

Protease Inhibitors: Summary and Applications

| Protease Inhibitor | Ord. No. | Solubility | Conc. of Stock Sol. | Stability of Stock Sol. ^(1,2,3) | Working Conc. | Target Molecules / Application |
|---------------------------------|----------|---------------------------------------|-----------------------------------|---|----------------------------------|--|
| AEBSF hydrochloride | 2931 | H ₂ O | 5 - 50 mg/ml (20 - 200 mM) | at -20 °C max. 6 months | 0.1 - 5 mM | Serine proteases. Irreversible inhibition of Trypsin, Chymotrypsin, Plasmin, Kallikrein, Thrombin. Low toxicity, hence good alternative for PMSF. Alkali-labile, don't go to pH values over 7. |
| Amastatin hydrochloride | 2932 | H ₂ O, Methanol | 1 mM (511 µg/ml) | at -20 °C max. 1 month | 1 - 10 µM | Amino-peptidases (AP). Slowly, but strongly, binding inhibitor of cytosolic aminopeptidase, microsomal aminopeptidase M and bacterial Leucin-aminopeptidase. Lower inhibition of aminopeptidase A. |
| Aminobenzamidin dihydrochloride | CN71 | H ₂ O, Ethanol | 100 - 200 mg/ml (0.5 - 1 M) | at -20 °C few days | 0.5 - 2 mM | Serine/Cysteine proteases, Trypsin-like proteases. Competitive inhibition of Trypsin, Trypsin-like enzymes, Thrombin, Plasmin. Oxidation sensitive! Prepare stock solutions freshly and store only for few days. |
| Antipain dihydrochloride | 2933 | H ₂ O, DMSO, Ethanol | 50 mg/ml (75 mM) | at -20 °C max. 1 month | 10 - 50 µg/ml (15 - 75 µM) | Serine/Cystein proteases, Trypsin-like proteases. Reversible inhibition of Papain, Trypsin, Cathepsin A, B, D, Plasmin, Chymotrypsin, Pepsin, Calpain I. Reaction profile similar to that of Leupeptin. |
| Aprotinin | A162 | H ₂ O | 10 mg/ml (1.54 mM) | at +4 °C ca. 1 month, at -20 °C several years | 1 - 10 µg/ml | Serine proteases, Esterases. Competitive, reversible inhibition of Trypsin, Chymotrypsin, Plasmin, Kallikrein. pH-optimum: 7-8. Activity: ≥3.0 PEU/mg (Ph. Eur. Units), 1 PEU equals 1 TIU (Trypsin inhibitory units), equals ca. 13000 KIU (Kallikrein inhibitory units). |
| Bacitracin | 5655 | H ₂ O, Ethanol | 100 - 1000 mg/ml (68 - 680 mM) | at -20 °C several months | 0.1 - 1 mg/ml (0.07 - 0.7 mM) | Inhibitor of special proteases like Glutathion-Insulin-Transhydrogenase, some endopeptidases. Stable at pH 4-5, unstable at pH >5 at room temperature. |
| Benzamidin hydrochloride | CN38 | H ₂ O, Ethanol | 100 - 150 mg/ml (0.65 - 1 M) | at -20 °C few days | 1 - 5 M (150 - 750 µg/ml) | Trypsin, Trypsin-like enzymes, Serine proteases. Strong, competitive, reversible inhibition of Trypsin, Thrombin, Plasmin. Oxidation sensitive! Prepare stock solutions freshly and store only for few days. |
| Bestatin hydrochloride | 2937 | Methanol | 1 - 15 mM (0.35 - 5 mg/ml) | at -20 °C max. 1 month | 10 - 100 µM | Amino-peptidases. Competitive and specific inhibition of Aminopeptidase B, Leucin-Amino-peptidases, Triamino-peptidases. No inhibition of Aminopeptidase A, Trypsin, Chymotrypsin, Elastase, Papain, Pepsin, Thermolysin. Not bactericidal, not fungicidal, low toxicity. |
| Calpain Inhibitor I | 2934 | DMSO, Methanol | 10 mg/ml (25 mM) | at -20 °C few days | 0.1 - 10 µM | Calpain (Calcium dependent Cystein proteases). Strong, competitive inhibitor of Calpain I and Calpain II (lower inhibition), Papain, Cathepsin B and L. No inhibition of Trypsin. |
| E-64 | 2935 | H ₂ O, DMSO, Ethanol | 1 mM (360 µg/ml) | at -20 °C max. 3 months | 1 - 20 µM | Cystein proteases. Irreversible, strong and highly selective inhibition. No inhibition of Serine proteases (exception: Trypsin). Low toxicity and high cell permeability, hence good alternative for Leupeptin or Antipain. |

