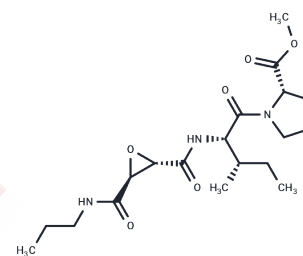


## CA-074 methyl ester

### Chemical Properties

CAS No. :	147859-80-1
Formula:	C19H31N3O6
Molecular Weight:	397.47
Storage:	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	CA-074 methyl ester (Cathepsin B Inhibitor IV) is a selective inhibitor of Cathepsin B (IC <sub>50</sub> =36.3 nM). CA-074 methyl ester has neuroprotective, anti-inflammatory and anti-cancer effects.
Targets(IC <sub>50</sub> )	Cysteine Protease
In vitro	<b>METHODS:</b> C57BL/6J BMM cells were treated with CA-074 methyl ester (50 μM) for 24, 48 and 72 hours, and the target protein expression was detected by Western Blot. <b>RESULTS:</b> CA-074 methyl ester inhibited RANKL-stimulated c-FOS upregulation and subsequent NFATc1 self-amplification. [1]
In vivo	<b>METHODS:</b> To study the neuroprotective effect of CA-074 methyl ester, CA-074 methyl ester (1 μg, 10 μg) was administered to the hippocampus induced by global cerebral I/R injury. <b>RESULTS:</b> CA-074 methyl ester prevented necroptosis in hippocampal CA1 neurons induced by global cerebral I/R injury. CA-074 methyl ester pretreatment strongly inhibited lysosomal membrane disruption and cathepsin-B leakage. CA-074 methyl ester inhibited the overexpression and nuclear translocation of RIP3 and the reduction of NAD <sup>+</sup> levels after I/R injury. Pretreatment with CA-074 methyl ester enhanced the upregulation of Hsp70. [2] <b>METHODS:</b> To study the effect of CA-074 methyl ester on osteoclastogenesis, CA-074 methyl ester (10, 30 mg/kg) was injected into the skull of mice. <b>RESULTS:</b> CA-074 methyl ester inhibited osteoclastogenesis in vivo. [1] <b>METHODS:</b> To study the anti-inflammatory activity of CA-074 methyl ester, CVB+CA-074 methyl ester (4 mg/kg) was injected intramuscularly into guinea pigs. <b>RESULTS:</b> Inflammation scores were significantly lower in the CVB+None group than in the CVB+ NONE group. The number of CD8 <sup>+</sup> T cells was reduced in the CVB+CA-074 methyl ester group compared with the sham-operated group. [3]
Kinase Assay	After seven days of cell culture and osteoclast generation, the media is removed and washed three times with PBS. BMMs are fixed with a fixing solution supplied by the manufacturer. The cells are incubated at 37°C with a solution containing deionized water, Fast Garnet GBC, Naphthol phosphate, Acetate, and Tartrate for 1 h. The staining solution is removed, washed with PBS (3x), and air-dried. TRAP positive cells with three or more nuclei across whole culture area are counted as multinucleated osteoclasts using light microscopy.

Cell Research	HL-60 cells are pre-treated for 24 h with 200 $\mu$ M BSO followed by 60 min with 1 mM DEM. Thereafter, the cells( $1 \times 10^6$ /ml) are incubated with 100 $\mu$ M CA-074 or CA-074Me, in the presence of 1% DMSO, 200 $\mu$ M BSO and 1 mM DEM at 37°C. Control cultures are treated with 1% DMSO alone or with 100 $\mu$ M Z-FA-DMK in the presence of 200 $\mu$ M BSO and 1 mM DEM. Untreated HL-60 cells are incubated with 100 $\mu$ M CA-074 or CA-074Me, in the presence of 1% DMSO at 37°C. Untreated control cultures are incubated with 1% DMSO alone or with 100 $\mu$ M Z-FA-DMK. After 2 h incubation, cells are washed three times with PBS/1% glucose and lysed in 100 mM citrate, pH 5.0, 2% Chaps (10 <sup>6</sup> cells/100 $\mu$ l). Subsequently, the lysate is centrifuged and the clarified supernatant used to assay for proteolytic activity.(Only for Reference)
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### Solubility Information

Solubility	DMSO: 257.5 mg/mL (647.85 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble) Ethanol: 73 mg/mL (183.66 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 5 mg/mL (12.58 mM),Sonication is recommended. <i>Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.</i>

### Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.5159 mL	12.5796 mL	25.1591 mL
5 mM	0.5032 mL	2.5159 mL	5.0318 mL
10 mM	0.2516 mL	1.258 mL	2.5159 mL
50 mM	0.0503 mL	0.2516 mL	0.5032 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

- Patel N, et al. CA-074Me compound inhibits osteoclastogenesis via suppression of the NFATc1 and c-FOS signaling pathways. J Orthop Res. 2015 Oct;33(10):1474-86.
- Wang Y, Chen Y Y, Gao G B, et al.Polyphyllin D "Punctures" Hypertrophic Lysosomes to Reverse Drug Resistance of Hepatocellular Carcinoma by Targeting Acid Sphingomyelinase.Molecular Therapy.2023
- Xu Y,et al. Protective mechanisms of CA074-me (other than cathepsin-B inhibition) against programmed necrosis induced by global cerebral ischemia/reperfusion injury in rats. Brain Res Bull. 2016 Jan;120:97-105.
- Li M, Bei Z C, Yuan Y, et al.In-cell bioluminescence resonance energy transfer (BRET)-based assay uncovers ceritinib and CA-074 as SARS-CoV-2 papain-like protease inhibitors.Journal of Enzyme Inhibition and Medicinal Chemistry.2024, 39(1): 2387417.
- Zhang L, et al. Treatment with CA-074Me, a Cathepsin B inhibitor, reduces lung interstitial inflammation and fibrosis in a rat model of polymyositis. Lab Invest. 2015 Jan;95(1):65-77.

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