

## Leupeptin Hemisulfate

## Chemical Properties

CAS No. :	103476-89-7
Formula:	C <sub>20</sub> H <sub>38</sub> N <sub>6</sub> O <sub>4</sub> ·1/2H <sub>2</sub> SO <sub>4</sub>
Molecular Weight:	475.59
Storage:	Keep away from moisture, Store at low temperature Powder: -20°C for 3 years   In solvent: -80°C for 1 year <small>Actual storage temperature shall be subject to the COA.</small>

## Biological Description

Description	Leupeptin hemisulfate is a protease inhibitor with cell membrane-permeable, reversible, competitive, and oral activities. Leupeptin hemisulfate inhibits the activity of Cathepsin B, Cathepsin H, and Cathepsin L, and blocks fusion of amphipathic lysosomes. Leupeptin hemisulfate also has anti-inflammatory activity.
Targets(IC50)	Cysteine Protease, Serine Protease, Virus Protease
In vitro	<p><b>METHODS:</b> SARS-CoV-2 infected Vero cells were treated with Leupeptin hemisulfate (0.06-200 μM) for 72 h, and the viral RNA level was detected by RT-PCR.</p> <p><b>RESULTS:</b> Leupeptin hemisulfate inhibited SARS-CoV-2 RNA levels in Vero cells with an EC<sub>50</sub> value of 42.34 μM. [1]</p> <p><b>METHODS:</b> SARS-CoV-2 pseudovirus-infected Huh7 cells were treated with Leupeptin hemisulfate (0.1-100 μM) for 24 h. Pseudovirus infection was detected by luciferase activity assay.</p> <p><b>RESULTS:</b> Leupeptin hemisulfate inhibited pseudovirus infection in Vero cells with an EC<sub>50</sub> value of 39.29 μM. [2]</p>
In vivo	<p><b>METHODS:</b> To determine macrophage autophagic flux, Leupeptin hemisulfate (9-40 mg/kg in 0.5 mL PBS) was administered to C57BL/6NCRl mice by single intraperitoneal injection.</p> <p><b>RESULTS:</b> Leupeptin hemisulfate treatment resulted in the highest accumulation of LC3b in the liver and the lowest in the spleen. LC3a, ATG8/LC3b homologs, and the LC3b-interacting protein p62 were degraded with kinetics similar to those of LC3b. [3]</p> <p><b>METHODS:</b> To examine whether cold activates autophagic flux, Leupeptin hemisulfate (40 mg/kg) was administered intraperitoneally to C57B6 mice in a single injection, and then the mice were cold-exposed at 4 °C for 1 h. The mice were then exposed to cold for 1 h.</p> <p><b>RESULTS:</b> LC3-II flux in brown fat was increased &gt;2-fold in cold-exposed mice. [4]</p>
Kinase Assay	Determination of BET Protein Binding Affinities to I-BET726: For determination of binding affinities to BET protein bromodomains, I-BET726 is titrated against truncates containing both BD1 and BD2 of BRD2 (10 nM), BRD3 (10 nM), and BRD4 (10 nM) in 50 mM HEPES pH7.5, 150 mM NaCl, 5% Glycerol, 1 mM DTT and 1 mM CHAPS in the presence of an Alexa 647 derivative (50 nM) of fluorescent ligand. After equilibrating for 1 h, the bromodomain protein: ligand interaction is detected using Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) following the addition of 1.5 nM europium chelate

Kinase Assay	labeled anti-6His antibody. Plates are read using an Envision Plate reader ( $\lambda_{EX} = 337$ nm, $\lambda_{EM} = 615$ nm, $\lambda_{EM} = 665$ nm; dual dichroic = 400 nm & 630 nm). These data are fitted to a four parameter IC50 model using Graphit data analysis software.
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### Solubility Information

Solubility	H2O: 87 mg/mL (182.93 mM), Sonication is recommended. DMSO: 60 mg/mL (126.16 mM), Sonication is recommended. Ethanol: 88 mg/mL (185.03 mM), Sonication is recommended. ( $< 1$ mg/ml refers to the product slightly soluble or insoluble)
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### Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.1027 mL	10.5133 mL	21.0265 mL
5 mM	0.4205 mL	2.1027 mL	4.2053 mL
10 mM	0.2103 mL	1.0513 mL	2.1027 mL
50 mM	0.0421 mL	0.2103 mL	0.4205 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

### Reference

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