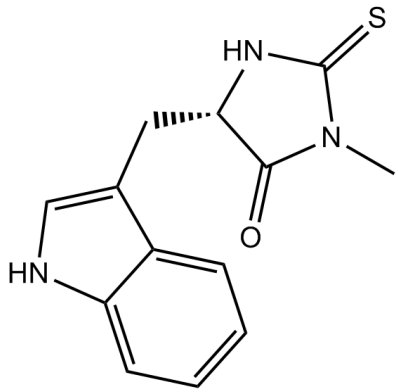
Product Name: Necrostatin-1

Revision Date: 6/30/2016

Product Data Sheet

Chemical Properties



Product Name:	Necrostatin-1
Cas No.:	4311-88-0
M.Wt:	259.33
Formula:	C13H13N3OS
Synonyms:	MTH-DL-Tryptophan,Nec-1
Chemical Name:	5-(1H-indol-3-ylmethyl)-3-methyl-2-sulfanylideneimidazolidin-4-one
Canonical SMILES:	CN1C(=O)C(NC1=S)CC2=CNC3=CC=CC=C32
Solubility:	>13mg/mL in DMSO
Storage:	Store at -20°C
General tips:	For obtaining a higher solubility , please warm the tube at 37° C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20° C for several months.
Shopping Condition:	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request

Biological Activity

Targets :	TNF-α
Pathways:	Apoptosis >> TNF-α

Description:

IC50: Necrostatin-1 (Nec-1),
(R)-5-([7-chloro-1H-indol-3-yl]methyl)-3-methylimidazolidine-2,4-dione (Nec-1a) (Figure 1A) (Degterev et al., 2008), exhibited an inhibitory constant (IC50) of 0.32 mM for RIP1 [1]. Necroptosis is a cellular mechanism of necrotic cell death induced by apoptotic stimuli in the form of death domain receptor engagement by their respective ligands under conditions where apoptotic execution is prevented. Necrostatin-1, identified as a small-molecule inhibitor of

necroptosis, is also a selective allosteric inhibitor of the death domain receptor–associated adaptor kinase RIP1.
In vitro: Previous study indicated that necrostatin-1 was a selective allosteric inhibitor of the death domain receptor–associated adaptor kinase RIP1 in vitro. In this study, RIP1 was found to be the primary cellular target responsible for the antinecroptosis activity of necrostatin-1. In addition, two other necrostatins, necrostatin-3 and necrostatin-5, were also shown to target the RIP1 kinase step in the necroptosis pathway, but through different mechanism compared with that of necrostatin-1. The findings established necrostatins as the first-in-class inhibitors of RIP1 kinase, the key upstream kinase involved in the activation of necroptosis [2].
In vivo: A previous study was designed to investigate the protective effects and mechanisms of Nec-1 in concanavalin A-induced hepatitis in mice. It was found that in Nec-1-treated mice the amelioration in liver functions and histopathological changes and the suppression of inflammatory cytokine production were observed. Western blotting analyses showed that the expression of TNF-α, IFN-γ, IL2, IL6, and RIP1 was significantly reduced in the Nec-1-treated mice, which was further confirmed by immunofluorescence and immunohistochemistry. In addition, autophagosome formation was significantly reduced by Nec-1 treatment. These results indicated that Nec-1 could prevent concanavalin A -induced liver injury via RIP1-related and autophagy-related pathways [3].
Clinical trial: Up to now, Necroptosis is still in the preclinical development stage.

***Reference**:*

[1] Xie T, Peng W, Liu Y, Yan C, Maki J, Degterev A, Yuan J, Shi Y. Structural basis of RIP1 inhibition by necrostatins. Structure. 2013;21(3):493-9.
[2] Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, Abbott D, Cuny GD, Yuan C, Wagner G, Hedrick SM, Gerber SA, Lugovskoy A, Yuan J. Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol. 2008;4(5):313-21.
[3] Yingqun Zhou, Weiqi Dai, Chunlei Lin, Fan Wang, Lei He, Miao Shen, Ping Chen, Chenfen Wang, Jie Lu, Ling Xu, Xuanfu Xu, and Chuanyong Guo. Protective Effects of Necrostatin-1 against Concanavalin A-Induced Acute Hepatic Injury in Mice. Mediators of Inflammation. http://dx.doi.org/10.1155/2013/706156

