产品名称：CW 069
产品别名：CW-069

生物活性：

Description	CW-069 is an allosteric inhibitor of microtubule motor protein HSET with an IC_{50} of 75 μM.
IC_{50} & Target	HSET
	75 μM (IC_{50})
In Vitro	CW-069 is an allosteric inhibitor of HSET with an IC50 of 75 μM. CW-069 shows statistically significant selectivity over KSP. CW-069 potently suppresses N1E-115 cells, and less potently inhibits the NHDF cells, with IC50 of 86 ± 10 μM and 181 ± 7 μM, respectively. CW-069 (100 or 200 μM) causes increased multipolar spindles in N1E-115 cells with supernumerary centrosomes and shows no effect on altering bipolar spindle morphology in normal human dermal fibroblast cells. CW-069 (200 μM) cacuses multipolar anaphase and cell death induced in N1E-115 cells via transfection with HSET siRNA, and antagonizes inhibition of KSP by monastrol, but does not exert mitotic arrest in HeLa cells[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 52 mg/mL (103.93 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9987 mL	9.9934 mL	19.9868 mL
	5 mM	0.3997 mL	1.9987 mL	3.9974 mL
	10 mM	0.1999 mL	0.9993 mL	1.9987 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→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.00 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.00 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 (5.00 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.00 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。

	3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.00 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.00 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]. Watts CA, et al. Design, synthesis, and biological evaluation of an allosteric inhibitor of HSET that targets cancer cells with supernumerary centrosomes. Chem Biol. 2013 Nov 21;20(11):1399-410.

实验参考：

Cell Assay	NHDF cells, HeLa cells, BT549, MCF-7 and MDA-MB-231 cells are verified by STR genotyping and all tested negative for mycoplasma. Cells are cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) at 37°C and 5% CO_2. All compounds (CW-069) used in the Sulforhodamine B colorimetric (SRB) assay are dissolved in DMSO and diluted in culture medium to a final concentration of 0.2% DMSO. For the SRB assay and live-cell imaging, cells are seeded in 96-well plates at a density of 2,500 cells per well. After 24 hr, the cells are treated with compound (CW-069) for 72 hr, with triplicate wells for each concentration. For the SRB assay, the cells are then fixed with trichloroacetic acid (TCA) and stained with SRB. Fluorescence is quantified using an Infinite 200 PRO plate-reader at a wavelength of 545 nm. Compound (CW-069)-treated wells are compared with solvent control wells and the concentration of compound that resulted in 50% of the solvent-control cell growth is designated as the IC_{50} concentration, calculated using Graphpad PRISM 6. At least three biological replicates are performed for each assay[1].
Kinase Assay	The protocol is optimized for use with full-length, N-terminal, 6His-tagged HSET and KSP, and measured the MT-stimulated activity of the proteins. Inhibition of the Gsp synthetase activity of HSET/KSP is observed spectrophotometrically by coupling the hydrolysis of ATP to oxidation of NADH via pyruvate kinase/lactate dehydrogenase reactions. The assay is initiated by adding purified Gsp synthetase/amidase (12.8 nM) to an assay mixture containing the following components (final concentration): 6 nM protein, 0.07 mg/mL MTs, 1.56 mM glutathione, 10 mM spermidine, 2 mM ATP, 2.7 mM $MgCl_2$, 1 mM phospho(enol)-pyruvate, 0.2 mM NADH, 50 μg/mL lactate dehydrogenase, 100 μg/mL pyruvate kinase, and various concentrations of inhibitor (CW-069) all in 50 mM Na PIPES (pH 6.8) at 37°C. The ADP-Glo detection assay is performed. All compound additions are performed using a multidrop BioMek Nxp. Plates are read using a Pherastar microplate reader[1].
References	[1]. Watts CA, et al. Design, synthesis, and biological evaluation of an allosteric inhibitor of HSET that targets cancer cells with supernumerary centrosomes. Chem Biol. 2013 Nov 21;20(11):1399-410.