

Inhibitor	Target Protease/ Mechanism of Action Class	Effective Concentrations	Notes	Stock Solutions	Working Concentrations
Acetyl-Pepstatin M.W. 643.8	Aspartic/Reversible Inhibits HIV-1 and HIV-2 proteases.	50 - 200 nM	Soluble in 50% acetic acid. Ki for HIV-1 and HIV-2 proteases is 20 nM and 5 nM, respectively, at pH 4.7.	1 mg /1.553 ml of 50% acetic acid = 1 mM solution	dilute 1:1000 to obtain 1 μ M concentration
AEBSF, hydrochloride M.W. 239.5	Serine/ Irreversible Inhibits trypsin, chymotrypsin, plasmin, thrombin , and kallikrein.	< 1 mM	Solutions in H ₂ O are stable for 1 - 2 months at 4°C, pH 7. Will slowly hydrolyze at pH > 8.	50 mg/2.09 ml H ₂ O = 100 mM solution	Use at 1 mM or less.
Amastatin <i>Streptomyces sp</i> M.W.474.6	Metallo/Reversible Inhibits alanyl- aminopeptidase.	1 - 10 μ M	Aqueous solutions are stable for 1 day. Stock solutions 1 mM in EtOH are stable for 1 month at -20°C.	1 mg/2.1 ml anhydrous EtOH or 5 mg/10.5 ml anhydrous EtOH = 1 mM	Dilute 1:100 for a 10 μ M working solution
Antipain, hydrochloride M.W. 641.2	Serine and Cysteine/Reversible Inhibits papain and trypsin. Plasmin is inhibited to a small extent. More specific than leupeptin for papain and trypsin.	1 - 100 μ M	Stable for several hours. Stock solutions of 10 mM in H ₂ O or buffer are stable for 1 week at 4°C, 1 month at -20°C. Also soluble in MeOH and DMSO	5 mg/8.27 ml H ₂ O = 10 mM	Dilute 1:100 for a 100 μ M working solution
Antithrombin III Human Plasma M.W. 65,000	Serine/ Reversible Inhibits thrombin, factor Xa ,trypsin, and other trypsin-like serine proteases	use at equimolar concentration	Aqueous stock solutions are stable at -20°C.	1 mg in 17.24 ml 150 mM NaCl, 100 mM sodium citrate, 10 mM Tris-HCl, pH 8.3 = 1 mM	use at concentration equimolar with protease

p-APMSF Hydrochloride MW 252.7	Serine/ Irreversible Inhibits trypsin-like serine proteases. No effect on acetylcholine esterase	10 - 100 μ M	Must be prepared fresh. Half- life of 6 min in pH 7.0 7.0 buffer systems. Stock solutions of 50 mM in H ₂ O are stable when aliquoted and stored at -20°C.	5 mg/346 μ l in H ₂ O = 50 mM	Dilute 1:500 for a 100 μ M solution.
Aprotinins Bovine Lung M.W. 6512	Serine/Reversible Inhibits serine proteases, including plasmin, kallikrein, trypsin, and chymotrypsin. Does not inhibit thrombin or factor Xa.	0.6 - 2 μ g/ml	Very stable. Inactive at pH > 12.8. Very soluble in water 10 mg/ml. Aliquots are stable at - 20°C.	10 mg/ml in PBS	Dilute 1:5000 for a 2 μ g/ml final concentration
Arphamenine A <i>Chromoboderium violetum</i> M.W. 387.4	Metallo A highly specific inhibitor of aminopeptidase B.	100 - 500 nM	Soluble in water. Stock solutions are stable for up to 1 month at -20°C	1 mg/2.58 ml H ₂ O = 1 mM solution	Dilute 1:1000 to obtain 1 μ M concentration.
Bestatin M.W. 308.4	Metallo/ Reversible Inhibits alanyl- aminopeptidase	1 - 10 μ M	Stable for 1 day. Stock solutions of 1 mM MeOH are stable for at least 1 month at - 20°C.	2 mg/6.48 ml MeOH = 1 mM	Dilute 1:100 for 10 μ M working solution.
Calpeptin M.W. 362.5	Cysteine Cell- permeable inhibitor of calpain I and II and papain.	0.3 - 1.0 μ M	A cell-permeable calpain inhibitor. Soluble in DMSO and DMF. Dilute in aqueous medium just before use.	5 mg/13.8 ml DMSO = 1 mM solution	Dilute 1:1000 to obtain 1 μ M concentration.
Cathepsin Inhibitor I M.W. 475.5	Cysteine/ Irreversible Inhibits cathepsin B, cathepsin L, cathepsin S, and papain	10 -100 μ M	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 25 hours at 30°C and about 2 weeks at -20°C.	1 mg/210 μ l DMSO or EtOH = 10 mM solution	Dilute 1:50 to obtain 200 μ M concentration
Cathepsin Inhibitor II M.W. 489.5	Cysteine/Irreversible Inhibits cathepsin B, cathepsin L, cathepsin S and papain	10 -100 μ M	Protect from light. Half- life in a buffered solution (pH 5 - 9) is 27 hours at 30°C and about 2 weeks at 0°C	1 mg/204 μ l DMSO or EtOH = 10 mM solution concentration.	Dilute 1:50 to obtain 200 μ M

