Protease Inhibitors

Application Table

The following table gives an overview on the use and application of frequently used protease inhibitors in biochemistry and cellbiology. It summarizes information on the mechanism of action, target protease class, solubility, concentration, and lists corresponding references.

Cat.No.	Product	M.W.	Description/Specifity of Inhibitor	Solubility Stability	Concentration Range [a)]	References
12745	AEBSF-HCl	239.7	Irreversible inhibitor of Thrombin and other serine proteases. Inhibits by acylation of the active site of the enzyme. Much less toxic than PMSF and DFP	H_2O, Aqueous solutions are stable between pH 5-6	0.1 - 2 µM	39, 61, 62
12548	(epsilon)-Aminocaproic acid	131.2	Highly active inhibitor of fibrinolysin and chymotrypsin.	H_2O	1 - 20 µM	1,2
13685	Antipain-HCL	678.2	Reversible inhibitor of serine and cysteine proteases. Inhibits papain and trypsin more specificly than leupeptin. Plasmin is inhibited only slightly. Also involved in inhibition of RNA synthesis	H_2O Methanol DMSO	1 - 100 µM	4,5,6
13718	Aprotinin (Trypsin inhibitor from bovine lung)	ca. 6500	Basic single-chain polypeptide that inhibits numerous serine proteases by binding to the active site of the enzyme, form ing tight complexes. It inhibits above all plasmin, kallikrein, trypsin, chymotrypsin and urokinase, but not carboxypeptidase A and B, papain, pepsin, subtilisin, thrombin and factor X. Used in cell culture to prevent proteolytic damage to cells and to extend lifetime of cells.	H_2O, Aqueous buffers. Sterile filtered solutions at pH 5-8 are stable for several months. Denatures at pH > 12	In cell culture: 0.01 - 3 µg/ml; in other applications: 10 - 250 µg/ml	9,10,11,12
14525	Benzamidine-HCl	174.6	Potent inhibitor of thrombin and trypsin	H_2O	0.1 - 50 µM	13,14,15
14980	Bestatin-HCl	344.8	Metalloprotease inhibitor with multi-pharmacological functions. Inhibits cell surface aminopeptidases (notably B) and leucine aminopeptidase. Inhibitor of leukotriene A4 hydrolase and of enkephalin degradation in cell preparations from brain. Has anti-carcinogenic and immunomodulating properties.	Methanol (5 mg/ml)	1 - 150 µM Mitogenic effects at nmolar concentrations	16,17,18,19,20
17158	Chymostatin	ca. 600	Peptide-derived aldehyde (mixture of 3 components). Reversible inhibitor of chymotrypsin-like serine and some cysteine proteases	DMSO Acetic acid	10 - 100 µM	4,21,22,23
77205	DFP (Diisopropylfluoro-phosphate)	184.2	A potent irreversible inhibitor of serine proteases and acetyl choline esterase. Highly toxic!	Isopropanol; aqueous solutions are unstable	10 - 100 µM	15,24,25
21100	E-64	357.4	Non-competitive irreversible inhibitor of papain and other cysteine proteases. Forms a thioether bond with the sulfhydryl group in the active center of the enzyme. Useful for active site titration: one mole of E-64 inhibits one mole of protease.	H_2O, DMSO Mixtures of water and ethanol	1 - 10 µM	29,30,31
21102	E-64d (EST)	342.4	Membrane-permeable analog of E-64c	DMSO	1 µM	63,69,70

