A Review of the Development of Novel HIV Protease Inhibitors and Their Potential in Modern Medicine

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In the United States alone, it is estimated that 1.3 million people are infected with the Human Immunodeficiency Virus (HIV) with about one-fifth of these individuals unaware of their status. HIV is the causative agent of acquired immune deficiency syndrome (AIDS), which is a condition characterized by a progressive failure of the immune system that makes an individual highly susceptible to opportunistic infections and cancers (Eissenstat 2012). Modern medical approaches towards prolonging the life of infected individuals use the viral protease enzyme as one potential drug target. An understanding of the viral life cycle and mechanisms of action are needed in order to understand how HIV leads to AIDS and how modern medicine attempts to deal with it.

HIV is a slowly replicating retrovirus known as a lentivirus that is transferred most commonly via bodily fluids such as blood, semen, or breast milk. Immune cells containing CD4 receptors such as helper T-cells, macrophages, and dendritic cells are the primary targets of HIV. The high affinity of HIV glycoprotein GP120 for the CD4 surface receptors of some immune cells allows the virus to easily adhere to and infect these cells (Spaltenstein et al 2010). Once absorbed into the cell, the viral RNA genome is reverse transcribed into double stranded DNA (ds-DNA). This ds-DNA is then taken into the nucleus and integrated into the cells’ DNA by the virus encoded integrase enzyme and help from host cell co-factors. The virus may be latent for a period, allowing it to exist undetected by the immune system, or it may be transcribed resulting in new RNA genomes and viral proteins. However, most of the viral genes are translated into large polypeptides that must be cleaved into their individual peptide components in order to form an intact and functional virus particle. As previously mentioned, the primary targets of the virus are human immune cells. The virus leads to diminishing levels of CD4 T-cells by inducing apoptosis of neighboring cells, directly killing infected cells by lysis upon their release, or
causing the infected cells to be targeted by the body’s own cytotoxic T cells. As the numbers of CD4 T-cells drop below a critical level, the body’s cell-mediated immunity is no longer functioning and the infected person is progressively more susceptible to opportunistic infections and cancers that ultimately define AIDS (Eissenstat 2012).

Modern medicine aims at disrupting the action of either of the virus’s key enzymes which include its reverse transcriptase, integrase, or protease. The mechanism used by the viral protease and how modern medicine works in disrupting this process will be addressed in this paper. The HIV protease is an aspartyl protease that uses two Aspartate-25 residues at the active site for general base catalysis that allow water to attack the polypeptide at the peptide bond between phenylalanine and proline residues (Spaltenstein et al 2010). The active site of the enzyme contains a pocket for binding to aromatic groups adjacent to the peptide bond being cleaved. Once water has attacked the carbonyl carbon, a highly unstable tetrahedral intermediate is formed. The tetrahedral intermediate then collapses and releases a carboxylic acid and the peptide fragment (a schematic representation of the mechanism is shown in figure 1). By utilizing this mechanism, the translated polypeptide can be cleaved and ultimately used to form functional viral particles. Medical scientists however, use the virus’s dependence upon this protease for replication as a target site to slow the number of viruses being produced in an infected individual. HIV protease inhibitors act as transition state analogs as they mimic the structure of the reaction transition state. These protease inhibitors are synthesized having a core structure containing a hydroxyl group adjacent to a branch containing a benzyl group. The benzyl group helps properly position the inhibitor into the active site by being attracted to the aromatic binding pocket. Once positioned, the adjacent hydroxyl group acts as the transition state analog by mimicking the negatively charged oxygen in the tetrahedral intermediate of the normal
reaction. The remainder of the inhibitor structure is designed to have induced affinity for pockets along the enzyme active site. Although these interactions are noncovalent, the collective affinity of the inhibitor for the enzymes active site is so high (some $K_i$ values of 0.1-0.3nM), the drugs are considered to act as irreversible inhibitors. These inhibitors reduce the available active sites for viral polypeptide binding, resulting in fewer polypeptides being cleaved, and ultimately fewer viral particles being formed (Huff 2010).

Unfortunately, the majority of commonly prescribed HIV PIs are associated with negative side effects such as abnormal glucose regulation, dyslipidemia, liver dysfunction and kidney stones. HIV PIs such as ritonavir are hypothesized to disrupt glucose regulation by targeting and inhibiting the insulin-sensitive glucose transporter GLUT4 in fat and muscle cells. Vyas et al studied the effects of PIs on glucose homeostasis in order to test this hypothesis. If the unwanted effects of PIs on glucose homeostasis are principally mediated through disruption of GLUT4, then the negative effects should be most noticeable in wild type mice positive for GLUT4 and least apparent in GLUT4 knock out (G4KO) mice. In the control group of wild type mice, the administration of ritonavir led to defective glucose regulation and elevated blood glucose levels. Importantly, administration of ritonavir to G4KO mice did not further elevate blood glucose levels. These results support the conclusion that PIs target GLUT4 \textit{in vivo} and disrupt normal glucose regulation.

