产品名称：6-溴-3-(1-甲基-1H-吡唑-4-基)-5-(3R)-3-哌啶基吡唑并[1,5-a]嘧啶-7-胺

产品别名： SCH900776；MK-8776

生物活性：

Description

SCH900776 is a potent, selective and orally bioavailable inhibitor of checkpoint kinase1 (Chk1) with an IC_{50} of 3 nM. SCH900776 shows 50- and 500-fold selectivity over CDK2 and Chk2, respectively.

IC_{50} & Target

Chk1	Chk2	CDK2
3 nM (IC_{50})	1500 nM (IC_{50})	160 nM (IC_{50})

In Vitro

SCH900776 (300 nM) shows potent inhibitory activities against phosphorylation at ser296-Chk1. SCH900776 (1 μM) causes a 30-fold decrease in the IC_{50} for hydroxyurea in MDA-MB-231 cells[1].. The K_d value of SCH 900776 for the CHK1 kinase domain is 2 nM. SCH 900776 exhibits an approximate EC_{50} of 60 nM in cells exposure to hydroxyurea. SCH 900776 induces dose-dependent suppression of CHK1 pS296 and concomitant accumulation of phospho-RPA signal in U2OS cells[2].

In Vivo

SCH 900776 induces the γ-H2AX biomarker at 4 mg/kg (i.p.), and enhances tumor pharmacodynamic and regression responses in A2780 xenograft model. SCH 900776 (16 and 32 mg/kg, i.p.) induces incremental improvements in tumor response. Escalation of SCH 900776 dose to 20 and 50 mg/kg in combination with gemcitabine results in improvements in TTP 10× in the A2780 xenograft systems[2].

Solvent&Solubility

In Vitro:

DMSO : ≥ 100 mg/mL (265.78 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.6578 mL	13.2890 mL	26.5781 mL
5 mM	0.5316 mL	2.6578 mL	5.3156 mL
10 mM	0.2658 mL	1.3289 mL	2.6578 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 (6.64 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.64 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 (6.64 mM); Clear solution

	此方案可获得 ≥ 2.5 mg/mL (6.64 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 (6.64 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.64 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]. Montano R, et al. Preclinical development of the novel Chk1 inhibitor SCH900776 in combination with DNA-damaging agents and antimetabolites. Mol Cancer Ther. 2012 Feb;11(2):427-38. [2]. Guzi TJ, et al. Targeting the replication checkpoint using SCH 900776, a potent and functionally selective CHK1 inhibitor identified via high content screening. Mol Cancer Ther. 2011 Apr;10(4):591-602.

实验参考：

Cell Assay	For cell growth assays, cells are seeded at low density (500-1000 cells) in 96-well plates and then incubated with drug for 24 h (8 wells per concentration). Following treatment, cells are washed and grown in fresh media for 5-7 days at 37°C. Prior to attaining confluence, cells are washed, lysed, and stained with Hoechst 33258. Fluorescence is read on a microplate spectrofluorometer. Results are expressed as mean and standard error for the concentration of drug that inhibited growth by 50%.[1]
Animal Administration	For tumor implantation, specific cell lines are grown in vitro, washed once with PBS and resuspended in 50% Matrigel in PBS to a final concentration of 4×10^7 to 5×10^7 cells per mL. Nude mice are injected with 0.1 mL of this suspension subcutaneously in the flank region. Tumor length (L), width (W), and height (H) are measured by a caliper twice a week on each mouse and then used to calculate tumor volume using the formula: (L×W×H)/2. Animals (N=10) are randomized to treatment groups and treated intraperitoneally with either SCH 900776 (formulated in 20% hydroxypropyl β-cyclodextrin) or individual chemotherapeutic agents, formulated as recommended. Tumor volumes and body weights are measured during and after the treatment periods. Data are recorded as means±SEM before being normalized to starting volume. Time to progression to 10x starting volume (TTP 10x) is monitored in some experiments. For pharmacodynamic marker analyses in mice, tumors and adjacent skin are collected at necropsy, fixed overnight in 10% formalin, and washed/stored in 70% ethanol. For skin punch biopsies, an area of approximately 4 square inches is shaved. Rats are anesthetized using inhaled isofluorane and dogs are locally anesthetized using subcutaneous administration of lidocaine. Samples are collected using a 4 mm biopsy punch. Skin punches are fixed in 10% formalin overnight before washing/storage in 70% ethanol. [2]
Kinase Assay	The Kinase Profiler service is used to generate general selectivity data for SCH 900776 against a broad range of serine/threonine and tyrosine kinases. Assays are typically run at two concentrations of SCH 900776 (0.5 and 5 μM), at a fixed (10 μM) concentration of ATP. [2]
References	[1]. Montano R, et al. Preclinical development of the novel Chk1 inhibitor SCH900776 in combination with DNA-damaging agents and antimetabolites. Mol Cancer Ther. 2012 Feb;11(2):427-38.

	[2]. Guzi TJ, et al. Targeting the replication checkpoint using SCH 900776, a potent and functionally selective CHK1 inhibitor identified via high content screening. Mol Cancer Ther. 2011 Apr;10(4):591-602.