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HIV Protease Inhibitor: Past Endeavors and Future Developments

In 1981, the world was introduced to a lethal killer in our midst that has forever changed the lives of both infected as well as non-infected individuals through its impact on our health, society, and culture. Acquired Immune Deficiency Syndrome (AIDS) is caused by the HIV that has acted as an incredible catalyst for scientific breakthroughs in the attempt to find a cure. The most common form of treatment up to date is Combination Therapy or Highly Active Antiretroviral Therapy (HAART); this method uses a combination of two or more reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs) (9). This paper will focus on the latter that has proven to be the most successful in slowing down the progression of AIDS. The increasing knowledge of the biochemical pathways of the HIV as well as the effects of current drugs available will lead to the discovery of a potent inhibitor against the HIV protease of the present as well as the future.

The survival of the HIV (Human Immunodeficiency virus) involves many biochemical pathways from its entrance into a cell to its replication and release. One of the pathways involves the HIV-encoded protease without which the new viral particles inside the host cell cannot mature (Figure 1). The protease, expressed as a part of the gag-pol polyprotein, is encoded in the 5' end of the pol gene that encodes a 99-amino-acid aspartic acid protease (3). Each of the monomers contains a conserved sequence of amino acids(Asp-Thr-Gly) that are essential in the formation of the active site as a result

of the dimerization process. The aspartyl protease activity in the active homodimers of the protein allows the cleaving of the gag and gag-pol polyproteins into functional units (8).

The cleavage sites on the gag and gal-pol polyproteins consist of phenylalanine-proline or tyrosine-proline bonds. Cleavage by a functional protease results in three large proteins and three small proteins. The large proteins (p24, p17, and p7) lead to functional RNA packaging and virion structure. Without these cleaved proteins, the virions will not mature into infectious viral particles (2).

In 1988, the structure of the HIV-1 protease was determined through crystallization, and computer modeling has been essential in designing inhibitors that structurally fit into the active site pocket of the protease (6). With the knowledge of this step in the life cycle of the HIV, scientists are using HIV-protease inhibitors to block the essential step. The inhibitors are synthesized to contain analogues of the phyenylalanine-proline sequences at positions 167 and 168 of the gag-pol polyprotein that the protease cleaves. By binding to the active sites on the enzyme, the protease is not able to interact with the actual proteins. Containing these analogues, many inhibitors have diverse chemical backbones such as hydroxyaminopentane amides, hydroxyethylamino sulfonamides (141W94), allophenylnorstatines (KNI-272), cyclic sulfones (GS-3333), irreversible-binding cis-epoxide compounds (LB-71148) and LB-71262), and nonpeptidomimetic dihydropyrones (PNU-140690) (3).

Drug-resistance, common to other drug therapies, is a major problem in the use of protease inhibitors. This resistance is due to specific mutations in the target protein, the HIV protease, that prevents correct binding of the inhibitor, making the inhibitors

ineffective. There are two types of mutations: primary and secondary. First of all, primary mutations involve changes in amino acid sequence within the active site of the enzyme and significantly affect the binding of the inhibitor to the mutant active site. Secondary mutations take place outside of the active site and change the enzymatic function of the mutant protease, increasing the growth rate (8).

Development of resistance due to the primary and secondary mutations against a certain protease inhibitor not only protects the enzyme from that inhibitor but may provide resistance from others as well. Cross-resistance results when the HIV protease mutates against the binding site for multiple inhibitors. In order to prevent this multi-drug resistance, HIV protease treatments usually involve combination of two or more protease inhibitors (4). When the HIV protease mutates to become resistant to one inhibitor, there is another inhibitor at work to disable the protease before the strain can mutate again to become resistance to both.

Currently, there are several such inhibitors available for those infected with the HIV. Each drug has proven to be potent against HIV-1 and has increased the level of CD4+ T lymphocytes and decreased plasma viral RNA levels (2). Some of the most common inhibitors include saquinavir, indinavir, ritonavir, and nelfinavir as well as amprenavir (Figure 2). Each inhibitor has different characteristics and optimal method of dosage. For example, while ritonavir is the only protease inhibitor that provides the convenient twice-a-day dosage, it has a wide range of side effects as well; it is associated with moderate diarrhea, nausea, anorexia, headaches, vomiting, and fatigue. All of the approved inhibitors have gastrointestinal side effects (7). Other such side effects include abnormal fat distribution, hyperlipidemia, glucose intolerance, and rare hemorrhaging in

hemophiliacs. Besides side effects, a rise in resistance to these inhibitors has been a serious problem for patients with multi-resistant HIV-1. For example, treatment with nelfinavir has resulted in a mutation in position 30 in the protease gene sequence that provides resistance to the inhibitor (3). .

.   As mentioned above, resistance to all the above inhibitors is a major problem that leaves a patient defenseless. In order to combat such multi-resistant HIV-1 type virus, the scientists are studying a new type of inhibitor that is effective against various mutants. The scientific community is taking advantage of the fact that there exists subsite residues that do not mutate for a particular drug. By targeting these sites, a novel protease inhibitor should be effective against the HIV-1 variants that have become resistant to other inhibitors (9). These sites have to be well conserved and do not have any previously known drug-selected mutations. Sequence alignment of retroviral proteases have shown such regions to be the active site consisting of amino acids 22-34, the flap consisting of amino acids 47-52, and the region with a single alpha helix consisting of amino acids 84-94 (Gly(86)-Arg(87)-(Asn/Asp(88)).

