

产品名称：HTH-01-015
产品别名：HTH-01-015

生物活性:				
Description	HTH-01-015 is a selective NUA1/ARK5 inhibitor (IC50 is 100 nM). HTH-01-015 inhibits NUA1 with >100-fold higher potency than NUA2 (IC50 of >10 μM).			
IC50 & Target	NUAK1			
	100 nM (IC50)			
In Vitro	HTH-01-015 is a specific NUA1 inhibitor. The related NUA1 and NUA2 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 NUA1 with an IC50 of 100 nM, but does not significantly inhibit NUA2 (IC50 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 NUA1 at Ser445. In U2OS cells, HTH-01-015 suppresses proliferation and phosphorylation of MYPT1 to the same extent as shRNA-mediated NUA1 knockdown. In mouse embryonic fibroblasts (MEFs), treatment with 10 μM HTH-01-015 suppresses proliferation and phosphorylation of MYPT1 to the same extent as NUA1-knockout. To test whether NUA1 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].			
Solvent&Solubility	In Vitro: DMSO : 100 mg/mL (213.42 mM; Need ultrasonic)			
	Preparing Stock Solutions	<div><div>Solvent</div><div>Mass</div><div>Concentration</div></div>	1 mg	5 mg
		1 mM	2.1342 mL	10.6712 mL
		5 mM	0.4268 mL	2.1342 mL
		10 mM	0.2134 mL	1.0671 mL
	*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。 储备液的保存方式和期限：-80℃，6 months；-20℃，1 month。-80℃ 储存时，请在 6 个月内使用，-20℃ 储存时，请在 1 个月内使用。			
	In Vivo: 请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂： ——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶			
	1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: ≥ 2.5 mg/mL (5.34 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.34 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。			
	2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (5.34 mM); Clear solution			

	<p>此方案可获得 ≥ 2.5 mg/mL (5.34 mM, 饱和度未知) 的澄清溶液。</p> <p>以 1 mL 工作液为例, 取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水溶液中, 混合均匀。</p> <p>3.请依序添加每种溶剂: 10% DMSO \rightarrow 90% corn oil</p> <p>Solubility: ≥ 2.5 mg/mL (5.34 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (5.34 mM, 饱和度未知) 的澄清溶液, 此方案不适用于实验周期在半个月以上的实验。</p> <p>以 1 mL 工作液为例, 取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中, 混合均匀。</p>
References	<p>[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.</p>
实验参考:	
Cell Assay	<p>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% CO₂ 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 ($\times 10$ magnification) are captured. For cell invasion assays, statistical significance is assessed using GraphPad Prism 5.0[1].</p>
Kinase Assay	<p>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 \pm S.D. or in the form of comparative histograms[1].</p>
References	<p>[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.</p>