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产品名称：Inauhzin
产品别名：INZ

生物活性：

Description	Inauhzin is a dual SirT1/IMPDH2 inhibitor, and acts as an activator p53, used in the research of cancer.		
IC_{50} & Target	SIRT1	MDM-2/p53	IMPDH2
In Vitro	Inauhzin (10 μM) induces p53 levels as effectively as actinomycin D (10 nM), and mediates p53-dependent cytotoxicity through its specific functional groups in human lung carcinoma H460 cells. Inauhzin (2 μM) induces p53 level and activity as well as p53-dependent apoptosis. Inauhzin also stabilizes p53 and inhibits its ubiquitylation. Inauhzin induces acetylation of p53 in H460 cells, but not tubulin, which is affected by knockdown of SIRT1[1]. Inauhzin (0-2 μM) significantly enhances the expression level and activity of p53 in HCT116$^{p53+/+}$ cells and enhances the expression level and activity of p53 in H460 cells in a dose-dependent manner. Inauhzin and Nutlin-3 demonstrate synergistic cytotoxicity in the Nutlin-3 low-sensitive cells. Inauhzin and Nutlin-3 synergistically induce p53-dependent apoptosis[2]. Inauhzin targets both SirT1 and IMP dehydrogenase 2 (IMPDH2), and acts as a potent p53 activator[3].		
In Vivo	Inauhzin (30 mg/kg, i.p.) effectively induces apoptosis and suppresses tumour growth of H460 xenograft harbouring p53[1]. Inauhzin (30 mg/kg, i.p.) reduces the HCT116 tumor volume by appr 70%. Inauhzin (15 mg/kg) in combination with 150 mg/kg of Nutlin-3 demonstrates a significant synergy on p53 induction, apoptosis and tumor suppression of HCT116$^{p53+/+}$ xenografts[2].		

Solvent&Solubility

In Vitro:

DMSO : 21 mg/mL (44.72 mM; Need ultrasonic and warming)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1296 mL	10.6478 mL	21.2956 mL
	5 mM	0.4259 mL	2.1296 mL	4.2591 mL
	10 mM	0.2130 mL	1.0648 mL	2.1296 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 (5.32 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.32 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。

References	[1]. Zhang Q, et al. A small molecule Inauhzin inhibits SIRT1 activity and suppresses tumour growth through activation of p53. EMBO Mol Med. 2012 Apr;4(4):298-312. [2]. Zhang Y, et al. Inauhzin and Nutlin3 synergistically activate p53 and suppress tumor growth. Cancer Biol Ther. 2012 Aug;13(10):915-24. [3]. Nguyen D, et al. Reviving the guardian of the genome: Small molecule activators of p53. Pharmacol Ther. 2017 Oct;178:92-108.

实验参考：

Cell Assay	The cell counting kit is used to assess cell growth. Cell suspensions are seeded at 5000 cells per well in 96-well culture plates and incubated overnight at 37°C. Compounds are added into the plates and incubated at 37°C for 72 h. Cell growth inhibition is determined by adding WST-8 at a final concentration of 10% to each well, and the absorbance of the samples is measured at 450 nm using a Microplate Reader[1].
Animal Administration	Five-weeks-old female SCID mice are housed in a BSL2 environment. Mice are subcutaneously inoculated with 5×10^6 H460 or 3×10^6 HCT116 cells. Tumour growth is monitored every other day with electronic digital calipers in two dimensions. Tumour volume is calculated with the formula: tumour volume (mm^3) = (length × $width^2$)/2. When the mean tumour volume reaches approximately 100 mm^3 after 7-9 days, animals are dosed by i.p. injection with vehicle (5% DMSO) or Inauhzin. Inhibition of tumour growth is calculated on the last day of treatment. To detect p53 activation in vivo, tumours are harvested and disrupted in RIPA buffer containing a protease inhibitor mixture. Tumour lysates are analysed by IB. Cell proliferation in tumours is assessed by BrdU labeling followed by Immunostaining. 200 mg/kg body weight of BrdU is administrated to mice via i.p. injection 2 h before mice are sacrificed. Apoptosis is examined by TUNEL staining, using the Fluorescein In situ cell death detection kit[1].
References	[1]. Zhang Q, et al. A small molecule Inauhzin inhibits SIRT1 activity and suppresses tumour growth through activation of p53. EMBO Mol Med. 2012 Apr;4(4):298-312. [2]. Zhang Y, et al. Inauhzin and Nutlin3 synergistically activate p53 and suppress tumor growth. Cancer Biol Ther. 2012 Aug;13(10):915-24. [3]. Nguyen D, et al. Reviving the guardian of the genome: Small molecule activators of p53. Pharmacol Ther. 2017 Oct;178:92-108.