Proteases And Protease Inhibitors: A Comprehensive Review of Biological Roles and Therapeutic Advances

Mukhtiyar Ahmed Mir[1], Uzma Rahman[2], Ruksana Banoo[3], Ghulam Rasool[4], Mushtaque Kausar[5], Vishali Pandita[6]

[1,2,4,5,6]*Department Of Environmental Science, Alpine Institute of Management and Technology, Dehradun, Uttarakhand, India-248007*

[3]*Department Of Zoology, Doon (PG) College Agriculture Science and Technology, Dehradun, Uttarakhand, India-248007*

***Abstract*—Protease inhibitors (PIs) are widely distributed across living systems and occur in a broad range of organisms, including bacteria, plants, and animals. In plants, these small proteins are predominantly localized in storage tissues such as roots and seeds, although they are also present in aerial parts. Their primary biological role is defence, particularly against phytophagous insects and pathogenic microorganisms.**

Mutations affecting protease inhibitors are associated with several hereditary and acquired disorders, including emphysema, epilepsy, neurotic edema, AIDS, and Netherton syndrome. Owing to their strong insecticidal and pest-deterrent properties, protease inhibitors represent one of the most important classes of bioactive compounds and have been extensively exploited as bioinsecticides, notably through the development of transgenic plants.

Proteases play critical roles in cellular signalling pathways and are implicated in the pathogenesis of numerous diseases, ranging from cardiovascular disorders to cancer, thereby serving as valuable diagnostic and therapeutic targets. They are also involved in host defence mechanisms against viruses, parasites, and other pathogens. Inhibitors targeting well-characterized proteases, such as angiotensin-converting enzyme (ACE) and HIV protease, have achieved substantial clinical success. This highlights the broad therapeutic potential of protease inhibitors, which is briefly reviewed here.

***Index Terms*—Protease Inhibitors, Therapeutics, Target Proteases, Review**

I. INTRODUCTION

Proteolysis under normal physiological conditions is tightly regulated through a dynamic balance between protease synthesis and their degradation and/or inactivation. One of the principal mechanisms of regulation involves the interaction of proteases with endogenous inhibitors, leading to enzyme inactivation. Disruption of either protease regulation or inhibitor function can result in the onset or progression of disease (Mathien et al. 2021; Nixon and Wood 2006). Consequently, the modulation of aberrant proteolysis has emerged as an important therapeutic strategy, which may be achieved either by supplementing deficient endogenous inhibitors or by administering specific inhibitors targeting overexpressed proteases.

Proteases are ubiquitous in nature and are abundantly distributed across plants, animals, and microorganisms. These enzymes catalyze proteolysis by cleaving peptide bonds that link amino acids within polypeptide chains, thereby playing a central role in protein metabolism. Proteases constitute one of the largest enzyme classes and account for more than 70% of commercially utilized enzymes, owing to their broad substrate specificity and diverse catalytic properties (Bafna et al. 2021; Srilakshmi et al. 2014).

Protease inhibitors function by binding to the active or receptive sites of target proteases, resulting in the formation of stable protease–inhibitor complexes and subsequent suppression of enzymatic activity (Pathak et al. 2020; Syeda Rakashanda et al. 2012). These inhibitors have attracted considerable attention due to

their extensive applications in medicine and biotechnology. Numerous plant-derived compounds have been identified as potent protease inhibitors, and species belonging to the families Gramineae (Poaceae), Malvaceae, Leguminosae (Fabaceae), Rutaceae, and Moringaceae have been systematically evaluated for such activity.

Among these, *Moringa oleifera* (family Moringaceae) has been reported to exhibit notably high protease inhibitory activity following ammonium sulphate fractionation. Owing to the absence of earlier reports on the isolation of protease inhibitors from tropical populations of *M. oleifera*—despite its wide range of industrial and medicinal applications—this species was selected as the source material for the present investigation. Extracts obtained from seeds and leaves showed the highest inhibitory activity against trypsin compared to other tested components of *M. oleifera*. Furthermore, phosphate buffer was identified as the most effective extraction medium for maximizing protease inhibitor yield.