Protocol

Cell experiment:

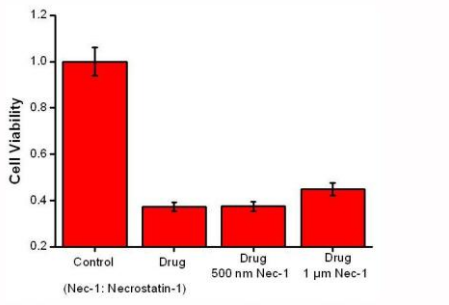
Cell lines	Mouse osteocyte cell line (MLO-Y4)
Preparation method	The solubility of this compound in DMSO is >10 mM. General tips for obtaining a higher concentration: Please warm the tube at 37℃ for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20℃ for several months.
Reacting conditions	
Applications	Necrostatin-1 (30 mmol/L) inhibited the mouse osteocyte cell line (MLO-Y4) necroptosis induced by TNF-α in vitro.

Animal experiment [3]:

Animal models	Rats underwent the ovariectomized surgery; Eight-week-old mice underwent sham surgery or contrast-induced AKI treatment;
Dosage form	1.65 mg/kg/d; intraperitoneal injection; once per day for 4 weeks
Applications	Treatment with Necrostatin-1 (1.65 mg/kg/d) significantly decreased RIP1 and RIP3 expression in ovariectomized rats. Moreover, necrostatin-1 prevented osmotic nephrosis and contrast-induced AKI in mice.
Other notes	Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.

***Reference**:*
1Degterev, A., Hitomi, J., Germscheid, M., Ch'en, I. L., Korkina, O., Teng, X., Abbott, D., Cuny, G. D., Yuan, C., Wagner, G., Hedrick, S. M., Gerber, S. A., Lugovskoy, A. and Yuan, J. (2008) Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol. 4, 313-3212Cui, H., Zhu, Y., Yang, Q., Zhao, W., Zhang, S., Zhou, A. and Jiang, D. (2016) Necrostatin-1 treatment inhibits osteocyte necroptosis and trabecular deterioration in ovariectomized rats. Sci Rep. 6, 338033Linkermann, A., Heller, J. O., Prokai, A., Weinberg, J. M., De Zen, F., Himmerkus, N., Szabo, A. J., Brasen, J. H., Kunzendorf, U. and Krautwald, S. (2013) The RIP1-kinase inhibitor necrostatin-1 prevents osmotic nephrosis and contrast-induced AKI in mice. J Am Soc Nephrol. 24, 1545-1557
2Cui, H., Zhu, Y., Yang, Q., Zhao, W., Zhang, S., Zhou, A. and Jiang, D. (2016) Necrostatin-1 treatment inhibits osteocyte necroptosis and trabecular deterioration in ovariectomized rats. Sci Rep. 6, 33803
3Linkermann, A., Heller, J. O., Prokai, A., Weinberg, J. M., De Zen, F., Himmerkus, N., Szabo, A. J., Brasen, J. H., Kunzendorf, U. and Krautwald, S. (2013) The RIP1-kinase inhibitor necrostatin-1 prevents osmotic nephrosis and contrast-induced AKI in mice. J Am Soc Nephrol. 24, 1545-1557

Product Validation



The drug used in this experiment is a small molecule that selectively inhibits tumor cell growth,and it has been reported to induce necrosis in target carcinoma cell.The addition of Necrostation-1 at a certain dose can partly improve the cell viability of MCF-7 cells in the presence of drug treatment.

Methed:MTT assay;Cell Lines: MCF-7;Concentration:0.5-4 μM;Incubation Time:24-48 h.

Caution

FOR RESEARCH PURPOSES ONLY.

NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Specific storage and handling information for each product is indicated on the product datasheet. Most ApexBio products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Shortterm storage of many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality of the reagents. Upon receipt of the product, follow the storage recommendations on the product data sheet.

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