E-64

Cat. No.: HY-15282

CAS No.: 66701-25-5

Molecular Formula: $C_{15}H_{27}N_5O_5$

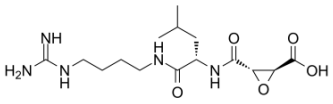
Molecular Weight: 357.41

Target: Cathepsin; Autophagy; Bacterial

Pathway: Metabolic Enzyme/Protease; Autophagy; Anti-infection

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (139.90 mM; Need ultrasonic)

H_2O : 36 mg/mL (100.72 mM; Need ultrasonic and warming)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.7979 mL	13.9895 mL	27.9791 mL
5 mM	0.5596 mL	2.7979 mL	5.5958 mL
10 mM	0.2798 mL	1.3990 mL	2.7979 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (5.82 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (5.82 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (5.82 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

E-64 (Proteinase inhibitor E 64) is a potent irreversible inhibitor against general cysteine proteases with IC_{50} of 9 nM for papain.

IC_{50} & Target

IC50: 9 nM (Papain)[1]

In Vitro

E-64 (Proteinase inhibitor E 64) is a cathepsin B-specific inhibitor, and its binding modes with papain, actinidin, cathepsin L,

	and cathepsin K have been reviewed at the atomic level. E-64 has been widely used as a potent and irreversible (covalent-type) inhibitor for many cysteine proteases such as papain, ficin, actinidin, cathepsin B and L[1]. The S.cervi adult parasites are incubated in the Kreb's Ringer bicarbonate (KRB) maintenance medium for 8 h at 37°C, 5% CO_2 with 5, 10, 20 and 40 μM concentration of E-64. E-64 shows a concentration and time dependent decrease in motility and viability of the parasites (EC_{50}=16 μM)[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	A broad spectrum of expression of CD4 and CD19 is found exists in both the islets and pancreatic lymph nodes (PLNs) and that anti-serpin B13 mAb exposure causes a significant shift that favored cells expressing low-to-intermediate amounts of these markers. However, this shift is abolished in animals that receive anti-serpin B13 mAb in the presence of the protease inhibitor E-64 (Proteinase inhibitor E 64), which maintains its blocking activity under the experimental conditions used[3]. Dahl salt-sensitive (SS) rats are fed an 8% high salt NaCl diet and intravenously infused with the irreversible cysteine cathepsin inhibitor E-64 (1 mg/day) or the vehicle (control). Both the control and E-64 infused groups develope significant hypertension and kidney damage, and no difference of the mean arterial pressure and the hypertension-associated albuminuria is observed between the groups[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [2]	The Cathepsin B activity is determined using Z-Arg-Arg-4mβNA as substrate with slight modifications. The crude extract is pre-incubated at 37°C for 5 min in 50 mM sodium acetate buffer, pH 5.0 containing 1 mM EDTA and 5 mM DTT. The substrate (final concentration, 100 μM) is added to make the final assay volume of 1 mL. The reaction mixture is incubated at 37°C for 30 min. The reaction is terminated by adding equal volume of stopping reagent containing Fast Garnet GBC salt (1 mg/mL), 10 mM pHMB and 50 mM EDTA, pH 6.0. The extraction of product, β-napthylamine (β-NA), is carried out with n-butanol. After complete layer separation, the absorbance is measured in n-butanol layer and activity is calculated using molar extinction coefficient of β-napthylamine solution as 31.5 M/cm per sec at 520 nm. One unit of enzyme activity is defined as the amount of enzyme liberating 1 μmol of βNA per minute at 37°C. The half maximal inhibitory concentration (IC_{50}) is calculated by plotting the graph between the different concentration of E-64 and the % inhibition in cathepsin B activity. Here, IC_{50} indicates the concentration of the E-64 required to inhibit the parasitic cathepsin B activity by half[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3][4]	Mice[3] The NOD/LtJ and BDC2.5 T cell receptor (TCR) transgenic NOD mice are used to study the effects of treatment with anti-serpin B13 monoclonal antibody (mAb). Four-week-old female NOD/LtJ mice are injected intravenously four times over a period of 10 days with anti-serpin B13 mAb (100 μg/injection). In addition, during the same period, some animals are also injected intraperitoneally with the protease inhibitor E64 at 10 mg/kg/day for several days. Control mice are treated with diluent (a sterilized PBS solution containing 10% DMSO) and control IgG. The solutions containing E64 or DMSO are prepared immediately before use. Twenty-four hours after the last injection, the mice are killed, and cells from their lymphoid organs and pancreatic islets are subjected to FACS analysis. Rats[4] Seven-week old male Dahl Salt Sensitive rats (SS/JrHsdMcwi) are used. Briefly, 8-week old anesthetized SS rats have their left femoral artery and vein catheterized. Both catheters are fixed and exteriorized from the back of the neck and the arterial line is connected to a heparinized saline infusion pump that is in line with a blood pressure transducer, and the venous line is connected to a saline infusion pump. Animals are allowed 360° movement using a tether-swivel system. This preparation allowed chronic venous infusion and arterial blood pressure measurement in conscious, freely moving rats. A stable baseline blood pressure is obtained for 4 days prior to switching both groups to an 8.0% NaCl diet and the simultaneous addition of E-64 (1 mg/day; 280 mM stock in DMSO) or the vehicle (DMSO in saline) control to the venous catheter. Daily MAP is calculated by averaging MAP taken every min over the beginning 3 h period of the rat sleep cycle. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Prolif. 2019 May;52(3):e12609.
- LWT-FOOD SCI TECHNOL. 2018 Jan, 87; 186-193
- Int J Oncol. 2019 Jul;55(1):331-339.
- Dev Comp Immunol. 2018 Jan;78:114-123.
- FEBS Lett. 2020 Oct 27.

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REFERENCES

[1]. Matsumoto K, et al. Structural basis of inhibition of cysteine proteases by E-64 and its derivatives. Biopolymers. 1999;51(1):99-107.

[2]. Wadhawan M, et al. Inhibition of cathepsin B by E-64 induces oxidative stress and apoptosis in filarial parasite. PLoS One. 2014 Mar 25;9(3):e93161.

[3]. Baldzizhar R, et al. Anti-serpin antibody-mediated regulation of proteases in autoimmune diabetes. J Biol Chem. 2013 Jan 18;288(3):1612-9.

[4]. Blass G, et al. Chronic cathepsin inhibition by E-64 in Dahl salt-sensitive rats. Physiol Rep. 2016 Sep;4(17). pii: e12950.

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