

Product Data Sheet

Product Name: Chymostatin

Cat. No.: GC15682

Chemical Properties

Cas. No. 9076-44-2

Chemical Name (2R,6R,9R,12R)-6-((R)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl)-2,12-dibenzyl-9-isobutyl-4,7,10,13-tetraoxo-3,5,8,11-tetraazatridecan-1-oic acid

SMILES O=C(O)[C@H](NC(N[C@H]([C@H]1CCN=C(N)N1)C(N[C@H](CC(C)C)C(N[C@H](CC2=CC=CC=C2)C=O)=O)=O)CC3=CC=CC=C3

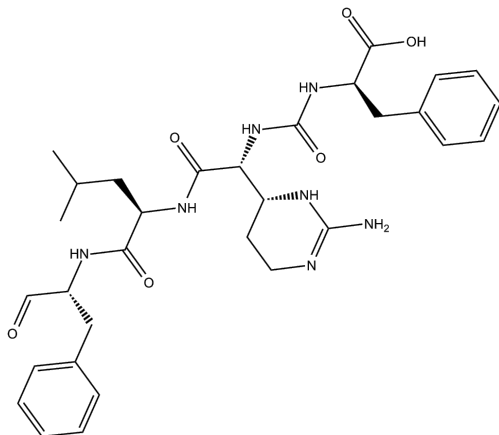
Formula $C_{31}H_{41}O_6N_7$ M.Wt 607.7

Solubility DMSO: 10 mg/ml, DMSO:PBS (pH 7.2) (1:1): 0.5 mg/ml 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.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

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Cell lines HK2 cells (human proximal tubule epithelial cells)

Preparation Method HK2 cells were cultured in DMEM F12 medium supplemented with 10% fetal bovine serum, penicillin (100U/ml), and streptomycin (100mg/ml) at 37°C under a humidified atmosphere with 5% CO₂. Cells were pretreated with Chymostatin (50μM) for 1 hour, followed by treatment with palmitic acid (0.6mM) for 24 hours.

Reaction
Conditions 50μM; 24h

Applications Chymostatin significantly prevented the upregulation of ER stress markers (BiP, IRE1α, p-eIF2α, ATF4, CHOP) and apoptosis markers (Bax/Bcl-2 ratio, cleaved caspase-3) induced by palmitic acid in HK2 cells. Chymostatin also reduced the increase in angiotensinogen and angiotensin type 1 receptor (AT1R) mRNA levels caused by palmitic acid treatment.

Animal experiment [2]:

Animal models Institute of Cancer Research (ICR) mice

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Preparation Method Mice were randomly divided into five groups: NS group (intraperitoneally injected with 0.9% saline solution), DMSO group (intraperitoneally injected with 0.05% DMSO), Chymostatin group (intraperitoneally injected with Chymostatin in 0.05% DMSO solution at a dosage of 2mg/kg), paraquat group (intraperitoneally injected with paraquat at a dosage of 25mg/kg), and Chymostatin treatment group (intraperitoneally injected with Chymostatin in 0.05% DMSO solution at a dosage of 2mg/kg 30 minutes before paraquat administration). Mice were sacrificed at different time points (0, 1, 2, 4, 8, 12, 24, and 48 hours) after treatment for lung tissue and blood sample collection.

Dosage form 2mg/kg; i.p.

Applications Chymostatin alleviated the pathological changes associated with acute alveolitis in mice, decreased the lung wet-to-dry ratio, the activity of cathepsin G, and the serum concentrations of TNF- α , IL-1 β , IL-6, and HMGB1, and increased the serum concentration of endocan. Chymostatin up-regulated endocan expression and down-regulated nuclear NF- κ Bp65 expression in the lung, reversing the inflammatory effects of paraquat-induced lung injury by inhibiting cathepsin G activity.

References:

- [1] Qiu M, Li S, Jin L, et al. Combination of Chymostatin and Aliskiren attenuates ER stress induced by lipid overload in kidney tubular cells. *Lipids Health Dis.* 2018 Jul 31;17(1):183.
- [2] Yang C, Song HW, Liu W, et al. Protective Effects of Chymostatin on Paraquat-Induced Acute Lung Injury in Mice. *Inflammation.* 2018 Feb;41(1):122-133.