Richmond et al hypothesized that HIV PIs impair skeletal muscle fatty acid transport and oxidation. HAART (highly active antiretroviral therapy) is a modern day approach at limiting the number of viral particles formed by administrating the patient with a “cocktail” of 3 or more PIs. The researchers set out to test what effects do the combination of multiple HIV PIs have on fatty acid transport and oxidation in murine skeletal muscle cells. Murine muscle cells were
exposed to a cocktail of commonly used HIV-PIs (ritonavir, lipinavir, atazanavir, and darunavir) overnight. For control, the researchers incubated murine muscle cells in absence of PIs and others incubated with only one PI. The results of the experiments showed that the combination of HIV PIs and high fatty acid media exposure reduces myotube palmitate transport and oxidation. It was found that the control samples incubated with only a single PI had little to no reduction in fatty acid uptake, while those exposed to a cocktail of PIs experienced severe reduction in the ability to transport and ultimately oxidize fatty acids. This study, along with other experiments, demonstrated that a cocktail of commonly used HIV PIs reduces fatty acid uptake and oxidation in murine muscle cells by lowering the number of CD36 proteins. CD36 are a member of intramembrane proteins with a variety of functions including the uptake of long-chain fatty acids and Low-density lipoproteins. Development of PIs that can be used in combination with others without reducing the number of CD36 proteins is hoped for. Modern PIs’ association with harmful side effects increases the need for research and development of HIV PIs that are free of such negative effects.

HIV protease inhibitors have shown long term inefficiency due to the drugs’ toxic side effects and acquired resistance by many mutant strains of HIV. Eissenstat et al designed a synthetic series of HIV protease inhibitors with enamino-oxindole substituents optimized to interact with the HIV protease binding pocket (Figure 2). Enamino-oxindole substituents induce the fit of the inhibitor to the HIV protease subsite and have already been used in pharmaceuticals as antibacterials and antimalarials. Eissenstat et al’s assays consisted of cells exposed to 50% tissue culture infective doses (TCID\textsubscript{50}) of virus in the presence of various concentrations of test enamino-oxindole protease inhibitor. The cells with the enamino-oxindole based inhibitor had up to 46% prolonged, virus free, lifespans compared to those control cells not inoculated with the
inhibitor. Enamino-oxindole substituents allow HIV protease inhibitors to be both effective against a wide variety of HIV strains and be free of toxic side effects, as determined from live tissue assays. Several compounds were efficient against even multidrug resistant mutant strains of HIV in both enzyme and cell assays, suggesting that preliminary studies in rats should follow in order to further determine their effectiveness and potential as improved HIV protease inhibitors.

In a subsequent study, Pawar et al tested novel linear and cyclic glycotetrapeptides as potential HIV PIs. The HIV proteases’ catalytic activity was monitored in the presence of the chromogenic substrate (substrate that mimics the conserved cleavage site of the normal HIV polypeptide) and increasing concentrations of PIs. The activity was then compared to that of control samples. One control sample consisted of only the HIV protease with the chromogenic substrate, while the second control included the HIV protease, the chromogenic substrate, and a common HIV PI (ritonavir). Both the linear and the cyclic glycopeptides resulted in decreased HIV protease activity. This increased efficiency as a competitive inhibitor is attributed to both the previously documented hydrophobic interactions of tripeptide side chains with the enzymes’ active site and the hydroxyl group at the C\textsubscript{3} position of the sugar hydrogen bonding with the Asp25/Asp25’ residues of the enzymes’ binding site. The increased efficiency \textit{in vitro} compared to a currently used PI suggests further testing in mice should follow.

HIV is a retrovirus that ultimately leads to the development of AIDS in infected individuals after diminishing the number of their body’s immune cells. Modern research and treatment methods focus on disrupting the action of viral enzymes responsible for the replication and pathology of the viral particles. In order to limit the number of viruses formed, HIV protease inhibitors are synthesized to mimic the transition state of the normal reaction and exhibit an even
higher affinity for the enzymes active site. By acting as competitive inhibitors of the viral protease, the enzyme ultimately cleaves fewer viral polypeptides into functional viral particles. Although HIV PIs have increased the life expectancies of infected individuals, prolonged usage of the HIV PIs themselves have brought about negative consequences such as glucose intolerance, altered fatty acid metabolism, and heart problems. These unwanted side effects enhance the need for development of potent and broad spectrum protease inhibitors with reduced toxicity for the treatment of wild type and multidrug resistant HIV strains. Novel PIs such as those containing enamino-oxindole and cyclic glycotetrapeptides have demonstrated potential as new and improved versions that not only increase the efficiency of the PI but also not being associated with harmful side effects.
Figure 1: Schematic representative mechanism of HIV protease.

Figure 2: Enamino-oxidole derived protease inhibitor.
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