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产品名称：CHR-6494
产品别名：CHR-6494

生物活性：

Description	CHR-6494 is a potent inhibitor of **haspin**, inhibiting histone H3T3 phosphorylation, with an **IC_{50}** of 2 nM.
IC_{50} & Target	IC50: 2 nM (haspin)[1]
In Vitro	CHR-6494 is a potent inhibitor of haspin, inhibiting histone H3T3 phosphorylation, with an IC50 of 2 nM. CHR-6494 does not modify H3S10 and H328 phosphorylation levels, and shows no significantly inhibitory effects on other protein kinases such as Aurora B kinase. CHR-6494 dose-dependently inhibits the growth of cancer cells, such as HCT-116, HeLa, MDA-MB-231, and Wi-38 cell, with IC50s of 500 nM, 473 nM, 752 nM and 1059 nM, respectively. CHR-6494 (500 nM) produces a mitotic catastrophe with abnormal morphology of the mitotic spindle and centrosome amplification, and upregulates the spindle assembly checkpoint protein BUB1 and the marker of mitotic arrest cyclin B1[1]. CHR-6494 exhibits inhibitory activities against melanoma cell lines, including BRAFV600E mutants, NRAS mutants, and wild type cells, with IC50s ranging from 396 nM to 1229 nM. CHR-6494 (300 nM and 600 nM) induces apoptosis, increases caspase 3/7 activity by 3- and 6-fold, respectively in COLO-792 cells, and to 8.5- and 16-fold in RPMI-7951 cells. CHR-6494 in combination with MEK inhibitors synergistically inhibits viability of melanoma cells, enhances apoptosis in melanoma cells, modulates cell cycle progression independently by arresting melanoma cells at different phases, and suppresses migration of melanoma cells[2].
In Vivo	CHR-6494 (50 mg/kg, i.p.) inhibits the growth of tumor and cuases no obvious body weight change in nude mice bearing HCT-116 human colorectal cancer cells[1].
Solvent&Solubility	***In Vitro:*** **DMSO : 50 mg/mL (171.03 mM;** Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.4207 mL	17.1034 mL	34.2067 mL
	5 mM	0.6841 mL	3.4207 mL	6.8413 mL
	10 mM	0.3421 mL	1.7103 mL	3.4207 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 (8.55 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (8.55 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% corn oil Solubility: ≥ 2.5 mg/mL (8.55 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (8.55 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]. Huertas D, et al. Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. Oncogene. 2012 Mar 15;31(11):1408-18. [2]. Han L, et al. Anti-Melanoma Activities of Haspin Inhibitor CHR-6494 Deployed as a Single Agent or in a Synergistic Combination with MEK Inhibitor. J Cancer. 2017 Aug 25;8(15):2933-2943.

实验参考：

Cell Assay	Cells are treated for 24, 48 and 72 h with the inhibitor or with DMSO as a control. Cell viability is assessed using the colorimetric XTT assay. Cells are seeded in 94-well plates at a density of 4 × 10^4 cells per well, and allowed to attach for 24 h. The medium is then exchanged with others containing different drug concentrations (0.001−10 μM). Eight wells for each concentration of the CHR-6494 compound are used. At the corresponding time, the culture medium is discharged, the XTT reagent is added and the final cell number and optical density are determined. Dose-response curves are generated and cell viability is evaluated after 72 h of treatment. The half-maximal inhibitory concentration (IC_{50}) is determined using GraphPad Prism software[1].
Animal Administration	Athymic nu/nu male mice, aged 4-5 weeks, are used for tumor xenograft assays. Animals are maintained in a sterile environment; their cages, food and bedding are sterilized by autoclaving. Mice are anesthetized and tumor cells are injected subcutaneously. In all, 3.5 × 10^6 exponentially growing HCT-116 cells diluted in 250 μL of sterile PBS are injected subcutaneously in each animal (n = 30). Body weight is recorded and tumor dimensions are measured twice weekly using digital calipers. Tumor volume (in mm^3) is estimated according to the formula V = D × d^2/2, where D is the long axis and d the short axis of tumor. When tumors reach an average volume of 200 mm^3 (15 days after injection), 24 mice harboring homogeneous tumor sizes are randomized into two groups: (1) control group (n = 8) treated with vehicle (solution of 10% DMSO/20% 2-hydroxypropyl-b-cyclodextrin; (2) treatment group (n = 16) mice is diary treated by intraperitoneal injection of 50 mg/kg of CHR-6494 diluted in a solution of 10% DMSO/20% 2-hydroxypropyl-b-cyclodextrin in two cycles of five consecutive days for 15 days. The treatment group is randomly divided into a short-time response group (n = 8), defined by tumor weight at the moment of killing of the control group, and a long-time response group (n = 8), defined by tumor regrowth after treatment. Mice are killed at the end of treatment, and tumors from both groups are excised and weighted. The mean volume of tumor mass is expressed as mean ± s.e.m. for each mouse group, and significance is assessed by means of the Mann-Whitney U-test. Values of P < 0.05 are considered statistically significant. Upon killing mice, colon, lung, liver and kidney tissues are obtained to analyze endogenous toxicity by hematoxylin and eosin[1].

Kinase Assay	The analysis of the enzymatic inhibitory capacity of the compound in a panel of 29 protein kinases is developed using a FRET assay based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to protein cleavage (Z′-LYTE Kinase Assay). In the primary reaction, the kinase transfers the γ-phosphate of ATP to a single tyrosine, serine or threonine residue in a synthetic FRET peptide. In the secondary reaction, a site-specific protease recognizes and cleaves non-phosphorylated FRET peptides. Phosphorylation of FRET peptides suppresses cleavage by the development reagent. Cleavage disrupts FRET between the donor (coumarin) and the acceptor (fluorescein) fluorophores on the FRET peptide, whereas uncleaved, phosphorylated FRET peptides maintain FRET. A ratiometric method, which calculates the ratio (the emission ratio) of donor emission to acceptor emission after excitation of the donor fluorophore at 400 n, is used to quantitate reaction progress[1].
References	[1]. Huertas D, et al. Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. Oncogene. 2012 Mar 15;31(11):1408-18. [2]. Han L, et al. Anti-Melanoma Activities of Haspin Inhibitor CHR-6494 Deployed as a Single Agent or in a Synergistic Combination with MEK Inhibitor. J Cancer. 2017 Aug 25;8(15):2933-2943.