The isolated protease inhibitors were effective against serine proteases, including chymotrypsin, thrombin, and elastase, as well as cysteine proteases such as cathepsin B and papain, all of which are of considerable pharmaceutical importance. Complete inhibition was observed against commercially available proteases from *Bacillus licheniformis* and *Aspergillus oryzae*, whereas inhibition of esperase, subtilisin, pronase E, and proteinase K was negligible. Additionally, the protease inhibitor demonstrated the ability to control proteolysis in the economically important shrimp *Penaeus monodon* during storage, suggesting its potential application as a natural seafood preservative.

The significance of proteases and their inhibitors in the pathophysiology of various diseases have been well established. The most critical roles are outlined in the following sections.

II. VIRAL INFECTION AND PROTEASES

Protease inhibitors (PIs) constitute an important class of antiviral chemotherapeutic agents, and several have been approved for the treatment of viral infections. Although their primary clinical application has been in the management of herpesvirus and human immunodeficiency virus (HIV) infections, protease-targeting antivirals have also demonstrated efficacy against other viral pathogens, including human respiratory syncytial virus and influenza A virus (Green et al. 2004). The clinical success of antiviral agents that target viral proteases has been particularly well documented in HIV-infected individuals.

In HIV, the viral protease plays a central role in viral maturation by cleaving large gag–pol polyprotein precursors into smaller functional proteins. These processed proteins subsequently assemble with the viral genome to form mature, infectious virions. Protease inhibitors block this enzymatic activity, thereby preventing viral replication and the production of infectious virus particles. Consequently, inhibition of HIV protease has become a cornerstone of antiretroviral therapy.

Beyond HIV, viral proteases have also been implicated in the replication of other medically significant viruses. For example, the viral protease N83 is critical for the replication of dengue virus, the causative agent of dengue fever and dengue haemorrhagic fever, which affect large populations in tropical regions. The Bowman–Birk inhibitor (BBI) derived from mung bean has been shown to inhibit this protease, highlighting its potential as a therapeutic lead compound (Murthy et al. 2000).

Recent studies indicate that protease inhibitors are integral components of modern antiretroviral combination therapies, significantly improving clinical outcomes and quality of life in HIV-infected patients (Bobbarala et al. 2009). In addition to synthetic inhibitors, plant-derived protease inhibitors have also been explored for their antimicrobial potential. For instance, protease inhibitors isolated from the leaves of *Coccinia grandis* exhibit strong inhibitory activity against *Klebsiella pneumoniae* and *Aspergillus flavus* (Satheesh and Murugan 2011).

Pharmacokinetic enhancement using ritonavir has further improved the clinical efficacy of protease inhibitor–based regimens. Ritonavir acts as a potent inhibitor of the cytochrome P450 enzyme CYP3A4, thereby increasing the bioavailability of co-administered protease inhibitors. Low-dose ritonavir (100–200 mg) achieves an optimal balance between enhanced efficacy and tolerability by increasing systemic exposure to protease inhibitors without significantly altering their adverse-effect profile. Clinical trials have demonstrated effective viral

suppression in both antiretroviral-naïve patients and those with prior treatment failure, without additional toxicity attributable to ritonavir boosting (Robert and Richard 2004).

In addition to CYP3A4 inhibition, ritonavir may influence cellular drug transport mechanisms. Efflux transporters such as multidrug resistance–associated proteins (MRP1 and MRP2), expressed in intestinal epithelial cells as well as hepatic and renal tissues, are thought to mediate the export of protease inhibitors from cells. Overexpression of these transporters in HIV patients may reduce intestinal absorption and enhance urinary and biliary excretion of protease inhibitors (Fromm 2000; Moyle and Back 2001). Similarly, P-glycoprotein and MRP transporters at the blood–brain barrier can restrict the penetration of protease inhibitors into the central nervous system (Olson et al. 2002). In vitro studies suggest that ritonavir may inhibit these efflux pathways, thereby increasing intracellular concentrations of co-administered protease inhibitors (Van der Sandt et al. 2001).

Intracellular concentrations of protease inhibitors within infected $CD4^+$ T cells are critical determinants of HIV replication. Expression levels of P-glycoprotein and MRP transporters in the cell membrane significantly influence intracellular drug accumulation, resulting in substantial variability in protease inhibitor efficacy. Several studies report that ritonavir inhibits these efflux transporters in $CD4^+$ cells, leading to increased intracellular drug levels (Meaden et al. 2002; Jones et al. 2001; Bossi et al. 2003).

