Product Data Sheet

Product Name: E-64
Cat. No.: GC13418

Chemical Properties

Cas. No.	66701-25-5		
Chemical Name	(2S,3S)-3-[[(2S)-1-[4-(diaminomethylideneamino)butylamino]-4-methyl-1-oxopentan-2-yl]carbamoyl]oxirane-2-carboxylic acid		
SMILES	CC(C)CC(C(=O)NCCCCN=C(N)N)NC(=O)C1C(O1)C(=O)O		
Formula	$C_{15}H_{27}N_5O_5$	M.Wt	357.41
Solubility	≥ 53.6mg/mL in DMSO	Storage	Store at 2-8°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	E-64		

Protocol

Cell experiment [1]:

Cell lines	MDA-MB-231 cells
Preparation Method	Confluent MDA-MB-231 cells were treated with E-64 concentrations ranging from 0 to 50μM for 24h. Cells were lysed and subjected to multiplex cathepsin zymography to detect changes in the amount of active material.
Reaction Conditions	0-50μM; 24h

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Applications	E-64 treatment significantly increased the amount of active cathepsin S, while significantly decreased the amount of cathepsin L in cell lysates.

Animal experiment [2]:

Animal models	Dahl Salt Sensitive rats (SS/JrHsdMcwi)
Preparation Method	Dahl Salt Sensitive rats (SS/JrHsdMcwi) had their left femoral artery and vein catheterized. Both catheters were fixed and exteriorized from the back of the neck and the arterial line was connected to a heparinized saline infusion pump that was in line with a blood pressure transducer, and the venous line was connected to a saline infusion pump. Animals were allowed 360° movement using a tether-swivel system. A stable baseline blood pressure was obtained for 4 days prior to switching both groups to an 8.0% NaCl diet and the simultaneous addition of N-[N-(L-3-trans-carboxyox-irane-2-carbonyl)-L-leucyl]-agmatine (E-64, 1mg/day; 280mM stock in DMSO) or the vehicle control to the venous catheter. Daily mean arterial pressure (MAP) was calculated by averaging MAP taken every min over the beginning 3 h period of the rat sleep cycle.
Dosage form	1mg/day; i.v.
Applications	A significant increase in the renal cortical mature form of Cath B and Cath L were measured in E-64 treated rats.

References:

[1]Wilder C L, Walton C, Watson V, et al. Differential cathepsin responses to inhibitor-induced feedback: E-64 and cystatin C elevate active cathepsin S and suppress active cathepsin L in breast cancer cells[J]. The international journal of biochemistry & cell biology, 2016, 79: 199-208.

[2]Blass G, Levchenko V, Ilatovskaya D V, et al. Chronic cathepsin inhibition by E-64 in Dahl salt-sensitive rats[J]. Physiological reports, 2016, 4(17): e12950.

Background

E-64 is a potent and irreversible inhibitor of cysteine proteases with an IC_{50} of 9nM for papain[1]. E-64 can inhibit the cysteine proteases cathepsin B, H, L and papain, but has no effect on serine proteases or metalloproteinases[2]. E-64 has antiparasitic activity in vitro and can induce oxidative stress and apoptosis in filarial parasites[3]. E-64 can

improve the preimplantation development of bovine somatic cell nuclear transfer embryos[4].

In vitro, E-64 (0-50μM) treatment of breast cancer MDA-MB-231 cells for 24h increased the amount of intracellular active cathepsin S and reduced the amount of cathepsin L in a dose-dependent manner[5]. E-64 (4mM) treatment of porcine alveolar macrophages (PAM) for 48h upregulated the mRNA levels of IFNγ, IL-12 and IFN-α in cells without cytotoxicity[6].

In vivo, daily intravenous infusion of E-64 (1mg) in Dahl salt-sensitive rats significantly increased the mature forms of the lysosomal proteases Cath B and Cath L in the renal cortex of rats, but had no effect on high-salt diet-induced hypertension and renal damage[7].

References:
[1] Matsumoto K, Mizoue K, Kitamura K, et al. Structural basis of inhibition of cysteine proteases by E-64 and its derivatives[J]. Peptide Science, 1999, 51(1): 99-107.
[2] Barrett A J, Kembhavi A A, Brown M A, et al. L-trans-Epoxysuccinyl-leucylamido (4-guanidino) butane (E-64) and its analogues as inhibitors of cysteine proteinases including cathepsins B, H and L[J]. Biochemical Journal, 1982, 201(1): 189-198.
[3] Wadhawan M, Singh N, Rathaur S. Inhibition of cathepsin B by E-64 induces oxidative stress and apoptosis in filarial parasite[J]. PLoS One, 2014, 9(3): e93161.
[4] Min S H, Song B S, Yeon J Y, et al. A cathepsin B inhibitor, E-64, improves the preimplantation development of bovine somatic cell nuclear transfer embryos[J]. Journal of Reproduction and Development, 2014, 60(1): 21-27.
[5] Wilder C L, Walton C, Watson V, et al. Differential cathepsin responses to inhibitor-induced feedback: E-64 and cystatin C elevate active cathepsin S and suppress active cathepsin L in breast cancer cells[J]. The international journal of biochemistry & cell biology, 2016, 79: 199-208.
[6] Liu B, Cui Y, Lu G, et al. Small molecule inhibitor E-64 exhibiting the activity against African swine fever virus pS273R[J]. Bioorganic & Medicinal Chemistry, 2021, 35: 116055.
[7] Blass G, Levchenko V, Ilatovskaya D V, et al. Chronic cathepsin inhibition by E-64 in Dahl salt-sensitive rats[J]. Physiological reports, 2016, 4(17): e12950.