Cathepsin Inhibitor III M.W. 505.5	Cysteine/Irreversible Inhibits cathepsin B, cathepsin L, cathepsin S, and papain	10 – 100 μ M	Protect from light Half-life in a buffered solution (pH 5 - 9) is 37 hours at 30°C and about 2 weeks at 0°C.	1 mg/198 μ l DMSO or EtOH = 10 mM solution concentration.	Dilute 1:50 to obtain 200 μ M
Cathepsin/Subtilisin Inhibitor M.W.518.0	Cysteine and Serine/Irreversible Inhibits cathepsin L, subtilisin, and thermitase	10-100 μ M	Protect from light. Half- life in a buffered solution (pH 5 - 9) is 2.5 hours at 30°C and about 40 hours at 0°C.	1 mg/ 193 μ l in DMSO or EtOH = 10 mM solution	Dilute 1:100 to obtain 100 μ M concentration
Chymostatin	Cysteine and Serine/Reversible Inhibits chymotrypsin-like serine proteases including, and α -, β -, γ -, and δ -chymotrypsin		Stable for several hours. Soluble in DMSO and glacial acetic acid. Solutions are stable for months at -20°C	5 mg/825 μ l in DMSO = 10 mM solution	Dilute 1:100 for a 100 μ M working solution.
Cystatin, Egg White M.W. 12,700	Cysteine/ Reversible Inhibits cysteine proteases, including dipeptidyl peptidase I and III, papain. ficin. and cathepsin B.	Use at equimolar concentrations.	Very stable. Stable to heat. Freeze in the presence of 20% glycerol or buffered to pH 7.5	At least 1 mg/ml in 20 mM Tris, pH 7.5	Use at equimolar concentration.
3,4-Dichloroisocoumarin M.W.215.0	Serine/Irreversible Inhibits a wide range of serine proteases. Not active towards beta-lactamases.	5 – 100 μ M	Half-life of 20 minutes at pH 7.5. Stock solutions of 10 mM in DMF or DMSO are stable for months at -20°C.	10 mg/4.65 ml in DMSO = 10 mM solution	Dilute 1:100 for 100 μ M working solution
DFP M.W.184.2	Serine/Irreversible Inhibits serine proteases. Inactivates acetylcholinesterase	100 μ M	Very toxic: special handling required. Half-life of 1 hour at pH 7.5. Stock solutions 0.1 - 0.5 M in dry propanol stable for several months at -70°C.	1 g/10.86 ml in dry propanol = 0.5M	Dilute 1 :5000 for 100 μ M working solution

Dipeptidylpeptidase IV Inhibitor I M.W. 455.5	Serine/Proline Specific Inhibits dipeptidylpeptidase IV.	10-100 μ M	DMSO stock solutions are stable for up to 6 months at 20°C. Use only high quality, moisture free, DMSO. Aqueous solutions are stable for 1 day.	1 mg/219.5 μ l DMSO = 10 mM solution	Dilute 1:100 to obtain a 100 μ M working solution.
Dipeptidylpeptidase IV Inhibitor II M.W. 355.8	Serine/Proline Specific/Irreversible Acylating inhibitor of dipeptidylpeptidase II and IV	1 - 10 μ M	Protect from light. Half-life in a buffered solution (pH 7.6) is 24 hours at 30°C.	1 mg/2.81 ml DMSO or EtOH = 1 mM solution.	Dilute 1:100 to obtain 10 μ M concentration
Diprotin A M.W. 341.5	Metallo/Reversible	10-50 μ M	Stable for 1 day at working concentrations. Inhibits dipeptidylpeptidase IV. Stock solutions can be prepared 1 mM in water, MeOH. or EtOH. Stability has not been determined.	2 mg /5.86 ml = 1 mM solution	Dilute 1:20 for a 50 μ M working solution
EST M.W. 342.4	Cysteine/Irreversible Cell-permeable inhibitor of lysosomal cysteine proteases.	20 - 50 μ g/ml	A membrane-permeable calpain inhibitor. Stock solutions are stable for up to 6 months at -20°C.	1 mg/ml in EtOH	Use 20 to 40 μ l per ml of medium
E-64 Protease Inhibitor M.W. 357.4	Cysteine/Irreversible Inhibits cysteine proteases. Does not affect cysteine residues in other enzymes or react with low molecular weight thiols such as beta- mercaptoethanol, very specific, active site titrant.	1 - 10 μ M	Stable for days at neutral pH. Stock solutions of 1 mM in aqueous solutions are stable for months at -20°C.	5 mg/13.99 ml = 1 mM solution	Dilute 1:100 for a 10 μ M working solution.

