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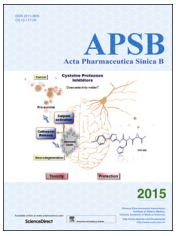

REVIEW

Cysteine proteases as therapeutic targets: does selectivity matter? A systematic review of calpain and cathepsin inhibitors

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Abstract Cysteine proteases continue to provide validated targets for treatment of human diseases. In neurodegenerative disorders, multiple cysteine proteases provide targets for enzyme inhibitors, notably caspases, calpains, and cathepsins. The reactive, active-site cysteine provides specificity for many inhibitor designs over other families of proteases, such as aspartate and serine; however, a) inhibitor strategies often use covalent enzyme modification, and b) obtaining selectivity within families of cysteine proteases and their isozymes is problematic. This review provides a general update on strategies for cysteine protease inhibitor design and a focus on cathepsin B and calpain 1 as drug targets for neurodegenerative disorders; the latter focus providing an interesting query for the contemporary assumptions that irreversible, covalent protein modification and low selectivity are anathema to therapeutic safety and efficacy.

Abbreviations: AD, Alzheimer's disease; Ala, alanine; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; APP/PS1, Aβ overexpressing mice APP (K670N/M671L) and PS1 (M146L) mutants; AppLon, London familial amyloid precursor protein mutation, APP (V717I); AppSwe, Swedish amyloid precursor protein mutation, APP (K670N/M671L); Arg, arginine; Aβ, amyloid β; Aβ1-42, amyloid β, 42 amino acid protein; BACE-1, β-amyloid cleaving enzyme; BBB, blood–brain barrier; CaMKII, Ca^{2+}/calmodulin-dependent protein kinases II; CANP, calcium-activated neutral protease; Cdk5/p35, activator of cyclin-dependent kinase 5; CNS, central nervous system; CREB, cyclic adenosine monophosphate response element binding protein; DTT, dithioerythritol; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated kinase 1/2; Gln, glutamine; Glu, glutamic acid; Gly, glutamine; GSH, glutathione; Hsp70.1, heat shock protein 70.1; Ile, isoleucine; isoAsp, isoaspartate; KO, knockout; Leu, leucine; Lys, lysine; MAP-2, microtubule-associated protein 2; Met, methionine; MMP-9, matrix metalloproteinase 9; NFT, neurofibrilliary tangles; Nle, norleucine; PD, Parkinson's disease; pGlu, pyroglutamate; Phe, phenylalanine; PK, pharmacokinetic; PKC, protein kinase C; Pro, proline; PTP1B, protein-tyrosine phosphatase 1B; pyroGluAβ, pyroglutamate-amyloid β; SP, senile plaques; TBI, traumatic brain injury; Thr, threonine; TNF, tumor necrosis factor; Tyr, tyrosine; Val, valine; WRX, Trp-Arg containing epoxysuccinate cysteine protease inhibitor; WT, wildtype

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1. Introduction

Proteases are enzymes that irreversibly hydrolyze a peptide bond in an amino acid sequence by nucleophilic attack and subsequent hydrolysis of a tetrahedral intermediate. Proteases are grouped according to the key catalytic group in the active site: serine (Ser), threonine (Thr), cysteine (Cys), aspartate (Asp), glutamate (Glu), or zinc in metalloproteases. Ser, Cys and Thr act directly as nucleophiles that attack an amide carbonyl C, whereas Asp, Glu and metalloproteases activate a water molecule that then acts as a nucleophile. The enzymes are also classified into exopeptidases and endopeptidases by the position of the peptide bond in a protein they cleave. Exopeptidases truncate one or several amino acids from either the N- or the C-terminus of a peptide, whereas endopeptidases cleave an internal peptide bond. The catalytic site of CA-clan papain-like cysteine proteases consists of Cys, histidine (His) and Asp residues and is highly conserved among members of the enzyme family[1]. This review will focus on approaches to inhibition of two families of protease enzymes, calpains and cathepsins, of interest in neurodegeneration and cancer therapy and the quixotic pursuit of selectivity.

2. Cysteine proteases

2.1. Cathepsins

Cathepsin inhibitors have been reviewed recently by Turk et al.[2] and earlier by Hernandez and Roush[3]. A review specific to cathepsin B inhibitors has also been published by Frlan and Gobec[4]. Cathepsins are a group of protease enzymes originally discovered in the cell lysosome, with several members ubiquitous in the human body. They are not catalytically conserved: cathepsins A, G are serine proteases; cathepsins D, E are aspartate proteases; and the remainder are lysosomal cysteine proteases, including the human isoforms B, C, F, H, K, L, O, S, V, X and W[2]. Cathepsins B, F, H and L occur throughout the CNS, while C, S, V and X are expressed in specific cell types within the CNS. The pH_{max} for optimum cathepsin activity is slightly acidic, corresponding to the environment found in the lysosome. Although they have been traditionally viewed as enzymes involved in terminal protein degradation, knockout (KO) mice have revealed major roles in cell regulation, *i.e.* of cell proliferation and adhesion, apoptosis, lipid metabolism and immune response[5,6].

