

Product Data Sheet

Product Name: E 64d
Cat. No.: GC13787

Chemical Properties

Cas. No. 88321-09-9

Chemical Name ethyl (2S,3S)-3-[[[(2S)-4-methyl-1-(3-methylbutylamino)-1-oxopentan-2-yl]carbamoyl]oxirane-2-carboxylate

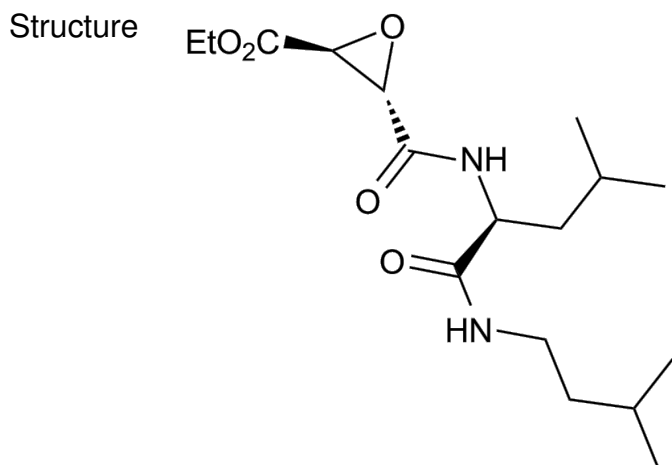
SMILES CCOC(=O)C1C(O1)C(=O)NC(CC(C)C)C(=O)NCCC(C)C

Formula $C_{17}H_{30}N_2O_5$ M.Wt 342.43

Solubility $\geq 17.1215\text{mg/mL}$ in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.



Protocol

Cell experiment [1]:

Cell lines SH-SY5Y cells

Caution: Product has not been fully validated for medical applications. For research use only.

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Preparation Method SH-SY5Y cells were pretreated with or without E 64d (3 μ M) for 30min and incubated with A β (0.001 or 0.01 μ M) for 5.5h, followed by incubation with methacholine (1mM) for 2h. Secreted sAPP α was analyzed by Western blotting using 6E10 antibody.

Reaction Conditions 3 μ M; 30min

Applications E 64d restores the A β -induced reduction of sAPP α secretion in SH-SY5Y cells.

Animal experiment [2]:

Animal models BALB/c mice

Preparation Method Arthritis was induced in 7-week-old female BALB/c mice with a mixture of anti-type II CL mAbs and LPS. 2 mg per body of mAb mixture was injected i.p. into each mouse, and 48h later, 50 μ g per body of LPS was injected i.p. in order to enhance the arthritis. A high dose (9mg/kg/day) or low dose (3mg/kg/day) of E 64d, recombinant cal pastatin (rCS) (9mg/kg/day) or control diluent was administered i.p. to the disease-affected mice for 10 days from the day before the injection of mAbs. Excise the right hind paw from each body and macroscopically assess the severity of arthritis in each paw. Evaluate histopathological severity based on findings of cellular infiltration, pannus formation, and bone erosion.

Dosage form 3、 9mg/kg/day for 10 days; i.p.

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Applications

The clinical arthritis score of the high-dose E 64d group was significantly lower than that of the untreated group, while there was no improvement in the low-dose E 64d group and the rCS-treated group. The cell infiltration histopathological score and total histopathological arthritis score of the high-dose E 64d group were significantly lower than those of the untreated group.

References:

[1]Tanabe F, Nakajima T, Ito M. The thiol proteinase inhibitor E-64-d ameliorates amyloid- β -induced reduction of sAPP α secretion by reversing ceramide-induced protein kinase C down-regulation in SH-SY5Y neuroblastoma cells[J]. Biochemical and Biophysical Research Communications, 2013, 441(1): 256-261.

[2]Yoshifuji H, Umehara H, Maruyama H, et al. Amelioration of experimental arthritis by a calpain-inhibitory compound: regulation of cytokine production by E-64-d in vivo and in vitro[J]. International immunology, 2005, 17(10): 1327-1336.

Background

E 64d is a cell-permeable, irreversible, broad-spectrum cysteine protease inhibitor with platelet aggregation inhibitory activity^[1]. E 64d is a non-selective covalent tissue protease inhibitor containing an epoxide ring as a warhead that can be nucleophilically attacked by Cys-SH and form a covalent bond at the catalytic site^[2]. E 64d inhibits the accumulation of protease-resistant prion protein (PrP-res) in ScNB cells with an IC₅₀ of 0.5±0.11 μ M^[3].

In vitro, pretreatment of SH-SY5Y cells with E 64d (3 μ M) for 30min improved the amyloid beta-induced decrease in sAPP α secretion and reversed the ceramide-induced downregulation of protein kinase C^[4]. Treatment of HT22 cells with E 64d (25 μ M) for 24h alleviated glutamate-induced apoptosis, reversed the decrease in mitochondrial membrane potential, and reduced oxidative stress^[5].

In vivo, E 64d (9mg/kg/day) was intraperitoneally injected into arthritic mice for 10 days, which

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significantly reduced the severity of clinical arthritis and histopathology, and decreased the mRNA levels of cytokines IL-6 and IL-1 β ^[6]. E 64d (1mg/kg) was intravenously injected into rats with spinal cord injury (SCI) for 7 days, which inhibited the inflammatory response caused by SCI, reduced the expression of calretinin 1 in spinal cord tissue, reduced neuronal apoptosis, and inhibited the activity of cyclooxygenase 2^[7].

References:

- [1] Chen Y, Li Q, Li Q, et al. p62/SQSTM1, a central but unexploited target: advances in its physiological/pathogenic functions and small molecular modulators[J]. Journal of medicinal chemistry, 2020, 63(18): 10135-10157.
- [2] Cannalire R, Stefanelli I, Cerchia C, et al. SARS-CoV-2 entry inhibitors: small molecules and peptides targeting virus or host cells[J]. International Journal of Molecular Sciences, 2020, 21(16): 5707.
- [3] Doh-Ura K, Iwaki T, Caughey B. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation[J]. Journal of virology, 2000, 74(10): 4894-4897.
- [4] Tanabe F, Nakajima T, Ito M. The thiol proteinase inhibitor E-64-d ameliorates amyloid- β -induced reduction of sAPP α secretion by reversing ceramide-induced protein kinase C down-regulation in SH-SY5Y neuroblastoma cells[J]. Biochemical and Biophysical Research Communications, 2013, 441(1): 256-261.
- [5] Xie R J, Li T X, Qiao X Y, et al. The Protective Role of E-64d in Hippocampal Excitotoxic Neuronal Injury Induced by Glutamate in HT22 Hippocampal Neuronal Cells[J]. Neural Plasticity, 2021, 2021(1): 7174287.
- [6] Yoshifuji H, Umehara H, Maruyama H, et al. Amelioration of experimental arthritis by a calpain-inhibitory compound: regulation of cytokine production by E-64-d in vivo and in vitro[J]. International immunology, 2005, 17(10): 1327-1336.
- [7] Zhang Z, Huang Z, Dai H, et al. Therapeutic Efficacy of E-64-d, a Selective Calpain Inhibitor, in Experimental Acute Spinal Cord Injury[J]. BioMed Research International, 2015, 2015(1): 134242.

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