6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (213.42 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1342 mL	10.6712 mL	21.3424 mL
	5 mM	0.4268 mL	2.1342 mL	4.2685 mL
	10 mM	0.2134 mL	1.0671 mL	2.1342 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 (5.34 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.34 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.34 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

HTH-01-015 is a selective NUAK1/ARK5 inhibitor (IC_{50} is 100 nM). HTH-01-015 inhibits NUAK1 with >100-fold higher potency than NUAK2 (IC50 of >10 μM).

IC_{50} & Target

NUAK1
100 nM (IC_{50})

In Vitro

HTH-01-015 is a specific NUAK1 inhibitor. The related NUAK1 and NUAK2 are members of the AMPK (AMP-activated protein

kinase) family of protein kinases that are activated by the LKB1 (liver kinase B1) tumor suppressor kinase. HTH-01-015 inhibits NUAK1 with an IC_{50} of 100 nM, but does not significantly inhibit NUAK2 (IC_{50} of >10 μM).? In all cell lines tested, HTH-01-015 inhibits the phosphorylation of the only well-characterized substrate, MYPT1 (myosin phosphate-targeting subunit 1) that is phosphorylated by NUAK1 at Ser^{445}. In U2OS cells, HTH-01-015 suppresses proliferation and phosphorylation of MYPT1 to the same extent as shRNA-mediated NUAK1 knockdown. In mouse embryonic fibroblasts (MEFs), treatment with 10 μM HTH-01-015 suppresses proliferation and phosphorylation of MYPT1 to the same extent as NUAK1-knockout. To test whether NUAK1 inhibition impaired the ability of the invasive U2OS cells to enter a matrix, 3D Matrigel Transwell invasion assays demonstrate that 10 μM HTH-01-015 markedly inhibits the invasiveness of U2OS cells in this assay[1].

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

PROTOCOL

Kinase Assay [1]

Kinase inhibitor specificity profiling assays are carried out against a panel of 140 protein kinases. Results are presented as a percentage of kinase activity in DMSO control reactions. Protein kinases are assayed in vitro with 0.1 or 1 μM of the inhibitors (e.g., HTH-01-015) and the results are presented as an average of triplicate reactions±S.D. or in the form of comparative histograms[1].

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Cell Assay [1]

Cell proliferation assays are carried out colorimetrically in 96-well plates using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay kit. Initially, 2000 cells per well are seeded for U2OS cells and 3000 cells per well are seeded for MEFs. The proliferation assays are carried out over 5 days in the presence or absence of 10 μM HTH-01-015 or WZ4003. The ability of U2OS cells to invade in the presence or absence of 10 μM HTH-01-015 or WZ4003 is tested in a growth-factor-reduced Matrigel invasion chamber. Cells are serum-deprived for 2 h, detached using cell-dissociation buffer, and 2.5×10^5 cells suspended in DMEM containing 1% (w/v) BSA are added to the upper chambers in triplicate and chemoattractant [DMEM containing 10% (v/v) FBS] is added to the lower wells. The chambers are kept at 37°C in 5% CO_2 for 16 h in the presence or absence of 10 μM HTH-01-015 or WZ4003 both in the upper and lower wells. Non-invaded cells are removed from the upper face of the filters by scraping, and cells that have migrated to the lower face of the filters are fixed and stained with Reastain Quick-Diff kit and images (×10 magnification) are captured. For cell invasion assays, statistical significance is assessed using GraphPad Prism 5.0[1].

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CUSTOMER VALIDATION

- J Cancer. 2023 Jul 24;14(12):2329-2343.

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REFERENCES

[1]. Banerjee S, et al. Characterization of WZ4003 and HTH-01-015 as selective inhibitors of the LKB1-tumour-suppressor-activated NUAK kinases. Biochem J. 2014 Jan 1;457(1):215-25.

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