Product Data Sheet

Product Name: Histatin 5
Cat. No.: GC31898

Chemical Properties

Cas. No.	115966-68-2		
SMILES	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr		
Formula	$C_{133}H_{195}N_{51}O_{33}$	M.Wt	3036.29
Solubility	Soluble in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ 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	Histatin 5		

Protocol

Cell experiment [1]:

Cell lines	Human gingival fibroblast cells

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Preparation Method	The cytotoxic effect of Histatin 5 was assessed on human gingival fibroblast cells grown in Dulbeccco's modified Eagle medium supplemented with antibiotic-fungal solution and 10% v/v fetal bovine serum. The incubation temperature was 37°C with 5% CO_2 in air and 95% air concentration. Cells were cultured to confluence (90%) and removed with trypsin (0.05%)/EDTA (0.02%) in 1×PBS. Medium was added to inactivate trypsin, and cells were then centrifuged at 2000rpm for 5min, resuspended, and re-seeded. The medium was changed two to three times a week. Total viable cell counts were performed in an incubator. Cell suspensions containing 2.0×10^4 cells/ml were incubated in 24-well plates for 48 hours at 37°C in a humidified environment with 5% CO_2. At the end of the culture, the medium was discarded, and adherent cells remained at the bottom of the plate. Gradient dilutions of Histatin 5 (100, 200, 400, 800, 1600, 3200, 6400, and 12800μg/mL) were performed with fresh medium. The plates were incubated at 37°C in an atmosphere of 5% CO_2 and 95% air for 24 hours. MTT assay was used to measure mitochondrial dehydrogenase activity. After the cells were grown in the control or test medium for 24h, 100μL of MTT stock solution was added to each well. The culture plates were incubated at 37°C in 5% CO_2 for 4 hours. At the end of incubation, the cultures were removed from the incubator and 100μL of MTT solubilization solution was added to dissolve the resulting formazan crystals. The plates were then shaken until the crystals were completely dissolved, and the absorbance was measured at a wavelength of 570nm by spectrophotometry; all experiments were performed three times.
Reaction Conditions	100, 200, 400, 800, 1600, 3200, 6400, and 12800μg/mL; 24h
Applications	Histatin 5 at concentrations above 3200μg/mL was significantly cytotoxic, resulting in an apparent reduction in gingival fibroblast cell viability.

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Animal experiment [2]:

Animal models	C57BL/6 mice
Preparation Method	The C57BL/6J mice aged between twelve and nineteen weeks received anesthesia through an intraperitoneal injection containing ketamine at 100mg/kg and xylazine at 5mg/kg. A circular 2.0-mm central epithelial segment was delineated with a 2-mm biopsy punch following 2 drops of topical 0.5% proparacaine, then removed with a brush. Histatin 5 (80μM), SHRGY (80μM) was applied to the cornea three times a day during the treatment (n = 7) or the control (n = 7) group. At 0, 18, and 24h, corneas were stained with fluorescein and imaged using a photo-slit lamp with a camera. The remaining wound areas were measured with the ImageJ software at each indicated time point and were compared with the baseline wound area for each mouse. The percentage of the remaining wound area was calculated for each time point.
Dosage form	80μM for three times a day; apply to the cornea
Applications	Histatin 5 treatment significantly promoted wound healing in a mouse corneal injury model.

References:

[1] Moffa E B, Mussi M C M, Xiao Y, et al. Histatin 5 inhibits adhesion of C. albicans to reconstructed

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human oral epithelium[J]. Frontiers in Microbiology, 2015, 6: 885.
[2] Shah D, Son K N, Kalmodia S, et al. Wound healing properties of histatin-5 and identification of a functional domain required for histatin-5-induced cell migration[J]. Molecular Therapy Methods & Clinical Development, 2020, 17: 709-716.

Background

Histatin 5 suppresses the activities of matrix metalloproteinases MMP-2 and MMP-9 with IC_{50} values of 0.57 and 0.25μM, respectively[1]. Histatin 5 significantly inhibited the growth of a variety of Candida species by binding to Candida cell wall proteins (Ssa1/2) and glycans with MIC_{50} values of 10-20μg/ml[2]. Histatin 5 has been widely used in

metal ion binding studies and antifungal studies[3].

In vitro, Histatin 5 at a concentration of 3200μg/mL for 24 hours was significantly cytotoxic to human gingival fibroblasts and dramatically inhibited cell viability[4]. Treatment of human gingival fibroblasts with 10μg/ml Histatin 5 for 20 minutes inhibited the production of inflammatory cytokines (IL-6 and IL-8) induced by P. gingivalis and lipopolysaccharides[5].

In vivo, Histatin 5 gel at a dose of 100μg/mL three times daily applied to the tongues of mice for three days prevented the occurrence of oral ulcers and significantly inhibited Candida albicans[6]. Administration of Histatin 5 (80μM) to the cornea three times daily for one day promoted wound healing in a mouse corneal injury model[7].

References:
[1] Gusman H, Travis J, Helmerhorst E J, et al. Salivary histatin 5 is an inhibitor of both host and bacterial enzymes implicated in periodontal disease[J]. Infection and immunity, 2001, 69(3): 1402-1408.
[2] Puri S, Edgerton M. How does it kill?: understanding the candidacidal mechanism of salivary histatin 5[J]. Eukaryotic cell, 2014, 13(8): 958-964.
[3] Zolin G V S, Fonseca F H, Zambom C R, et al. Histatin 5 metallopeptides and their potential against Candida albicans pathogenicity and drug resistance[J]. Biomolecules, 2021, 11(8): 1209.
[4] Moffa E B, Mussi M C M, Xiao Y, et al. Histatin 5 inhibits adhesion of C. albicans to reconstructed human oral epithelium[J]. Frontiers in Microbiology, 2015, 6: 885.
[5] Imatani T, Kato T, Minaguchi K, et al. Histatin 5 inhibits inflammatory cytokine induction from human gingival fibroblasts by Porphyromonas gingivalis[J]. Oral microbiology and immunology, 2000, 15(6): 378-382.
[6] Kong E F, Tsui C, Boyce H, et al. Development and in vivo evaluation of a novel histatin-5 bioadhesive hydrogel formulation against oral candidiasis[J]. Antimicrobial agents and chemotherapy, 2016, 60(2): 881-889.
[7] Shah D, Son K N, Kalmodia S, et al. Wound healing properties of histatin-5 and identification of a functional domain required for histatin-5-induced cell migration[J]. Molecular Therapy Methods & Clinical Development, 2020, 17: 709-716.

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