

Product Information

trans-EPOXYSUCCINYL-L-LEUCYLAMIDO-(4-GUANIDINO)BUTANE

Sigma Prod. No. E 3132

CAS Number 66701-25-5

SYNONYMS: E 64¹; Proteinase Inhibitor E 64¹; N-[N-(L-3-transcarboxyirane-2-carbonyl)-L-Leucyl]-agmatine²

PHYSICAL DESCRIPTION:

Appearance: White powder³
 Molecular Formula: C₁₅H₂₇N₅O₅
 Molecular Weight: 357.4

METHOD OF PREPARATION:

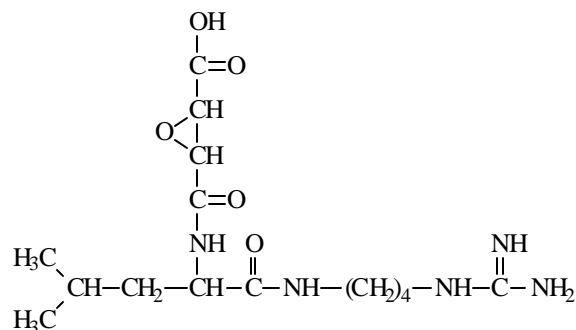
E-64 is synthetically prepared.⁴ Synthetic⁵ and natural⁶ methods of preparation have been reported.

SOLUBILITY / SOLUTION STABILITY:

E-64 is soluble in water. A 20 mg/ml solution can be prepared in deionized water (heat may be needed)³. A suggested stock solution is a 1 mM aqueous solution. The effective concentration for use as a protease inhibitor is 1-10 μM.² Aqueous stock solutions are stable for months at -20 °C; diluted solutions are stable for days at neutral pH.² E-64 is stable from pH 2-10 but is unstable in ammonia or in HCl⁶. E-64 is also soluble in DMSO⁷; a 10 mM solution was prepared in dry DMSO and stored at -20 °C. Subsequent dilutions were in culture medium.⁸ Solutions for injection were prepared by dissolving E-64 in 0.9% sodium chloride or in a minimum amount of saturated sodium bicarbonate followed by dilution with 0.9% sodium chloride (after adjusting the pH to 7.0 with acetic acid).⁹

USAGE / APPLICATIONS:

Some enzymes E-64 inhibits at the indicated concentrations are: Actinidin;¹⁰ Ananain¹¹ (pineapple stem); Bromelain (stem, 10 μM and fruit)^{6,12}; Calpain (chicken skeletal muscle)¹³; Cathepsin B (human and rat liver, 10 μM)^{6,9,12,14-16}; Cathepsin B1 (squid, 10 μM)¹⁷; Cathepsin H (human liver, 10 μM)^{12,14,15}; Cathepsin L (human, 10 μM, and rat liver)^{9,12,14-16,18}; Cathepsin (rat liver, 2.8 mM, about 82% inhibition)¹⁹; Clostripain (100 μM, 81% reversible competitive inhibition)^{12,20}; Comosain (pineapple stem)¹¹; CMP-Sialic Acid:Lactosylceramide α(2-3) Sialyltransferase (SAT-1)²¹; Ficin (10 μM)¹²;



α-Ginivain²⁰; Papain (10 μM)^{6,12,22} (E-64 was not overcome by high levels of cysteine, by dialysis or by gel filtration⁶); α- and β-Trypsin (the latter by a reversible competitive mechanism). E-64 is reported to be one of the most effective low molecular weight inhibitors of trypsin catalyzed hydrolysis.²⁰ E-64 inhibited the activity of bleomycin hydrolase and blocked the activity of a yeast cysteine protease gene (YCP1) which induces an increase in bleomycin metabolism (this may be the cause of bleomycin resistance during bleomycin therapeutic treatment).²³ E-64 (100 μg/ml) promoted heat-induced apoptosis in mouse mammary carcinoma FM3A cells.⁷ E-64 (≥10 μM) inhibited neutrophil movement (chemotaxis) induced by C5a suggesting that an active thiol protease is needed for chemotaxis to C5a.²⁴ E-64 (50-100 μM) selectively blocked T cell receptor-triggered programmed cell death in a mouse hybridoma.⁸ E-64 inhibited the ability of EJ human bladder carcinoma cells to invade through an artificial basement membrane (probably by inhibition of cathepsin B) and to degrade the human basement membrane laminin.²⁵

