Protease Inhibitors: Summary and Applications

Depending on the binding of the inhibitor, you may find both reversible and irreversible inhibition.

In ***reversible*** enzyme inhibition the inhibitor doesn't bind closely to the enzyme and can be separated or displaced in succession. It lowers its activity and/or the speed constant of the reaction of the substrate to the product. It also establishes a balance between the enzyme-inhibitor complexes with or without substrate. Metabolic processes are typical examples of this, which, depending on the physiological state of the cells, either are enhanced or inhibited.

Competitive inhibitors are reagents which compete with substrate (A) for the binding site in the enzyme's active centre and increase the Michaelis Menten constant of the enzyme. They are either extremely similar to the substrate (but are not converted and can be displaced again by the substrate) or even represent a second substrate and thereby competitively inhibit the conversion of substrate A.

In ***non-competitive*** inhibition the inhibitor does not bind in the active centre but through binding, it changes the enzyme's tertiary structure to such an extent that a product can no longer be produced/set free.

Uncompetitive inhibition is a special case where the inhibitor can only interact with the enzyme-substrate complex and it changes both the MM-constant as well as the maximum reaction speed.

In ***partial-competitive*** inhibition, binding of the inhibitor lowers the affinity of the enzyme to the substrate without influencing the speed constants.

In ***irreversible*** inhibition, the inhibitor binds so closely that it can no longer be separated from the enzyme and thus the activity of the enzyme is permanently reduced or destroyed. Typical examples of this are protein-synthesis inhibitive antibiotics or "suicide substrates" which bind covalently to the active centre.

Technical Info

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Protease Inhibitor	Ord. No.	Solubility	Conc. of Stock Sol.	Stability of Stock Sol.[1.2.3]	Working Conc.	Target Molecules / Application
AEBSF hydro-chloride	2931	H_2O	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 hydro-chloride	2932	H_2O, Methanol	1 mM (511 µg/ml)	at -20 °C max. 1 month	1 - 10 µM	Aminopeptidases (AP). Slowly, but strongly, binding inhibitor of cytosolic aminopeptidase, microsomal aminopeptidase M and bacterial Leucin-aminopeptidase. Lower inhibition of aminopeptidase A.
Aminoben-zamidin dihydro-chloride	CN71	H_2O, 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 dihydro-chloride	2933	H_2O, 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, Chymo-trypsin, Pepsin, Calpain I. Reaction profile similar to that of Leupeptin.
Aprotinin	A162	H_2O	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 1950 (Kallikrein inhibitory units). 1 PEU equals ca. 1.5 TIU (Trypsin inhibitory units). 1 TIU equals ca. 1300 KIU.
Bacitracin	5655	H_2O, 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 endopepti-dases. Stable at pH 4-5, unstable at pH >5 at room temperature.
Benzamidin hydro-chloride	CN38	H_2O, 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 prote-ases. Strong, competitive, reversible inhibi-tion of Trypsin, Thrombin, Plasmin. Oxidation sensitive! Prepare stock solutions freshly and store only for few days.
Bestatin hydro-chloride	2937	Methanol	1 - 15 mM (0.35 - 5 mg/ml)	at -20 °C max. 1 month	10 - 100 µM	Aminopeptidases. Competitive and specific inhibition of Aminopeptidase B, Leucin-Aminopeptidases, Triaminopeptidases. 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 prote-ases). Strong, competitive inhibitor of Calpain I and Calpain II (lower inhibition), Papain, Cathepsin B and L. No inhibition of Trypsin.
E-64	2935	H_2O, 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.
EDTA	8043	H_2O	0.5 M (186 mg/ml)	autoclaved at RT ca. 1 year	1 - 10 mM	Metallo-proteases. Inhibition through chelat-ing 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.).

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Technical Info

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Protease Inhibitor	Ord. No.	Solubility	Conc. of Stock Sol.	Stability of Stock Sol.[1.2.3]	Working Conc.	Target Molecules / Application
EGTA	3054	H_2O	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 hemi-sulphate	CN33	H_2O, 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_2O	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.
Phorspho-ramidon	-	DMSO, Methanol, (H2O, Ethanol)	up to 17 mM (10 mg/ml)	at -20 °C for max. 1 year	1 - 10 µM	Thermolysin and other bacterial Metalloendopeptidases. 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_2O	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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