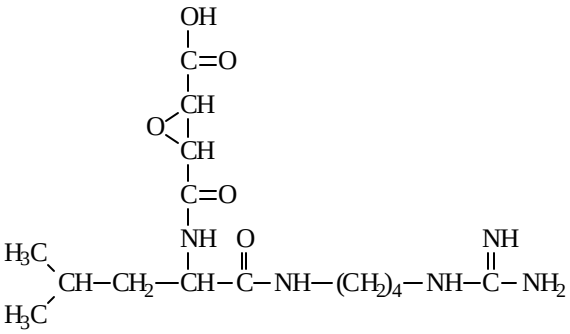
上海伊卡生物技术有限公司

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

Sigma Prod. No. E 0203

CAS Number 66701-25-5

SYNONYMS: E 64[1]; Proteinase Inhibitor E 64[1]; N-[N-(L-3-transcarboxyirane-2-carbonyl)-L-Leucyl]-agmatine[2]



PHYSICAL DESCRIPTION:
Appearance: White powder[3]
Molecular Formula: $C_{15}H_{27}N_5O_5$
Molecular Weight: 357.4

METHOD OF PREPARATION:
E-64 is synthetically prepared.[4] Synthetic[5] and natural[6] 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 HCl[6]. E-64 is also soluble in DMSO[7]; 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] Ananain[11] (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) Sialytransferase (SAT-1)[21]; Ficin (10 µM)[12]; α-Ginivain[20]; Papain (10 µM)[6,12,22] (E-64 was not overcome by high levels of cysteine, by dialysis or by gel filtration[6]); 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]

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