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| EDTA | 8043 | H ₂ O | 0.5 M (186 mg/ml) | autoclaved at RT ca. 1 year | 1 - 10 mM | Metallo-proteases. Inhibition through chelating of bivalent metal ions (e.g. calcium Ca ²⁺ , magnesium Mg ²⁺). Reversible inhibition of all enzymes needing bivalent ions for function (metallo-proteases, DNAses etc.). |
| EGTA | 3054 | H ₂ O | 0.5 M (190 mg/ml) | autoclaved at RT ca. 1 year | 1 - 10 mM | Unspecific protease inhibitor. Reversible inhibition through chelating of bivalent metal ions (e.g. calcium Ca ²⁺ , magnesium Mg ²⁺). Reversible inhibition of all enzymes needing bivalent ions for function (metallo-proteases, DNAses etc.). pK _a calcium (pH 7): ca. 6.9 |
| Genistein | 0716 | DMSO | 100 mg/ml (370 mM) | at +4 °C ca. 6 months, at -20 °C ca. 1 year | 1 - 100 µg/ml | Tyrosin-specific protein kinases. Competitive inhibition of ATP-binding. Insulin receptor-Tyrosinkinase and Serine-/Threonin-specific proteases are not inhibited. Inhibition of topoisomerases I and II. |
| Leupeptin hemisulphate | CN33 | H ₂ O, Ethanol | 1 - 10 mM (0.5 - 5 mg/ml) | at +4°C for max 7 days, at -20 °C for 6 months | 1 - 100 µM | Serine- and Cystein proteases, Trypsin-like proteases. Competitive inhibition of Calpain, Cathepsin B, Kallikrein, Papain, Plasmin and Trypsin. Inhibition of Thrombin is discussed. Low or no inhibition of Pepsin, Cathepsin A and D and Chymotrypsin. Note: Working solutions are stable for few hours only. Leupeptin may alter measured values during protein quantitation. |
| Pefabloc ^{®(4)} | A154 | H ₂ O | 5-50 mg/ml (20 - 200 mM) | at -20 °C max. 6 months | 0.1 - 5 mM | Serine proteases. Irreversible broad-band inhibition of Trypsin, Chymotrypsin, Plasmin, Kallikrein, Thrombin and others. Low toxicity, hence good alternative for PMSF. Alkali-labile, don't go to pH values over 7. |
| Pepstatin A | 2936 | DMSO, Methanol ⁽⁵⁾ | 1 mM (685 µg/ml) | at -20 °C max. 1 month, at +4 °C max. 1 week | 1 - 100 µM | Acidic proteases, Aspartate proteases. Strong, highly selective inhibition of Pepsin, Renin, Cathepsin D, Chymosin, Protease B, retroviral protease. Doesn't inhibit Thiolproteases, neutral Proteases and Serine proteases. |
| Phosphoramidon | - | DMSO, Methanol, (H ₂ O, Ethanol) | up to 17 mM (10 mg/ml) | at -20 °C for max. 1 year | 1 - 10 µM | Thermolysin and other bacterial Metallo-endopeptidases. Mammalian Enkephalinase and a few mammalian Metallo endopeptidases. Weak inhibition of Collagenase. No inhibition of Trypsin, Chymotrypsin, Papain, or Pepsin. |
| PMSF | 6367 | Ethanol, Methanol | 100 mM (17.4 mg/ml) ⁽⁶⁾ | at +4 °C for max. 6 months, at -20 °C for max. 2 years | 0.1 - 1 mM | Serine- and Cystein proteases. Irreversible inhibition of Chymotrypsin, Trypsin, Thrombin and of Dystin-protease Papain. Reversible inhibition of Cystein proteases. Not stable in aqueous solutions. Always prepare freshly. Activity is reduced in high salt concentrations. |
| Trypsin Inhibitor | 5279 und 2949 | H ₂ O | 1 - 10 mg/ml | at -20 °C ca. 3 years | 1 - 100 µg/ml ⁽⁷⁾ | Trypsin and Trypsin-like proteases. Strong inhibition of Trypsin, weaker inhibition of Chymotrypsin. Low inhibition of Plasmin, Kallikrein, Thrombin. pH-optimum: 8.0. |

(1) We recommend storage in small aliquots. Avoid frequent freeze-and-thaw cycles by all means!

(2) While preserving whole activity. Longer storage is possible while taking a loss in activity.

(3) Storage at -80 °C prolongs shelf life. Distinct data are not known.

(4) Registered trademark of Pentapharm AG, Basel.

(5) Addition of 10-50 % acetic acid may be necessary in order to enable complete dissolving of Pepstatin A

(6) Stock solutions of higher concentration, e.g. 200 – 250 mM, should be warmed to 30 °C for 30 mins. in order to completely dissolve PMSF.

(7) In order to stop trypsination during cell culture, use a stock solution of 1 mg/ml and a working concentration of 0.5 mg/ml.

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