The crystal structure of a number of cathepsins has been determined, among them cathepsin B[7]. Cathepsin B is unique among the cathepsins in that it has an occluding loop, a peptide sequence which when closed can hinder access to the primed side of the substrate pocket. Thus cathepsin B can function as an endo- or exopeptidase depending on pH[8]. The occluding loop has been targeted for the design of non-electrophilic cathepsin B inhibitors[9]. The lysosomal cathepsin K occurs in osteoclasts and is a major factor in bone resorption and a target for treating osteoporosis. Several inhibitors are in development, with one, odanacatib, having reached phase III clinical trials[10]. Table 1 shows residue preference of cathepsin B in peptide substrates in each position[11,12]. Fig. 1 shows primed and unprimed amino acid residues in protease substrates and inhibitors.

2.2. Calpains

Calpains are neutral, cytosolic cysteine proteases with 15 isoforms reported, of which 11 have been identified in humans[13,14]. The first reports characterizing members of the enzyme family emerged in 1964, naming the enzyme calcium-activated neutral protease (CANP)[15–17]. .

Table 1 Cathepsin B: residue preference in peptide substrates in each position[11,12].

Unprimed[a]	Preference	Primed[a]	Preference
P1	Gly > Ala, Met, Gln	P1′	Phe > Gly
P2	Val > Phe, Tyr	P2′	Val, Ile > Gly, Thr
P3	Gly > Lys, Phe	P3′	Gly

[a]See Fig. 1 for depiction of primed and unprimed sites. Ala, alanine; Gln, glutamine; Gly, glutamine; Ile, isoleucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Tyr, tyrosine; Val, valine.

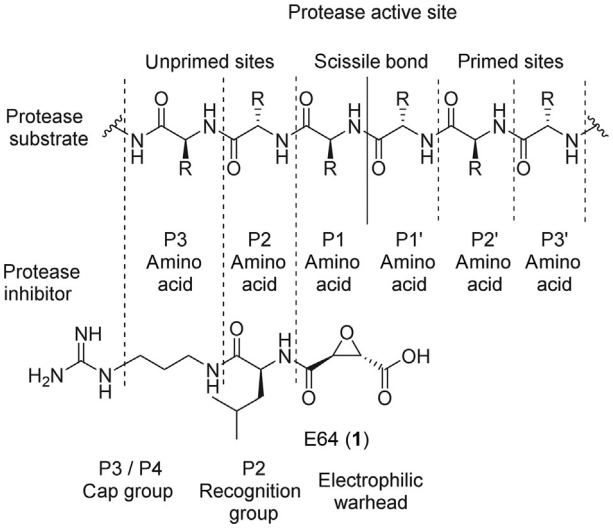

Figure 1 Nomenclature of primed and unprimed amino acid residues in protease substrates and inhibitors.

The enzymes consist of a catalytic subunit (82 or 80 kDa for calpains 1 and 2, respectively) and a Ca^{2+} binding subunit (28 kDa)[18]. The enzymes are unique among cysteine proteases in that the cytosolic proenzyme is activated by Ca^{2+} ions, inducing a conformational change. This change drives spatial proximity of the catalytic triad to the regulatory subunit, domain I, and subsequent autocatalytic cleavage[19]. The two most widely researched isoforms of calpain are ubiquitous, these are termed calpains 1 and 2, or μ- and m-calpain, requiring 5–30 μmol/L or millimolar Ca^{2+} for activation, respectively[18]. The presence of phospholipids or phosphoinositides can decrease the Ca^{2+} concentration required for the activation of calpain 2[20,21]. The expression of calpains 1 and 2 can vary greatly depending on cell types and conditions. Other members of the calpain family are tissue-specific. The active sites and substrates of calpains 1 and 2 are very similar, and specific inhibitors have not been developed.

Calpains and cathepsins regulate the activity of other biomolecules through limited proteolytic cleavage at specific sites. The products of these enzyme catalyzed reactions are often functional proteins and therefore these cysteine proteases constitute important regulatory enzymes. Protease activation is a necessary cog in the