Background

Chymostatin is a potent and selective inhibitor of the proteasome chymotrypsin-like activity, with K_i values of 9.36nM for chymotrypsin and 13.1nM for cathepsin D^[1]. Chymostatin also exhibits some inhibitory activity against trypsin and elastase^[2]. Chymostatin can easily cross cell membranes and

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is an important tool compound for studying the ubiquitin-proteasome pathway and protein degradation in various cellular processes^[3]. Additionally, Chymostatin has potential therapeutic applications in neurodegenerative diseases and cancer ^[4]

In vitro, pretreatment of HK2 cells with Chymostatin (50 μ M) for 1 hour, followed by treatment with palmitic acid (0.6mM) for 24 hours, significantly inhibited the protein expression of endoplasmic reticulum (ER) stress markers and reduced the levels of pro-apoptotic factors, thereby significantly alleviating cell apoptosis. Moreover, co-pretreatment of HK2 cells with Chymostatin (50 μ M) and Aliskiren (10nM) for 1 hour, followed by treatment with cholesterol (10 μ g/ml) for 6 days, also significantly inhibited the protein expression of ER stress markers and decreased the levels of apoptosis-related proteins, protecting cells from cholesterol-induced damage^[5]. Pretreatment of a co-culture system of HEK-293T cells and RBL-2H3 cells with Chymostatin (100 μ M) for 1 hour, followed by infection with SARS-CoV-2 pseudovirions, resulted in a significant inhibition of viral entry into cells by 52.1% compared to untreated cells^[6].

In vivo, Chymostatin (2mg/kg) was administered intraperitoneally 30 minutes after paraquat-induced acute lung injury in mice. Chymostatin significantly alleviated the pathological changes in lung tissues, decreased the lung wet-to-dry ratio, tissue cathepsin G activity, and serum concentrations of TNF- α , IL-1 β , IL-6, and HMGB1, while increasing the serum concentration of endothelial cell-specific molecule-1 (endocan). Additionally, Chymostatin up-regulated endocan expression and down-regulated nuclear NF- κ Bp65 expression in the lung, thereby inhibiting the inflammatory response^[7]. Chymostatin (2mg/kg/h) was administered via intravenous infusion to rats with hypertension induced by unilateral nephrectomy and high-salt diet (UNX HS) and the Goldblatt renal artery stenosis (2K1C) model. Chymostatin significantly reduced plasma and tissue levels of angiotensin II (Ang II). In Goldblatt hypertensive rats, Chymostatin increased renal and hindlimb vascular resistance. In UNX HS hypertensive rats, Chymostatin blocked the solvent-induced decrease in outer medullary blood flow^[8].

References:

- [1] Umezawa, H., Aoyagi, T., Morishima, H., et al. Chymostatin, a new chymotrypsin inhibitor produced by actinomycetes. J Antibiot (Tokyo). 1970 Aug;23(8):425-7.
- [2] Akahoshi, F., Ashimori, A., Sakashita, H., et al. Synthesis, structure-activity relationships, and pharmacokinetic profiles of nonpeptidic difluoromethylene ketones as novel inhibitors of human chymase. J Med Chem. 2001 Apr 12;44(8):1297-304.

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- [3] Sacchetta P, Battista P, Santarone S, et al. Purification of human erythrocyte proteolytic enzyme responsible for degradation of oxidant-damaged hemoglobin. Evidence for identifying as a member of the multicatalytic proteinase family. *Biochim Biophys Acta*. 1990 Mar 1;1037(3):337-43.
- [4] Satoh M, Yokosawa H, Ishii S. Membrane-bound trypsin-like enzyme functioning in degradation of dynorphin in neuroblastoma cells. Purification and characterization. *J Biochem*. 1988 Mar;103(3):493-8.
- [5] Qiu M, Li S, Jin L, et al. Combination of Chymostatin and Aliskiren attenuates ER stress induced by lipid overload in kidney tubular cells. *Lipids Health Dis*. 2018 Jul 31;17(1):183.
- [6] Liu S, Suzuki Y, Takemasa E, et al. Mast cells promote viral entry of SARS-CoV-2 via formation of chymase/spike protein complex. *Eur J Pharmacol*. 2022 Sep 5;930:175169.
- [7] Yang C, Song HW, Liu W, et al. Protective Effects of Chymostatin on Paraquat-Induced Acute Lung Injury in Mice. *Inflammation*. 2018 Feb;41(1):122-133.
- [8] Roszkowska-Chojecka MM, Walkowska A, Gawryś O, et al. Effects of chymostatin, a chymase inhibitor, on blood pressure, plasma and tissue angiotensin II, renal haemodynamics and renal excretion in two models of hypertension in the rat. *Exp Physiol*. 2015 Sep;100(9):1093-105.

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