Cat.No.	Product	M.W.	Description/Specifity of Inhibitor	Solubility Stability	Concentration Range [a]	References
11280	EDTA-Na_2	372.3	Reversible inhibitor of metalloproteases	H_2O (pH 8)	1 - 10 µM	26,27
11290	EGTA	380.4	Inhibits metalloproteases.. Reveals high selectivity for Ca2+ over Mg2+ ions.	NH_4OH, NaOH	1 - 10 µM	28
20947	Elastatinal	512.6	Inhibitor of Elastase	H_2O, MeOH, DMSO	K_i: 0.21 µM (with Ac-Ala-Ala-Ala-OMe as substrate)	4
51867	Leupeptin-hemisulfate	475.6	Tripeptide aldehyde. Reversible competitive inhibitor of serine and cysteine proteases. Inhibits also phospholipase D and C activation in rat hepatocytes.	H_2O Stable for sereval months when stored in aliquots at -20 °C	1 - 100 µM	4,34,35,36
28301	(alpha)2-Macroglobulin from human plamas	725000	Glycoprotein composed of 4 identical subunits. Broad-range endoproteinase inhibitor. Inhibits by forming a »trap« around the enzyme allowing only small substrate molecules to enter and to be cleaved by the entrapped protease.	H_2O Stable for several months when stored in aliquots at -20 °C	Used at equimolar concentrations	3,37
31682	PEFABLOC® SC [b] 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride	239.5	Water-soluble and relatively non-toxic irreversible inhibitor ofthrombin and other serine proteases. Inhibits by acylation of the active site of the enzyme.	H_2O (20 g/100 ml) Stable for several months between pH 5 - 6; limited stability above pH 7.5	0.1 - 5 mM in cell culture: 0.1 - 0.25 µM	38,39,40
52682	Pepstatin A	685.9	Pentapeptide derivative. Reversible inhibitor of aspartic proteases, e.g. pepsin, cathepsin D, chymosin, renin	Methanol (1 mg/ml) DMSO	1 - 10 µM	4,41,42,43
31985	Phebestin	441.5	Inhibitor of Aminopeptidase N	H_2O, DMSO, MeOH	IC_{50}: 0.18 µg/ml	72
32395	PMSF Phenylmethyl sulfonyl fluoride	174.2	Irreversibly inhibits serine proteases by sulfonylation of the serine residue in the active site of the protease. Inhibits also papain (reversible by DTT treatment) and acetylcholin-esterase. Does not inhibit metallo-, aspartic- and most cysteine proteases.	Isopropanol, ethanol, methanol. (100 – 200 mM) Unstable in aqueous solution	0.1 - 1 µM	44,45,46
32753	Phosphoramidon	587.5	Specific inhibitor of thermolysin and neutral endopeptidase-24.11 (ANP Degradation Enzyme). Inhibits also the activity of Endothelin Converting Enzyme, collagenase and metallo-endoproteinases from many microorganisms. Does not inhibit serine, cysteine and aspartic proteases	H_2O (20 mg/ml) DMSO Methanol	1 - 100 µM	4,47,48,49,50
17013	TLCK (1-Chloro-3-tosylamido-7-amino-2-heptanone HCl)	369.3	Irreversibly inhibits trypsin but not chymotrypsin by alkylating the histidine residue in the active site of the enzyme. Inhibits also some other serine and cysteine proteases like bromelain, ficin and papain. TLCK does not react with zymogens or in active protease-inhibitor complexes.	1 mM HCl, DMSO H_2O (20 mg/ml). Aqueous solutions are unstable above pH 7	10 - 1000 µM	51,52,53,54
17016	TPCK (1-Chloro-3-tosylamido-4-phenyl-2-butanone)	351.8	Irreversibly inhibits chymotrypsin but not trypsin by specifically reacting with histidine. Inhibits also other serine and cysteine protease such as bromelain, ficin and papain.	Ethanol (20 mg/ml) sparingly soluble in water; unstable at alkaline pH	10 - 1000 µM	40,55
37310	Trypsin inhibitor from egg white (Ovomucoid)	ca. 28000	Monomeric Glycoprotein. Inhibits bovine (but not human) trypsin in a 1:1 molar ratio. Inhibition is reversible and pH dependant.	H_2O, 1 mM HCl (1 mg/ml) Very stable between pH 3 - 7 against heat and 9 M urea. Unstable at alkaline pH	Used at equimolar concentrations (10-100 µg/ml)	56,57,58

Cat.No.	Product	M.W.	Description/Specifity of Inhibitor	Solubility Stability	Concentration Range [a]	References
37328 37329 37330	Trypsin inhibitor from soybean	ca. 22000	Monomeric protein. Reversible serine protease inhibitor. Inhibits trypsin, factor Xa, plasmin and plasma kallikrein, but not tissue kallikrein.	H_2O (1 mg/ml) Sensitive to heat and high pH	Used at equimolar concentrations (10-100 µg/ml)	59,60

a) Concentration range refers to data frequently used in the literature. The optimal concentration depends very much on the test system under investigation and has to be determined in each case empirically.
b) PEFABLOC is a registered trademark of Pentapharm/Basel

