产品名称：**WZ4003**
产品别名：**WZ4003**

生物活性：

Description	WZ4003 is the first potent and highly specific NUAK kinase inhibitor with IC50 of 20 nM/100 nM for NUAK1 (ARK5)/NUAK2, without significant inhibition on other 139 kinases.

IC_{50} & Target	NUAK1	NUAK2
	20 nM (IC_{50})	100 nM (IC_{50})

In Vitro	WZ4003 (3-10 μM) markedly suppresses NUAK1-mediated MYPT1 phosphorylation, in HEK-293 cells expressing wild-type NUAK1. Moreover, WZ4003 (10 μM) inhibits MYPT1 Ser445 phosphorylation as well as cell migration, invasion and proliferation to a similar extent as knock out in MEFs or knock down in U2OS cells of NUAK1[1]. WZ4003 also exhibits a high, specific affinity to the L858R/T790M mutant EGFR, while a significantly reduced cellular IC50 against T790M containing Ba/F3 cells[2].

Solvent&Solubility

In Vitro:

DMSO : 33.33 mg/mL (67.06 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0121 mL	10.0606 mL	20.1211 mL
	5 mM	0.4024 mL	2.0121 mL	4.0242 mL
	10 mM	0.2012 mL	1.0061 mL	2.0121 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°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 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→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 2.5 mg/mL (5.03 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.03 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 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。

2.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 2.5 mg/mL (5.03 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.03 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。

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。

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. [2]. Zhou W, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature. 2009 Dec 24;462(7276):1070-4

实验参考：

Cell Assay	Cell proliferation assays are carried out colorimetrically in 96-well plates. 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. [1]
Kinase Assay	In vitro activities of purified GST-NUAK1 and GST-NUAK1[A195T] are measured using Cerenkov counting of incorporation of radioactive ^{32}P from [γ-^{32}P]ATP into Sakamototide substrate peptide. Reactions are carried out in a 50 μL reaction volume for 30 min at 30°C and reactions are terminated by spotting 40 μL of the reaction mix on to P81 paper and immediately immersing in 50 mM orthophosphoric acid. Samples are washed three times in 50 mM orthophosphoric acid followed by a single acetone rinse and air drying. The kinase-mediated incorporation of [γ-^{32}P]ATP into Sakamototide is quantified by Cerenkov counting. One unit of activity is defined as that which catalysed the incorporation of 1 nmol of [^{32}P]phosphate into the substrate over 1 h. [1]
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. [2]. Zhou W, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature. 2009 Dec 24;462(7276):1070-4