

## Protease Inhibitors - Application Table

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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/Specificity of Inhibitor	Solubility Stability	Concentration Range a)	References
<a href="#">12309</a>	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 not aminopeptidase B. Inhibits also leucine aminopeptidase.	Ethanol 0.5 % AcOH	1 - 100 $\mu$ M	1,2,3,4
<a href="#">12548</a>	(epsilon)-Aminocaproic acid	131.2	Highly active inhibitor of fibrinolysin and chymotrypsin.	H <sub>2</sub> O	1 - 20 mM	5,6
<a href="#">13684</a>	(alpha)1-Antichymotrypsin from human plasma	68000	Glycoprotein that inhibits chymotrypsin-like proteases (above all human neutrophil cathepsin G) by forming stable complexes. Acute phase protein; concentration in plasma increases after events like inflammation or tissue damage	Aqueous buffers	Used at equimolar concentration	7,8,9,10
<a href="#">13685</a>	Antipain-HCl	678.2	Reversible inhibitor of serine and cysteine proteases. Inhibits papain and trypsin more specifically than leupeptin. Plasmin is inhibited only slightly. Also involved in synthesis inhibition of RNA	H <sub>2</sub> O Methanol DMSO	1 - 100 $\mu$ M	11,12,13
<a href="#">13695</a>	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 <sub>2</sub> O	Used at equimolar concentrations	8,14 15,16
<a href="#">13694</a>	(alpha)1-Antitrypsin from human plasma	ca.	Glycoprotein that is mainly involved in the	H <sub>2</sub> O	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	
<u>12320</u>	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 <sub>2</sub> O (20 mg/ml). Aqueous solutions are stable when stored in aliquots at -20 °C	10-50 µM	20,21
<u>13718</u>	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 <sub>2</sub> O 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
<u>14525</u>	Benzamidine-HCl	174.6	Potent inhibitor of thrombin and trypsin	H <sub>2</sub> O	0.1 - 50 mM	26,27,28
<u>14980</u>	Bestatin-HCl	344.8	Metalloprotease inhibitor with multi-pharmacological functions. Inhibits cell surface aminopeptidases (notably B) and leucine aminopeptidase. Inhibitor of leukotriene A <sub>4</sub> hydrolase and of enkephalin degradation in cell preparations from brain. Has anticarcinogenic and immunomodulating properties.	Methanol (5 mg/ml)	1 - 150 µM Mitogenic effects at nmolar concentrations	29,30,31,32, 33
<u>15700</u>	Calpain inhibitor I	367.2	Tripeptide aldehyde. Specific inhibitor of the Ca <sup>2+</sup> -dependent cysteine protease calpain I and of cathepsin B and L.	Ethanol, Methanol, DMF, DMSO (10 mg/ml)	1 - 50 µM	34,35,36
<u>15710</u>	Calpain Inhibitor II	385.2	Tripeptide aldehyde. Specific inhibitor of the Ca <sup>2+</sup> -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.			
<a href="#">17158</a>	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 $\mu$ M	11,39,40,41
<a href="#">77205</a>	DFP (Diisopropylfluoro-phosphate)	184.2	A potent irreversible inhibitor of serine proteases and acetylcholine esterase. Highly toxic!	Isopropanol; aqueous solutions are unstable	10 - 100 $\mu$ M	28,42,43
<a href="#">21000</a>	Ebelactone A	338.5	Non-toxic inhibitor for esterases, acylpeptide hydrolase, lipase and N-formylmethionine aminopeptidase	Methanol (200 mg/ml)	1 - 10 $\mu$ M	43,44,45
<a href="#">21001</a>	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 $\mu$ M	44,46,47
<a href="#">11280</a>	EDTA-Na <sub>2</sub>	372.3	Reversible inhibitor of metalloproteases	H <sub>2</sub> O (pH 8)	1 - 10 mM	48,49
<a href="#">11290</a>	EGTA	380.4	Inhibits metalloproteases.. Reveals high selectivity for Ca <sup>2+</sup> over Mg <sup>2+</sup> ions.	NH <sub>4</sub> OH, NaOH	1 - 10 mM	50
<a href="#">21100</a>	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 <sub>2</sub> O, DMSO Mixtures of water and ethanol	1 - 10 $\mu$ M	51,52,53
<a href="#">24730</a>	Hirudin	7027	Inhibits thrombin by blocking substrate binding groups	H <sub>2</sub> O Aqueous buffers	Used at equimolar concentrations	54,55
<a href="#">51867</a>	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 <sub>2</sub> O Stable for several months when stored in aliquots at -20 °C	1 - 100 $\mu$ M	11,56,57,58
<a href="#">28301</a>	( $\alpha$ ) <sub>2</sub> -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 <sub>2</sub> O 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.			
<a href="#">31682</a>	PEFABLOC® SC b) 4-(2-Aminoethyl)-benzenesulfonyl-fluoride hydrochloride	239.5	Water-soluble and relatively non-toxic irreversible inhibitor of thrombin and other serine proteases. Inhibits by acylation of the active site of the enzyme.	H <sub>2</sub> O (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
<a href="#">52682</a>	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
<a href="#">32395</a>	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 - 200mM) Unstable in aqueous solution	0.1 - 1 mM	66,67,68
<a href="#">32753</a>	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 <sub>2</sub> O (20 mg/ml) DMSO Methanol	1 - 100 µM	11,69,70,71, 72
<a href="#">17013</a>	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 <sub>2</sub> O (20 mg/ml). Aqueous solutions are unstable above pH 7	10 - 1000 µM	73,74,75,76
<a href="#">17016</a>	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 <sub>2</sub> O, 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 <sub>2</sub> O (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 investigation and has to be determined in each case empirically.

b) PEFABLOC is a registered trademark of Pentapharm/Basel

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