Highly active antiretroviral therapy (HAART), which combines nucleoside reverse transcriptase inhibitors (NRTIs) with HIV protease inhibitors, remains the standard treatment for AIDS. HAART effectively suppresses viral replication and facilitates immune system reconstitution, despite moderate toxicity and associated side effects. HIV protease inhibitors specifically target the viral aspartyl protease, which cleaves the gag–pol polyprotein at nine defined sites to generate functional viral proteins. Notably, several of these cleavage sites involve phenylalanine- or tyrosine–proline bonds, a specificity not commonly observed in mammalian endopeptidases. This unique cleavage pattern is mimicked in the design of most HIV protease inhibitors, contributing to their selectivity and clinical utility (Deeks et al. 1997).

However, prolonged PI-containing HAART regimens have been associated with metabolic and systemic complications, including insulin resistance, hyperbilirubinemia, hyperlipidemia, lipodystrophy, osteopenia, and osteoporosis. While the incidence of AIDS-associated cancers such as Kaposi's sarcoma has declined since the introduction of PI-based HAART, changes in the prevalence and progression of non-Hodgkin's lymphomas have been reported (Powderly 2002; Sgadari et al. 2002). The underlying mechanisms, involving metabolic dysregulation, immune modulation, and tissue remodeling, remain incompletely understood.

III. PROTEASES AND HIV

Commonly prescribed HIV protease inhibitors—such as ritonavir, saquinavir, indinavir, and nelfinavir—have also been shown to exert non-antiretroviral effects. These include modulation of cellular enzymes and signalling pathways implicated in immune activation and inflammation. HIV protease inhibitors influence T-cell and endothelial cell function, alter antigen presentation, suppress inflammatory cytokine production (e.g., TNF-α, IL-6, and IL-8), and reduce adhesion molecule expression (Phenix et al. 2001; Chavan et al. 2001). Additionally, they may impair dendritic cell development and function, thereby affecting T-cell priming. Inhibitory effects against fungal aspartyl proteases have also been reported, suggesting broader antimicrobial potential (Gruber et al. 2001; Cassone et al. 2002).

Advances in the molecular biology of HIV have greatly enhanced understanding of viral enzymes essential for replication and maturation. While inhibitors of viral reverse transcriptase, such as nucleoside analogues (e.g., zidovudine), have proven effective, long-term use is limited by toxicity and the emergence of resistant viral strains. Viral proteases have therefore emerged as attractive alternative targets. These enzymes catalyse the cleavage of viral polyprotein precursors into mature structural and functional components, a process essential for viral infectivity. Protease inhibitors disrupt viral maturation and have been rationally designed as non-hydrolysable peptide analogues that mimic the transition state of substrate cleavage. If comparable selectivity and efficacy are achieved in vivo, protease inhibitors represent a powerful and durable class of antiviral

agents for the treatment of AIDS and other viral diseases (Jamjoom 1991).

IV. BINDING MECHANISM OF HIV-1 PROTEASE

The interaction between HIV-1 protease and a short peptide substrate has been investigated in detail using advanced computational approaches. Extended molecular dynamics simulations totaling 1.6 microseconds were performed with an accurate explicit-solvent force field, combined with bias-exchange metadynamics to enhance sampling across seven distinct reaction coordinates. This strategy enabled repeated observation of ligand approach, binding, and stabilization within the enzyme active site.

The resulting protease–ligand complex closely reproduces available crystallographic structures, demonstrating excellent structural agreement. Based on these simulations, a kinetic model was constructed to characterize the stability of intermediate states and the transition rates between them. Both the calculated binding free energy and the predicted association and dissociation rate constants showed strong concordance with experimental measurements.

Notably, the binding process was found to occur without a prerequisite opening of the protease flaps, indicating that the displacement of water molecules from the enzyme's active-site cavity represents a key kinetic barrier. Insights gained into this binding pathway provide valuable guidance for the rational design of more potent HIV-1 protease inhibitors. Ongoing efforts aim to simplify and generalize such binding free-energy calculations, making them broadly accessible to users of molecular dynamics simulation platforms (Pietrucci et al. 2009).

