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产品名称：Ro 5126766
产品别名：CH5126766

生物活性：

Description	Ro 5126766 is a first-in-class dual MEK/RAF inhibitor that allosterically inhibits $BRAF^{V600E}$, CRAF, MEK, and BRAF(IC_{50}: 8.2, 56, 160 nM, and 190 nM, respectively).			
IC_{50} & Target	MEK	$BRaf^{V600E}$	Braf	CRAF
	160 nM (IC_{50})	8.2 nM (IC_{50})	190 nM (IC_{50})	56 nM (IC_{50})
In Vitro	Ro 5126766 (RO5126766) is an allosteric inhibitor that binds directly to MEK and prevents its phosphorylation by RAF through the formation of a stable RAF-MEK complex. Ro 5126766 inhibits both the phosphorylation of MEK by RAF and the activation of ERK by MEK. In cell-free MEK and RAF kinase assays, Ro 5126766 effectively inhibits activation of ERK2 by MEK1 with an IC_{50} of 160 nM (SD=±0.043) and inhibits the phosphorylation of MEK1 protein by *BRAF* (IC_{50}=190 nM, SD=±0.003), $BRAF^{V600E}$ (IC_{50}=8.2 nM, SD=±0.0015), and *CRAF* (IC_{50}=56 nM, SD=±0.016). Ro 5126766 effectively inhibits both MEK and ERK phosphorylation in a panel of human tumor cell lines including *KRAS*/*HRAS* and *BRAF* mutant cell lines and *KRAS*/*HRAS* and *BRAF* wild-type cells[1]. In order to investigate whether the mevalonate pathway affects the sensitivity to MEK inhibitors, human breast cancer MDA-MB-231 cells harboring *KRAS* and *BRAF* mutations are treated Ro 5126766 (CH5126766), with or without statins, which inhibits HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway. The combined treatment of Ro 5126766 with Fluvastatin demonstrates more significant reduction in cell growth in a dose-dependent manner than the single treatment of Ro 5126766. The marked combined effects of Ro 5126766 at 40 nM and Fluvastatin at 0.3 μM is also confirmed on the suppression of the colony formation of the cells[2].			
In Vivo	In *KRAS*-mutant xenograft models, Ro 5126766 (RO5126766) inhibits growth and causes tumor regressions more effectively than another allosteric MEK inhibitor, PD0325901. Preclinical data from a series of human tumor mouse xenograft models indicates an ED_{50} for Ro 5126766 of 0.03 to 0.23 mg/kg and an ED_{90} of 0.15 to 1.56 mg/kg. These effective doses are associated with target trough concentrations of 17 to 133 ng/L and 87 to 901 ng/mL, respectively.[1]. In this experiment, Ro 5126766 (CH5126766) or PD0325901 is administrated at their maximum tolerated dose (MTD) in the HCT116 model (1.5 and 25 mg/kg, respectively). These doses inhibit pERK and ERK signaling output at similar degrees in the tumors from the drug-treated mice at 4 hours from the first drug administration. Moreover, in HCT116 models, the ED_{50} for Ro 5126766 and PD0325901 are 0.056 and 0.80 mg/kg, respectively. Therefore, the doses used for this experiment are 26.8- and 31.3-fold higher doses than the 50% effective doses, respectively. Daily oral administration of either drug causes significant tumor regression of each these tumors. However, whereas inhibition of tumor growth is maintained for the entire 28-day treatment period in Ro 5126766-treated mice, tumor models receiving PD0325901 become refractory after 10 days of treatment[3].			
	In Vitro: **DMSO： 100 mg/mL (212.11 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)			

Solvent&Solubility

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.1211 mL	10.6054 mL	21.2107 mL
5 mM	0.4242 mL	2.1211 mL	4.2421 mL
10 mM	0.2121 mL	1.0605 mL	2.1211 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: ≥ 2.75 mg/mL (5.83 mM); Clear solution

此方案可获得 ≥ 2.75 mg/mL (5.83 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 27.5 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: ≥ 2.75 mg/mL (5.83 mM); Clear solution

此方案可获得 ≥ 2.75 mg/mL (5.83 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 27.5 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。

References

[1]. Martinez-Garcia M, et al. First-in-human, phase I dose-escalation study of the safety, pharmacokinetics, and pharmacodynamics of RO5126766, a first-in-class dual MEK/RAF inhibitor in patients with solid tumors. Clin Cancer Res. 2012 Sep 1;18(17):4806-19.

[2]. Iizuka-Ohashi M, et al. Blockage of the mevalonate pathway overcomes the apoptotic resistance to MEK inhibitors with suppressing the activation of Akt in cancer cells. Oncotarget. 2018 Apr 13;9(28):19597-19612.

[3]. Ishii N, et al. Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. Cancer Res. 2013 Jul 1;73(13):4050-4060.

实验参考：

Cell Assay

The number of viable cells is assessed with a Cell Counting Kit-8 assay. Human breast cancer MDA-MB-231 cells, human melanoma SK-MEL-28 cells, and human non-small cell lung cancer A549 cells are seeded at a density of 2,000 cells per well in 96-well plates and incubated for 24 h, and then treated with Ro 5126766 (10, 20, 40, and 80 nM) for 72 h. After a further 4 h incubation with the kit reagent, the absorbance at 450 nm of the samples is measured using a multi-plate

	reader[2].
Animal Administration	Mice[3] Female BALB-*nu*/*nu* mice (CAnN.Cg-Foxn1nu/CrlCrlj nu/nu) are given access to standard mouse chow and water ad libitum. A total of 5×10^6 (HCT116) or 1×10^7 (Calu-6 and COLO205) tumor cells per mouse are injected subcutaneously into the right flank of the 7- to 9-week-old mice. When tumor volume reaches to 200 mm^3 (day 0), the mice are randomized and vehicle [5% DMSO and 10% 2-hydroxypropyl-β-cyclodextrin (HPCD) solution in distilled water], Ro 5126766 (1.5 mg/kg or 2.0 mg/kg) or PD0325901 (25 mg/kg) is administered orally once a day. Drugs are administrated at the maximum tolerated dose (MTD). Tumor growth inhibition (TGI) is calculated. The value of the 50% effective dose (ED_{50}) for each compound is calculated[3].
References	[1]. Martinez-Garcia M, et al. First-in-human, phase I dose-escalation study of the safety, pharmacokinetics, and pharmacodynamics of RO5126766, a first-in-class dual MEK/RAF inhibitor in patients with solid tumors. Clin Cancer Res. 2012 Sep 1;18(17):4806-19. [2]. Iizuka-Ohashi M, et al. Blockage of the mevalonate pathway overcomes the apoptotic resistance to MEK inhibitors with suppressing the activation of Akt in cancer cells. Oncotarget. 2018 Apr 13;9(28):19597-19612. [3]. Ishii N, et al. Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. Cancer Res. 2013 Jul 1;73(13):4050-4060.