Ebelactone B <i>Streptomyces sp.</i> M.W. 352.5	Serine A specific inhibitor of carboxypeptidase-Y-like exopeptidase	0.1 - 2 mg/ml	Soluble in MeOH (200 mg/ml), EtOH and chloroform. Solutions are stable for 1 week at 3°C and 1 month at -20°C.	1 mg/2.84 ml = 1 mM solution	Use 2.84 or 5.08 µl/ml for 1 or 2 ug, respectively.
Ecotin, <i>E. coli</i> M.W. 32,200	Serine A broad range serine protease inhibitor. Inhibits chymotrypsin, elastase, Factors Xa and XIIa, and trypsin	0.5 to 1.5 nM	Avoid freeze/thaw cycles. Store the stock solution at -70°C.	Provided as a solution in 1 mM HCl	check for lot specific volume information and dilute accordingly
EDTA, Disodium salt, M.W. 372.2	May interfere with other metal-dependent biological processes	1-10 mM	stable in aqueous solution. Stock solutions of 0.5M in H ₂ O at pH 8.5 are stable for several months at RT.	1.9 g/10 ml = 0.5 M solution	Dilute 1:50 for a 10 mM working solution
EDTA, Tetrasodium salt, M.W. 380.2	Metallo/Reversible chelator May interfere with other metal-dependent biological processes	1-10 mM	stable in aqueous solution. Stock solutions of 0.5M in H ₂ O at pH 8.5 are stable for several months at RT.	1.9 g/10 ml = 0.5 M solution	Dilute 1:50 for a 10 mM working solution
Elastatinal M.W. 512.6	Serine/Reversible Inhibits elastase and elastase-like serine proteases	10-100 µM	Stable for several hours. Stock solutions of 10 mM in H ₂ O are stable for 1 week at 4°C and for several months at -20°C.	5 mg/975 µl = 10 µM solution	Dilute 1:100 for a 100 µM working solution.
leuhistin M.W. 241.3	Metallo/Reversible Competitive inhibitor of aminopeptidase M	1 - 2 µg/ml	Soluble in water, EtOH, and DMSO. Store stock solutions at -20°C.	1 mg/ml of H ₂ O	Use 1 to 2 µl/ml.

leupeptin, Hemisulfate MW 475.6	Serine and Cysteine/Reversible Inhibits trypsin-like proteases and some cysteine protease; including endoproteinase Lys-C, kallikrein, papain, thrombin, cathepsin B, and trypsin.	10 – 100 μ M	Stable for several hours. Stock solutions of 10 mM on H ₂ O are stable for 1 week at 4°C and 1 month at -20°C.	5 mg/1.054 ml = 10 mM solution	Dilute 1:100 for a 100 μ M working solution
α_2 - Macroglobulin, Human Plasma M.W. 725,000	Broad Range/ Irreversible Inhibits bromelain, chymotrypsin, elastase, endoproteinase, Glu-C, ficin, papain, plasmin, subtilisin, thermolysin, thrombin, and trypsin. Forms "trap" around most proteases.	Use at equimolar concentrations.	Aqueous stock solutions are stable for 6 months at -20°C at pH 6 - 7.5. Sensitive to acidic pH; denatured below pH 4.0. Ammonia, methylamine, and hydroxylamine (above pH 7. 0) cause irreversible conversion to the closed form. Do not use in the presence of DTT.	1 mg/13.8 μ l = 100 μ M solution	Use at equimolar concentrations.
NCO-700 M.W. 1141.3	Cysteine Inhibits calpain, cathepsin B, cathepsin L, and papain	0.5 - 100 mM	Prepare a solution on the day of use. In aqueous solution, this material is stable for up to 6 hours at room temperature. Stable for longer periods in EtOH.	1 mg/87.6 ml PBS. H ₂ O or EtOH = 10 mM solution	Dilute 1:100 to obtain a 100 μ M working solution.
Pepstatin M.W. 685.9	Aspartic/Reversible Inhibits cathepsin D, cathepsin G, pepsin, renin, and many microbial aspartic proteases. Weak binding to proteases.	1 μ M	Soluble in MeOH to 1 mg/ml. Soluble to 1 m/ml in EtOH if allowed to sit overnight and to 333 mg/ml in 6N acetic acid. Stable for 1 week at 4°C	5 mg/7.29 ml = 1 mM	Dilute 1:1000 for a 1 μ M working solution