GENERAL NOTES:

E-64 is an irreversible, potent and highly selective cysteine protease inhibitor, i.e., E-64 does not react with the functional thiol group of L-lactate dehydrogenase or creatine kinase, non-protease enzymes.^{6,9} E-64 does not inhibit serine proteases (except trypsin) like the cysteine protease

inhibitors, leupeptin and antipain.^{12,14} It does not react with low molecular weight thiol compounds such as 2-mercaptoethanol. E-64 has been used as an active site titrant.^{2,12,26} The trans-epoxysuccinyl group (active moiety) of E-64 irreversibly binds to an active thiol group of many cysteine proteases such as papain, actinidase, and cathepsins B, H and L^{14,20} to form a thioether linkage. Crystal structures of papain-E-64 and actinidum-E-64 complexes were reported.^{10,27} Mechanisms of inhibition of some cysteine proteases including cathepsins B and L and of trypsin were reported.^{9,20} E-64 is a very useful cysteine protease inhibitor for use in in vivo studies because it has a specific inhibition, it is permeable in cells and tissues, it has low toxicity, it is easily synthesized and it is stable.¹⁴

REFERENCES:

1. Material Safety Data Sheet
2. *Proteolytic Enzymes: A Practical Approach*, Beynon, R.J. and Bond, J.S., eds. IRL Press, Oxford, England, 1989, 244.
3. Sigma Quality Control data.
4. Supplier information.
5. Hanada, K. et al., *Agric. Biol. Chem.*, 42, 529 (1978).
6. Hanada, K. et al., *Agric. Biol. Chem.*, 42, 523 (1978).
7. Zhu, W.-G. et al., *Biochem. Biophys. Res. Commun.*, 225, 924 (1996).
8. Sarin, A. et al., *J. Expt. Med.*, 178, 1693 (1993).
9. Hashida, S. et al., *J. Biochem.*, 88, 1805 (1980).
10. Varughese, K.I. et al., *Biochem.*, 31, 5172 (1992).
11. Napper, A.D. et al., *Biochem. J.*, 301, 727 (1994).
12. Barrett, A.J. et al., *Biochem. J.*, 201, 189 (1982).
13. Sugita, H. et al., *J. Biochem.*, (Tokyo) 87, 339 (1980).
14. Katunuma, N. and Kominami, E., *Methods Enzymol.*, 251, 382 (1995).
15. Barrett, A.J. and Kirschke, H., *Methods Enzymol.*, 80, 535 (1981).
16. Inubushi, T. et al., *J. Biochem.*, 116, 282 (1994).
17. Inaba, T. et al., *Agric. Biol. Chem.*, 43, 655 (1979).
18. Nomura, T. et al., *Biochem. Biophys. Res. Commun.*, 228, 792 (1996).
19. Towatari, T. et al., *J. Biochem.*, 84, 659 (1978).
20. Sreedharan, S.K. et al., *Biochem. J.*, 316, 777 (1996).
21. Melkerson-Watson, L.J. and Sweeley, C.C., *Biochem. Biophys. Res. Commun.*, 175, 325 (1991).
22. Hanada, K. et al., *Agric. Biol. Chem.*, 42, 537 (1978).
23. Pei, Z. et al., *Mol. Pharm.*, 48, 676 (1995).
24. Barna, J.B. and Kew, R.R., *Inflammation*, 19, 561 (1995).
25. Redwood, S.M. et al., *Cancer*, 69, 1212 (1992).
26. Buttle, D.J. and Barrett, A.J., *Biochem. J.*, 223, 81 (1984).
27. Varughese, K.I., et al., *Biochem.*, 28, 1330 (1989).

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.