Rational Drug Design Approach to Synthesizing HIV-1 Protease Inhibitors

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Introduction

Human Immunodeficiency Virus (HIV), the causitive agent of AIDS, now infects an estimated 20 million people worldwide, and while a cure has not yet been found for this fatal disease, rapid advances in molecular biology along with the 3-D elucidation of HIV proteins have led to new drug targeting approaches for designing antiviral agents that specifically bind to key regulatory proteins that are essential for HIV replication. The HIV-1 protease is one such enzyme crucial for the maturation and assembly of infectious viral particles. Thus by inhibiting HIV-1 protease activity, a potential cure for AIDS is at hand. Armed with the 3D crystal structure of the HIV-1 protease, rational drug design approaches have been widely employed in the development of HIV protease inhibitors. While in clinical trials existent HIV-1 protease inhibitors show promise in causing an initial dramatic decline in viral plasma RNA in HIV-infected individuals, most patients taking protease inhibitors alone, however, by year's end will show an increase in plasma viral RNA to near baseline levels (Kaplan 1996). Failure of HIV-1 protease inhibitor therapy is attributed to the development of resistant viral protease. While techniques to combat resistance are currently being investigated, we have yet many obstacles to clear before discovering a cure for HIV.

Structure and Function of HIV-1 Protease: Potential Drug Target Identified

Through X-ray crystallography techniques, the 3D structure of HIV-1 protease has been extensively studied and characterized. In its mature form, the viral protease exists as a dimer, whose subunits each consist of 99 amino acids. The folded subunits together interact to form a core hydrophobic, cylindrical catalytic cavity (24Å long by 6-8 Å in diameter) and two flexible flaps (one per subunit) that can close around the substrate. Centered in the hydrophobic active site are two symmetrically disposed catalytic aspartyl residues (Asp25 and Asp25') that are involved in hydrolysis of the peptide bond (West 1995). Studies have shown that the hydrophobic cavern can hold six amino acids of the substrate in an extended conformation for cleavage (Molla 1998). Because structural and enzymatic proteins of the HIV virus are translated as part of two polyprotein precursors (*Gag* and *Gag//Pol)* cleavage of these precursors to generate gag matrix (p17), capsid (p24), nucleocapsid (p15) proteins, *pol* reverse transcriptase, integrase enzymes, and other viral proteins is vital for the production of mature, infectious viral particles (Molla 1998). Since this cleavage process is critical to viral propagation, inhibition of viral protease proves to be an attractive drug target.

Rational Drug Design: Structure and Receptor Based Approaches

Initial approaches to developing HIV-1 protease inhibitors were based on characterizing substrate-protease interactions that led to cleavage. Studies by Griffiths, J.T. *et al.* (1992) found preference for proteolysis of substrates with scissile hydrophobic-hydrophobic (usually involving leucine, alanine, or valine) or aromatic-proline (i.e. phenylalanine-proline) peptide bonds. Hydrogen bonding and electrostatic interactions along the cylindrical groove were also taken into account. But beyond optimal fitting of amino acid side chains into the binding groove, characterization of transition state structures has also guided the design of protease inhibitors. In a functional protease a water molecule is held between the two active apartic acids. During hydrolysis the water molecule is added across the cleaved peptides. With this information at hand, the initial inhibitors consisted of small polypeptides of seven amino acids in length that were designed to mimic normal cleavage sites but with the replacement of a nonhydrolyzable isostere at the cleavage site. Furthermore, the central carbonyl groups of these potent protease inhibitors also interact, via hydrogen bonding, with the water molecule held at the active site in order to stabilize the close formation of the flaps on the enzyme (West 1995).

While inhibitors such as Ro31-8959, LY289612, and other similar protease inhibitors designed with the above described criteria prove to be potent antivirals *in vitro,* they suffer from poor absorption, poor oral bioavailability, short serum half-life values, high susceptibility to hydrolysis by degradative enzymes, and other pharmacological problems (Reich 1995). Because of these limitations on peptidomimetic inhibitors, efforts have been made to minimize pharmacological problems by developing inhibitors that are structural mimics of peptides but with little or no peptidic character.

Reich *et al.* (1995) approached this problem by beginning with the cocrystal structure of HIV-1 protease with LY289612 (a peptidomimetic inhibitor). As their base design they retained only the non-peptidic hydroxyethyl-t-butylbenzamide portion of the original inhibitor. From here, the researchers synthesized and attached a variety of substituents to fit into the various active site pockets of the viral protease. By repeatedly solving the co-crystal structure of the protease and a newly designed non-peptide inhibitor (seven in all), they were able to improve upon their design and maximize binding affinities with each repetition. Further characterization *in vivo* showed improved bioavailability of up to 30% and maintenance in plasma levels, at or above, the antiviral IC_{90} for five or more hours in rats, dogs, and monkeys.

A different approach to designing non-peptide inhibitors involves the replacement of the water molecule at the catalytic site with a component of the inhibitor itself. In designing these compounds computer modeling techniques have been employed to generate a class of cyclic inhibitors (i.e. XM323). In XM323 and analogous compounds, the inhibitor's carbonyl oxygen is designed to displace the water molecule and hydrogen bond to Ile50. Cocrystalization of protease with the inhibitor confirmed computer generated predictions. While XM323 has 50% bioavailability and drug levels in plasma exceeds the IC_{90} for 4 to 16 hours, Phase I clinical trials