1	Steffen, L. a. Steffen, D. (1976) Clin.Chem. 22, 381-3	41	Hansen, J. et al. (1988) EMBO J. 7, 1785-91
2	Sano, M. et al. (1990) J. Nihon Univ.Sch. Dent. 32, 181-6	42	Lammers, G. a. Jamieson, J.C. (1988) Biochem. J. 256, 623-31
3	Travis, J. a. Salvesen, G.S. (1983) Ann.Rev. Biochem. 52, 655- 709	43	Tyagi, S.C. (1992) Biochem. Cell Biol. 70, 309-15
4	Umezawa, H. (1976) Meth. Enzymol. 45, 678-95	44	Fahrney, D.E. a. Gold, A.M. (1963) J. Am. Chem. Soc. 85, 997-1009
5	Miyata, S. et al. (1988) J. Exp. Zool. 246, 150-5	45	Prouty, W.F. a. Goldberg, A.L. (1972) J. Biol. Chem. 247, 3341-52
6	Cox, L.R. et al. (1991) Cancer Res. 51, 4810-4	46	James, G.T. (1978) Anal. Biochem. 86, 574-9
7	Laura, R. et al. (1980) Biochemistry 19, 4859-64	47	Rae, G.A. et al. (1993) Eur. J. Pharma col. 240, 113-9
8	Cole, T.C. et al. (1989) Biochim. Bio phys. Acta 990, 254	48	Fawzi, A.B. et al. (1994) Anal. Biochem. 222, 342-50
9	Kassel, B. (1970) Meth. Enzymol. 19, 844-52	49	Murphy, L.J. et al. (1994) Br. J. Pharmacol. 113, 137-42
10	Zyznar, E.S. (1981) Life Sci. 28, 1861- 66	50	Angus, R.M. et al. (1994) Clin. Sci. 86, 291-5
11	Hewlett, G. (June 1990) Biotechnology, 565-7	51	Shaw, E. et al. (1965) Biochemistry 4, 2219-24
12	Gray, E.S. a. Tsai, R.W. (1994) J. Exp. Zool. 268, 428-33	52	Walls, A.F. et al. (1992) Biochem. Pharmacol. 43, 1243-8
13	Ensinck, J.W. et al. (1972) J. Clin. Endocrinol.Metab. 35, 463-7	53	Akama, K. et al. (1994) J. Biochem. 116, 464-70
14	Hial, V. et al. (1974) Biochemistry 13, 4311	54	Bedi, G.S. (1995) Prp. Biochem. 25, 133.54
15	Bharadwaj, M. et al. (1996) Biochem. J. 313, 193-9	55	Ong, E.B. et al. (1965) J. Biol. Chem. 240, 694-8
16	Wilkes, S.H. a. Prescott, J.M. (1985) J. Biol. Chem. 260, 13154-62	56	Feeney, R.E. et al. (1963) J. Biol. Chem. 238, 1415
17	Burley, S.K. et al. (1991) Proc. Natl. Acad. Sci. USA 88, 6916-20	57	Kassel, B. (1970) Methods Enzymol. 19, 890-902
18	Mathe, G. (1991) Biomed. Pharmaco ther. 45, 49-54	58	Kato, I. et al. (1987) Biochemistry 26, 193
19	Kumano, N. a. Sugawara, S. (1992) J. Biol. Regul. Homeost. Agents 6, 116-20	59	Kassel, B. (1970) Methods Enzymol. 19, 853-62
20	Baker, J.R. et al. (1995) Biochem. Phar macol. 50, 905-12	60	Birk, Y. (1976) Methods Enzymol. 45, 700-7
21	Tsuboi, R. et al. (1988) J. Clin. Micro biol. 26, 1431-3	61	Marwardt, F. et al (1973) Thrombosis Res. 2, 343-8
22	Alfieri, S.C. et al. (1988) Mol. Biochem. Parasitol. 29, 191-201	62	Taylor, J.A. et al. (1995) Immunology 86, 629-35
23	Tokunaga, M. et al. (1993) Yeast 9, 379-87	63	Buttle, D.J. et al. (1992) Arch. Biochem. Biophys. 299, 377-80
24	Wilson, B.W. a. Walker, C.R. (1974) Proc. Natl. Acad. Sci. USA 71, 3194-8	64	Demuth, H.-U. et al. (1993) FEB5 Lett. 320, 23
25	Banerjee, S. et al. (1991) Cancer Res. 51, 1092-8	65	Hashida, 5. et al. (1980) J. Biochem. 88, 1805-11
26	Iizuka, K. et al. (1993) J. Mol. Cell Cardiol. 25, 1101-9	66	Tamai, M. et al. (1981) J. Biochem. 90 255-7
27	Janas, R.M. et al. (1994) Biochem. Biophys. Res. Commun. 198, 574-81	67	Barrett, A.J. et al. (1982) Biochem. J. 201, 189-98
28	Mortensen, A.M. a. Novak, R.F. (1992) Toxicol. Appl. Pharmacol. 117, 180-8	68	Suzuki, K. (1983) J. Biochem. 93 1305-12
29	Hamada, K. et al. (1978) Agric. Biol. Chem. 42, 529	69	Tamai, M. et al. (1986) J. Parmacobio. Dyn. 9, 672
30	Barrett, A.J. et al. (1982) Biochem. J. 201, 189	70	Tamai, M. et al. (1987) Chem. Pharm. Bull. 35, 1098-1104
31	Montenez, J.P. et al. (1994) Toxicol. Lett. 73, 201-8	71	Naganawa, H. et al. (1985) J. Antibiotics 38, 1813 -5
32	Marquard, F. (1970) Methods Enzymol. 19, 924-32	72	Nagai, M. et al. (1997) J. Antibiotics 50, 82-4
33	Walsmann, P. (1988) Pharmazie 43, 737-44		
34	Neefjes, J.J. a. Ploegh, H.L. (1992) EMBO J. 11, 411-6		
35	Benistant, C. et al. (1994) Biochim. Biophys. Acta 1223, 84-90		
36	Carlin, C. et al. (1994) J. Cell Physiol. 160, 427-34		
37	Barret, A.J. (1981) Methods Enzymol. 80, 737-54		
38	Waismann, P. et al. (1972) Acta Biol. Med. Germ. 28, 577-85		
39	Mintz, G.R. (1993) Biopharm 6, 34-38		
40	Hahm, B. et al. (1995) J. Virol. 69, 2534-9		