V. ROLE OF PROTEASES IN CANCER AND OTHER MALIGNANCIES

Proteases and their regulatory pathways play critical roles in cancer development, progression, and therapeutic response. Inhibition of the proteasome, a key proteolytic complex responsible for protein turnover, can profoundly influence multiple cellular pathways, including cell-cycle regulation and apoptosis. Bortezomib, a clinically approved proteasome inhibitor, has emerged as an effective therapeutic option for patients with relapsed or refractory multiple myeloma and has also improved outcomes in newly diagnosed cases. Evidence further suggests that proteasome inhibition may be beneficial in leukemia, as malignant cells appear to be more sensitive to proteasome blockade than normal hematopoietic cells (Cynthia et al. 2007). In addition, antifungal peptides derived from buckwheat have been shown to suppress the proliferation of hepatoma, leukemia, and breast cancer cells (Scott et al. 2008).

Epidemiological studies indicate that populations with high dietary intake of legumes exhibit lower incidences of skin, breast, colon, and prostate cancers (Noemí Eiró et al. 2013). This protective effect has been attributed, at least in part, to reduced protease activity in transformed cells (Kennedy 1998). Owing to their ability to inhibit proteolytic enzymes, protease inhibitors have long been considered promising agents for limiting tumor growth and metastasis. However, the role of serine protease inhibitors in cancer remains complex. Their presence within tumors has often been correlated with poor clinical prognosis, and accumulating evidence suggests that certain serine protease inhibitors may enhance tumor aggressiveness. These findings have opened new avenues for their potential application as prognostic or diagnostic cancer biomarkers (Billings Habres 1992).

In recent years, protease inhibitors have gained increased attention due to their demonstrated anti-carcinogenic effects under various in vitro and in vivo experimental conditions (Nick et al. 2003). While proteases are essential for maintaining normal physiological functions, specific proteases such as caspases are central to the induction of apoptosis in abnormal or damaged cells. Caspases are constitutively expressed and play a pivotal role in immune regulation and programmed cell death. Consequently, several proteases and protease-regulating pathways are currently being explored as targets for anticancer drug development (Gills and Lopiccolo et al. 2007).

Human immunodeficiency virus protease inhibitors (HIV PIs) have also been repurposed for cancer therapy based on two key observations. First, Akt represents a major oncogenic signalling molecule, yet no direct Akt inhibitors have been widely established in clinical use. Second, HIV PIs have been shown to inhibit Akt activation, a mechanism that may underlie

clinical side effects such as lipodystrophy and insulin resistance. Emerging evidence suggests that HIV PIs act as broad-spectrum anticancer agents through pleiotropic mechanisms affecting cancer cell survival, metabolism, and signalling pathways (Karacostas et al. 1989; Roberts et al. 1990).

protease inhibitors (lopinavir, ritonavir, darunavir, nelfinavir, indinavir, and saquinavir) have been evaluated for activity against coronaviruses. High sequence similarity between the main proteases of SARS-CoV-2 and SARS-CoV supports the feasibility of protease-targeted drug development. Several HIV protease inhibitors have demonstrated in vitro binding to coronavirus proteases, although clinical outcomes have been variable, warranting further investigation (Martinez 2020; Zhu et al. 2019).

VI. PLANT PROTEASES AS THERAPEUTIC AGENTS

Plant proteases are ubiquitous and play critical roles in growth, development, defense, and programmed cell death. Their high thermal stability and catalytic efficiency distinguish them from many animal proteases, making them attractive for therapeutic and industrial applications (Kurepa et al. 2009; Pacheco and Silva-López 2012). Advances in recombinant DNA technology have enabled the safe, standardized production of proteases, minimized contamination risks and ensured clinical suitability (Swiech et al. 2017). Plant-derived proteases are generally non-toxic, cost-effective, and easy to obtain, and they are widely used in food processing, wound healing, digestive disorders, cancer therapy, and infectious disease management (Rathnavelu et al. 2016; Matagne et al. 2017).

Collectively, these findings highlight the extensive involvement of proteases and protease inhibitors across diverse health and disease conditions, reinforcing their significance as therapeutic targets and bioactive agents in modern medicine.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to the conception, design, and preparation of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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