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Review

# Improving Viral Protease Inhibitors to Counter Drug Resistance

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**Drug resistance is a major problem in health care, undermining therapy outcomes and necessitating novel approaches to drug design. Extensive studies on resistance to viral protease inhibitors, particularly those of HIV-1 and hepatitis C virus (HCV) protease, revealed a plethora of information on the structural and molecular mechanisms underlying resistance. These insights led to several strategies to improve viral protease inhibitors to counter resistance, such as exploiting the essential biological function and leveraging evolutionary constraints. Incorporation of these strategies into structure-based drug design can minimize vulnerability to resistance, not only for viral proteases but for other quickly evolving drug targets as well, toward designing inhibitors one step ahead of evolution to counter resistance with more intelligent and rational design.**

## Drug Resistance and Viral Proteases as Drug Targets

Drug resistance is a major health burden in a wide range of diseases from cancer to bacterial and viral infections, causing treatment failure as well as severe economic impact on the healthcare system. Drug resistance can be conferred via various mechanisms, including decreased intracellular levels of drug (due to efflux pumps), altered gene expression, and changes in drug target [1–4]. The most common mechanism of resistance to drugs against quickly evolving targets involves mutation of the targeted protein, including resistance to small-molecule inhibitors of viral proteases.

Viral proteases are ideal drug targets as they are essential in the viral life cycle, and inhibiting the viral protease prevents the generation of new infectious viral particles. Most viruses that infect humans and cause disease encode at least one viral protease [5,6]. These proteases are responsible for cleaving the viral polyprotein precursors at specific sites to release individual functional proteins, including *cis* cleavage of the protease itself. Certain viral proteases have also been reported to cleave host cell proteins such as translation initiation factors (eIF4 and eIF3d) in HIV to inhibit host protein translation [7,8], and transcription factors in HCV to confound the innate immune response [9]. Although most viral proteases share general backbone folds and catalytic mechanisms with host cellular proteases, they are generally more compact likely due to evolutionary pressure to maintain a small genome [6]. Nevertheless, viral proteases are able to recognize and cleave diverse substrate sequences with distinct specificities.

## HIV-1 and HCV Protease Inhibitors

Among medically relevant viruses, the viral protease most extensively investigated is inarguably the aspartyl protease encoded by HIV-1, with hundreds of sequences in the Stanford Database and crystal structures in the Protein Data Bank [10,11]. HIV-1 protease is comprised of two identical chains of 99 amino acids each, with the active site located at the dimer interface and each monomer contributing a catalytic Asp to the active site (Figure 1A). In the unliganded state,

### Trends

Viral proteases recognize substrates through a conserved shape, defining the substrate envelope.

A variant that does not bind inhibitors efficiently but still processes substrates is resistant.

Resistance mutations occur where inhibitors protrude outside of the substrate envelope.

High-potency inhibitors that fit within the substrate envelope can avoid resistance.

Resistance-avoiding strategies need to be included in structure-based drug design.

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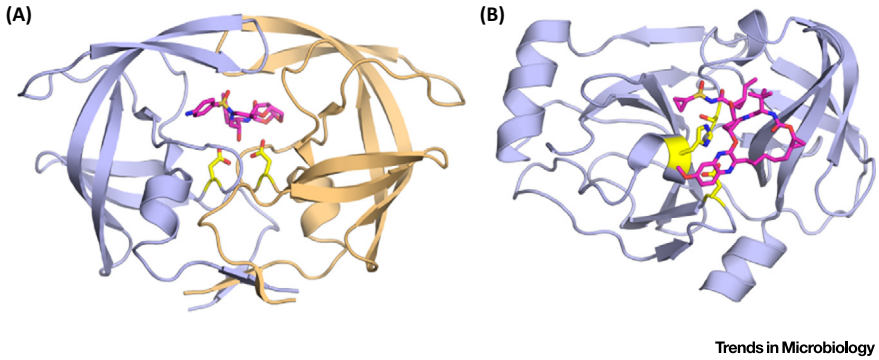


Figure 1. HIV-1 and HCV Protease Structures. (A) HIV-1 protease bound to darunavir (PDB: 1T3R). The two monomers of HIV-1 protease are in light purple and gold. (B) HCV NS3/4A protease bound to MK-5172 (PDB: 3SUD). The inhibitors are in magenta and the catalytic residues in yellow sticks.

HIV-1 protease is symmetric with highly flexible flaps that allow access to the active site. In the liganded state, these flaps close upon the bound substrate or inhibitor at the active site and become much more rigid. HIV-1 protease has been the target of extensive drug discovery and development efforts for decades, and has had a major role in launching the field of structure-based drug design. These efforts resulted in nine FDA-approved HIV-1 protease inhibitors (PIs). All HIV-1 PIs are competitive inhibitors that bind at the protease active site. Although these PIs are very effective in inhibiting the wild-type protease and have significantly contributed to clinical treatment outcomes in combination therapy [12–14], resistance has emerged to all HIV-1 PIs.

HCV, which infects millions of people worldwide and causes chronic liver disease, liver failure, and liver cancer [15,16], encodes a chymotrypsin-like serine protease, NS3/4A (Figure 1B). HCV NS3/4A protease is a prime therapeutic target for direct-acting antivirals, with four FDA-approved inhibitors [17–21] and several in various stages of clinical development. However, even for the drugs that have not yet been approved for the clinic, resistant viral variants have emerged [22–24]. Rapid emergence of resistance and low efficacy against genotypes other than HCV genotype 1 has mandated combination therapies, which also decreased treatment duration and increased cure rates, especially for genotype 1 [25–27].

## Mutations Confer Resistance by Selectively Weakening Inhibitor Binding but Retaining Specific Substrate Recognition and Cleavage

For a virus to become resistant to a PI, the viral genome acquires mutations that allow the protease to thwart inhibition by the drug but still retain the ability to cleave the viral polyprotein substrate at the required specific sites to allow viral maturation. HIV-1 evolves rapidly due to a high viral replication rate ($10^7$–$10^9$ newly infected cells/day in a patient [28]) and the error-prone mechanism of the viral reverse transcriptase, which generates a diverse pool of viral variants. This rapid evolution enables the targeted viral protease to acquire mutations that abrogate the efficiency of inhibitor–protein binding. Many mutations already pre-exist at low levels even before the start of therapy in infected patients, and quickly become selected under drug pressure. Critically, these selected protease variants still retain their substrate recognition and cleavage activity and allow viral propagation. HIV-1 protease needs to process the Gag and Gag-Pro-Pol polyproteins at nine distinct sites, while HCV NS3/4A protease cleaves viral polyprotein precursors at four cleavage sites and cleaves two human immune proteins to confound the innate immune response. However, these cleavage sites are highly diverse in amino acid sequence, and unlike most known human proteases, the viral proteases do not have simple substrate sequence recognition motifs.