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产品名称：(5R)-5-[(7-氯-1H-吲哚-3-基)甲基]-3-甲基-2,4-咪唑烷二酮
产品别名：Necrostatin 2

生物活性：

Description	Necrostatin 2 is a potent necroptosis inhibitor. EC50 for inhibition of necroptosis in FADD-deficient Jurkat T cells treated with TNF-α is 0.05 μM. Necrostatin 2 is also a RIPK1 inhibitor.
IC_{50} & Target	Necroptosis[1], RIPK1[4]
In Vitro	Evaluation of necroptosis inhibitory activity is performed using a FADD-deficient variant of human Jurkat T cells treated with TNF-α. Utilizing these conditions the cells efficiently undergo necroptosis, which is completely and selectively inhibited by Necrostatin 2 (EC50=50 nM). Necrostatin 2 shows activity in a broad range of necroptosis cellular systems[1]. Necrostatin 2 at 30 μM completely protects L929 cells from TNF-α-induced necroptosis. In addition to TNF-α, the pan-caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp(OMe)-fluoromethylketone (zVAD.fmk) has also been found to induce necrosis in L929 cells, which is efficiently inhibited by Necrostatin 2[2]. EC50 for inhibition of necroptosis in FADD-deficient Jurkat T cells treated with TNF-α is 0.05 μM[3].

Solvent&Solubility

In Vitro:

DMSO : ≥ 100 mg/mL (360.09 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.6009 mL	18.0044 mL	36.0088 mL
	5 mM	0.7202 mL	3.6009 mL	7.2018 mL
	10 mM	0.3601 mL	1.8004 mL	3.6009 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 (9.00 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (9.00 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 (9.00 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (9.00 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 (9.00 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (9.00 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]. Teng X, et al. Structure-activity relationship study of [1,2,3]thiadiazole necroptosis inhibitors. Bioorg Med Chem Lett. 2007 Dec 15;17(24):6836-40. [2]. Jagtap PG, et al. Structure-activity relationship study of tricyclic necroptosis inhibitors. J Med Chem. 2007 Apr 19;50(8):1886-95. [3]. Teng X, et al. Structure-activity relationship study of novel necroptosis inhibitors. Bioorg Med Chem Lett. 2005 Nov 15;15(22):5039-44. [4]. Takahashi N, et al. Necrostatin-1 analogues: critical issues on the specificity, activity and in vivo use in experimental disease models. Cell Death Dis. 2012 Nov 29;3:e437.
实验参考：	
Cell Assay	L929 cells (100000 cells/mL, 100 μL/well in a 96-well plate) are treated with 10 ng/mL human TNF-α or 100 μM zVAD.fmk in the presence of DMSO (control), 30 μM Necrostatin 2, or 8for 24 h at 37°C in a humidified incubator with 5% CO_2 followed by ATP-based viability assessment as described in the previous experiment. Stock solutions (30 mM) in DMSO are initially prepared and then diluted with DMSO to give testing solutions. Each sample is done in duplicate. The final DMSO concentration is 0.5%. Cell viability values are adjusted to account for nonspecific toxicity, which in most cases is <10%. The reported cell viability values (%)±SD are determined from two independent experiments[2].
Kinase Assay	Evaluation of necroptosis inhibitory activity is performed using an FADD-deficient variant of human Jurkat T cells treated for 24 h with TNF-α. Under these conditions the cells underwent necroptosis (the DMSO control had ~40% viability), which is inhibited by Necrostatin 2 (EC50=0.21±0.2 μM) as a positive control. For EC50 value determinations, cells are treated with 10 ng/mL human TNF-α in the presence of increasing concentrations of test compounds (0.029, 0.058, 0.12, 0.23, 0.46, 0.93, 1.9, 3.7, 11.1, 33, and 100 μM) in duplicate for 24 h followed by ATP-based viability assessment. EC50 values±SD are determined from at least two independent experiments[2].
References	[1]. Teng X, et al. Structure-activity relationship study of [1,2,3]thiadiazole necroptosis inhibitors. Bioorg Med Chem Lett. 2007 Dec 15;17(24):6836-40. [2]. Jagtap PG, et al. Structure-activity relationship study of tricyclic necroptosis inhibitors. J Med Chem. 2007 Apr 19;50(8):1886-95. [3]. Teng X, et al. Structure-activity relationship study of novel necroptosis inhibitors. Bioorg Med Chem Lett. 2005 Nov 15;15(22):5039-44. [4]. Takahashi N, et al. Necrostatin-1 analogues: critical issues on the specificity, activity and in vivo use in experimental disease models. Cell Death Dis. 2012 Nov 29;3:e437.