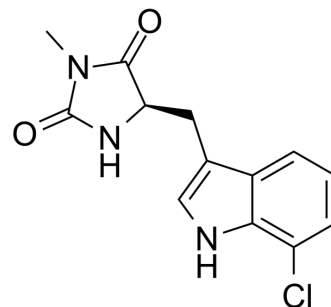


Necrostatin 2

Cat. No.:	HY-14622		
CAS No.:	852391-19-6		
Molecular Formula:	C ₁₃ H ₁₂ ClN ₃ O ₂		
Molecular Weight:	277.71		
Target:	RIP kinase		
Pathway:	Apoptosis		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (360.09 mM)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent	Mass	1 mg	5 mg	10 mg
	Concentration				
	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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
 Solubility: ≥ 2.5 mg/mL (9.00 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
 Solubility: ≥ 2.5 mg/mL (9.00 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
 Solubility: ≥ 2.5 mg/mL (9.00 mM); Clear solution

BIOLOGICAL ACTIVITY

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.

IC₅₀ & 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 (EC_{50} =50 nM). Necrostatin 2 shows activity in a broad range of necroptosis cellular systems^[1]. Necrostatin 2 at 30 μ M 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]. EC_{50} for inhibition of necroptosis in FADD-deficient Jurkat T cells treated with TNF- α is 0.05 μ M^[3].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[2]

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 (EC_{50} =0.21 \pm 0.2 μ M) as a positive control. For EC_{50} 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 μ M) in duplicate for 24 h followed by ATP-based viability assessment. EC_{50} values \pm SD are determined from at least two independent experiments^[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[2]

L929 cells (100000 cells/mL, 100 μ L/well in a 96-well plate) are treated with 10 ng/mL human TNF- α or 100 μ M zVAD.fmk in the presence of DMSO (control), 30 μ M Necrostatin 2, or 8for 24 h at 37°C in a humidified incubator with 5% CO₂ 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 (%) \pm SD are determined from two independent experiments^[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cancer Cell Int. 2024 Jun 3;24(1):194.
- Int Immunopharmacol. 2024 Apr 30;134:112159.
- Mol Carcinog. 2023 May 5.
- J Cancer. 2023 May 15;14(8):1336-1349.
- FEBS Open Bio. 2019 Feb 23;9(4):582-593.

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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.

Caution: Product has not been fully validated for medical applications. For research use only.

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