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
12309	Amastatin-HCl	511	Non-toxic reversible metallo-protease inhibitor. Inhibits many membrane-bound peptidases which are critical regulators of peptide hormones, e.g. aminopeptidase A and M, but notaminopeptidase B. Inhibits also leucine aminopeptidase.	Ethanol 0.5 % AcOH	1 - 100 µM	1,2,3,4
12548	(epsilon)-Aminocaproic acid	131.2	Highly active inhibitor of fibrinolysin and chymotrypsin.	H_2O	1 - 20 mM	5,6
13684	(alpha)1-Antichymotypsin from human plasma	68000	Glycoprotein that inhibits chymotrypsin-like proteases (aboveall human neutrophil cathepsin G) by forming stablecomplexes. Acute phase protein; concentration in plasmaincreases after events like inflammation or tissue damage	Aqueous buffers	Used at equimolar concentration	7,8,9,10
13685	Antipain-HCl	678.2	Reversible inhibitor of serine and cysteine proteases. Inhibits papain and trypsin more specificly than leupeptin. Plasmin isinhibited only slightly. Also involved in synthesis inhibition of RNA	H_2O Methanol DMSO	1 - 100 µM	11,12,13
13695	Antithrombin III from human plasma	ca. 60000	Glycoprotein that plays a major role in controlling serine proteases in the blood clotting cascade. Inactivates above all thrombin by forming an extremely stable complex, an effect which is enhanced by heparin. Inhibits also other proteases of the coagulation cascade like plasmin, kallikrein, factor IXa, Xa, XIa and XIIa.	H_2O	Used at equimolar concentrations	8,14 15,16
13694	(alpha)1-Antitrypsin from human plasma	ca.	Glycoprotein that is mainly involved in the	H_2O	Used at equimolar	8,9,17,18,19

	(alpha1-proteinase inhibitor)	53000	control of neutrophil elastase activity. Inhibits also most of other mammalian serine proteases but at a lower rate. Blocks the action of target enzymes by binding nearly irreversibly to their active site.	Aqueous buffers	concentrations	
12320	APMSF-HCl (4-Amidino-phenyl-methane-sulfonyl-fluoride)	252.7	Irreversible inhibitor of trypsin-like serine proteases. Stronger inhibitor than PMSF, but does not inhibit chymotrypsin and acetylcholine esterase.	H_2O (20 mg/ml). Aqueous solutions are stable when stored in aliquots at -20 °C	10-50 µM	20,21
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, forming 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	22,23,24,25
14525	Benzamidine-HCl	174.6	Potent inhibitor of thrombin and trypsin	H_2O	0.1 - 50 mM	26,27,28
14980	Bestatin-HCl	344.8	Metalloprotease inhibitor with multi-pharma-cological functions. Inhibits cell surface aminopeptidases (notably B) and leucine aminopeptidase. Inhibitor of leukotriene A4 hydrolase and of enkephalin degradation in cell preparations frombrain. Has anticarcinogenic and immunomodulating properties.	Methanol (5 mg/ml)	1 - 150 µM Mitogenic effects at nmolar concentrations	29,30,31,32, 33
15700	Calpainnhibitor I	367.2	Tripeptide aldehyde. Specific inhibitor of the Ca2+-dependent cysteine protease calpain I and of cathepsin B and L.	Ethanol, Methanol, DMF, DMSO (10 mg/ml)	1 - 50 µM	34,35,36
15710	Calpain Inhibitor II	385.2	Tripeptide aldehyde. Specific inhibitor of the Ca2+-dependent cysteine protease calpain II and of	Ethanol, Methanol, DMF, DMSO (10 mg/ml)	1 -50 µM	35,36,37,38

			cathepsin B and L.			
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	11,39,40,41
77205	DFP (Diisopropylfluoro-phosphate)	184.2	A potent irreversible inhibitor of serine proteases and acetylcholine esterase. Highly toxic!	Isopropanol; aqueous solutions are unstable	10 - 100 µM	28,42,43
21000	Ebelactone A	338.5	Non-toxic inhibitor for esterases, acylpeptide hydrolase, lipase and N-formylmethionine aminopeptidase	Methanol (200 mg/ml)	1 - 10 µM	43,44,45
21001	Ebelactone B	352.5	Non-toxic inhibitor for esterase, lipase and N-formyl-methionine aminopeptidase. Inhibits also carboxypeptidase Y-like exopeptidase.	Methanol (200 mg/ml)	1 - 10 µM	44,46,47
11280	EDTA-Na_2	372.3	Reversible inhibitor of metalloproteases	H_2O (pH 8)	1 - 10 mM	48,49
11290	EGTA	380.4	Inhibits metalloproteases.. Reveals high selectivity for Ca2+over Mg2+ ions.	NH_4OH, NaOH	1 - 10 mM	50
21100	E-64	357.4	Non-competitive irreversible inhibitor of papain and othercysteine proteases. Forms a thioether bond with the sulfhydrylgroup in the active center of the enzyme. Useful for activesite titration: one mole of E-64 inhibits one mole of protease.	H_2O, DMSO Mixtures of water and ethanol	1 - 10 µM	51,52,53
24730	Hirudin	7027	Inhibits thrombin by blocking substrate binding groups	H_2O Aqueous buffers	Used at equimolar concentrations	54,55
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	11,56,57,58
28301	(alpha)2-Macroglobulin from human plasma	725000	Glycoprotein composed of 4 identical subunits. Broad-range endo-proteinase inhibitor. Inhibits by forming a »trap« around the enzyme	H_2O Stable for several months when stored in aliquots at -20 °C	Used at equimolar concentrations	8,59

			allowing only small substrate molecules to enter and to be cleaved by the entrapped protease.			
31682	PEFABLOC® SC b) 4-(2-Aminoethyl)-benzenesulfonyl-fluoride hydrochloride	239.5	Water-soluble and relativly non-toxic irreversible inhibitor of thrombin 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 mMin cell culture: 0.1 - 0.25 mM	60,61,62
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	11,63,64,65
32395	PMSF Phenylmethyl-sulfonyl fluoride	174.2	Irreversibly inhibits serine proteases by sulfony-lation 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 - 200mM) Unstable in aqueous solution	0.1 - 1 mM	66,67,68
32753	Phosphoramidon	587.5	Specific inhibitor of thermolysin and neutral endopeptidase-24.11 (ANP Degradation Enzyme). Inhibits also the activityof Endothelin Converting Enzyme, collagenase and metallo-endoproteinases from many micro-organisms. Does not inhibit serine, cysteine and aspartic proteases	H_2O (20 mg/ml) DMSO Methanol	1 - 100 µM	11,69,70,71, 72
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 inactive protease inhibitor complexes.	1 mM HCl, DMSO H_2O (20 mg/ml). Aqueous solutions are unstable above pH 7	10 - 1000 µM	73,74,75,76
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	62,77

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)	78,79,80
37328 37329 37340	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)	81,82

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

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