Phosphoramidon , disodium salt M.W. 587.5	Metallo/Reversible Inhibitor of some metalloendopeptidases. A highly specific inhibitor of thermolysin	25 mg/ml (8.5 mM)	Stock solutions of 1 mg/ml in H ₂ O are stable for at least 1 month at -20°C.	1 mg/1.015 ml = 1.7 mM solution	Dilute 1:200 for a 8.5 μM working solution.
PMSF M.W. 174.2	Serine/Irreversible Inhibits carboxypeptidase Y, chymotrypsin, factor Xa, papain, plasmin, proteinase K, subtilisin, thrombin, and trypsin.	0.1 - 1 mM	Toxic: Must be prepared fresh and added at several steps during sample preparation. Half-life of 1 hour at pH 7.5. Stock solutions of 200 mM in anhydrous solvents (MeOH, EtOH) are stable for at least 9 months at 4°C.	100 mg/2.87 ml = 200 mM solution	Dilute 1:200 for a 1 μM working concentration.
Subtilisin Inhibitor I M.W. 379.4	Serine Inhibits subtilisin and thermolysin.	10- 100 μM	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 4 hours at 30°C, 60 hours at 0°C.	1 mg/264 μl DMSO or EtOH = 10 mM solution.	Dilute 1:100 to obtain 100 μM concentration.
Subtilisin Inhibitor II M.W. 475.5	Serine Inhibits subtilisin and thermolysin.	10- 100 μM	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 4 hours at 30°C, 60 hours at 0°C.	1 mg/210.3 μl DMSO or EtOH = 10 mM solution.	Dilute 1:100 to obtain 100 μM concentration.
Subtilisin Inhibitor III M.W. 505.5	Serine Inhibits subtilisin and thermolysin.	10- 100 μM	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 8 hours at 30°C, 120 hours at 0°C.	1 mg/198 μl DMSO or EtOH = 10 mM solution.	Dilute 1:100 to obtain 100 μM concentration.
Subtilisin Inhibitor V M.W. 552.6	Serine/Cysteine/Irreversible Inhibits subtilisin and elastase.	10- 100 μM	Protect from light. Half-life in a buffered solution (pH 5 - 9) is 4 hours at 30°C, 60 hours at 0°C.	1 mg/181 μl DMSO or EtOH = 10 mM solution.	Dilute 1:100 to obtain 100 μM concentration.

TLCK Hydrochloride M.W. 369.3	Serine/Irreversible Inhibits trypsin-like serine proteases including bromelain, endoproteinase Arg-C, endoproteinase Lys-C, ficin, papain, plasmin, thrombin, and trypsin.	10- 100 μ M	Very unstable above pH 7.5. Stock solutions of 10 mM in aqueous solutions (1 mM HCl, pH 3.0) or MeOH should be prepared fresh as needed.	5 mg/1.354 ml = 10 mM solution	Dilute 1:100 for a 100 μ M working solution
TPCK M.W. 351.5	Serine/Irreversible Inhibits chymotrypsin- like serine proteases including bromelain, chymotrypsin, ficin and papain.	10- 100 μ M	Stable for several hours. Stock solutions of 10 mM in MeOH are stable for several months at 4°C.	5 mg/1.42 ml = 10 mM solution	Dilute 1:100 for a 100 μ M working solution
Trypsin Inhibitor Soybean M.W. 20,000	Serine/Reversible Inhibits factor Xa and trypsin	Use at equimolar concentrations.	Dissociated at low pH. Stock solutions in dilute buffers are stable at -20°C.	20 mg/ml = 1 mM solution	Use at equimolar concentrations