The closest discovery to such a miracle drug is UIC-94003. It is nonpeptidic, containing 3(R), 3a(S), 6a(R)-bis-tetrahydrofuranyl urethane(bis-THF) and a sulfonamide isostere (Figure 3). Scientists are hoping that this new inhibitor would be able to stop the vicious cycle between the humans developing a new inhibitor and the HIV protease producing a new mutant resistant strain in response.

With this goal in mind, research has shown that UIC-94003 has proven to be potent against multi-PI-resistance HIV-1 strains, not affected by any of the existing mutations on the HIV protease. Unlike previous protease inhibitors (PI's), UIC-94003

shares a closer contact with the amino acids (Asp29 and Asp30), which are members of the main chains of the active site (Figure 4). This new inhibitor is novel in that previous mutations, and therefore resistance, in the binding sites of the HIV protease are not of concern since the main interaction takes place through Asp29 and Asp30. This interaction is made possible by the replacement of a THF group by a fused-ring bis-THF moiety in the UIC-94003 compared to previous PI's such as amprenavir. With this new bis-THF group, the amide hydrogen atoms from Asp29 and Asp30 in the main chain form hydrogen bonds in the S2 subsite (9). This interaction with the main chain atoms that do not mutate allow UIC-94003 to bind to multi-resistant proteases, regardless of other resistance the protease might have against other PI's.

Despite this interaction with the main chain, however, the expected development of a new mutation, and, therefore, a new resistance, was observed. Indeed, a new mutation A28S, unobserved in previous resistant proteases, led to the inability of UIC-94003 to bind to the active site. A probably reason is that the mutation results in a slightly more polar and larger side chain, decreasing the likelihood of UIC binding due to steric hindrance (9). However, the A28S mutation, while providing initial resistance to UIC-94003, also resulted in a decreased replication rate of the virus. In the attempt to mutate and become resistant to the inhibitor, the protease has actually caused a secondary mutation I50V. The combination of A28S and I50V, through an unknown biochemical pathway, has shown to decrease the replication rate and has proven to be disadvantageous for the virus.

While UIC-94003 offers a very hopeful and encouraging step towards stopping the HIV in its tracks, there are other methods to control HIV at other stop points in its life

cycle. For example, in a recent study, a group of scientists are attempting to disable the protease not by preventing cleavage but through its protein formation. Specifically, they are attacking the dimer interface in order to cause instability, and therefore, inefficiency, of the HIV protease dimer. As mentioned before, there are highly conserved regions such as that of Gly(86)-Arg(87)-Asn(88); it was discovered that a mutation R87K within the region causes folding of a different monomer that forms an unstable dimer interface with the other monomer. The substitution disturbs the inner C-terminal beta-sheet in the residues 96-99 which are critical for the dimer formation and, therefore, the function of the protease (6).

As seen above, there are various approaches to attacking and stopping the proliferation of the HIV. Despite the above study on the structure and folding of the protease protein, the most well understood and practical method of treating HIV is still protease inhibitors. As seen in this paper, protease inhibitors offer tremendous results and yet cause side effects as well as resistance that leave the inhibitors useless after time. With increasing understanding of the active sites from the already known structure of the HIV protease, it is important to target sites that are common against all possible mutant strains, while not causing many side effects - in other words, a protease inhibitor that offers no compromise in the fight against AIDS.   As effective as HIV protease inhibitors are, they can only treat and not cure the patient of the disease. It remains to be seen whether such a cure, and not only a treatment, will be discovered in the future.
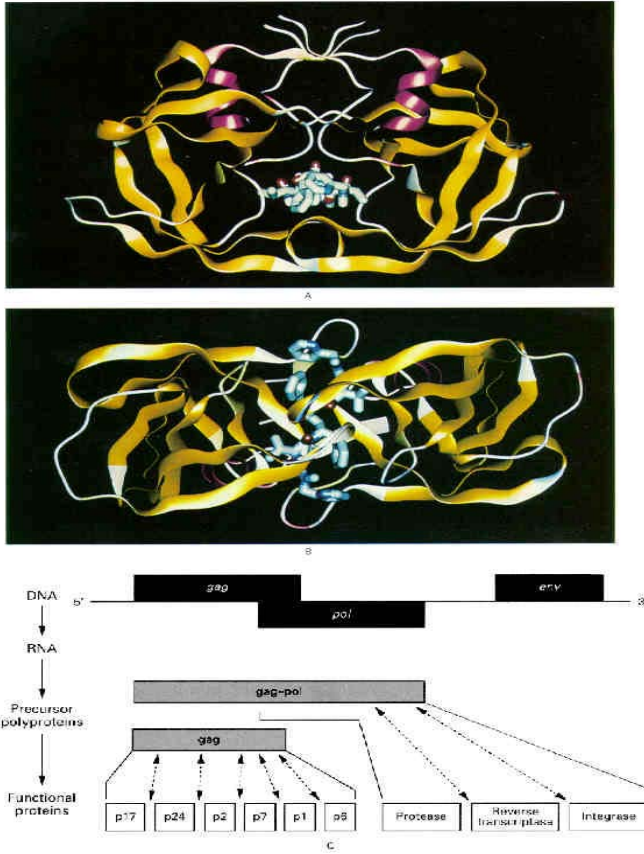

Figures

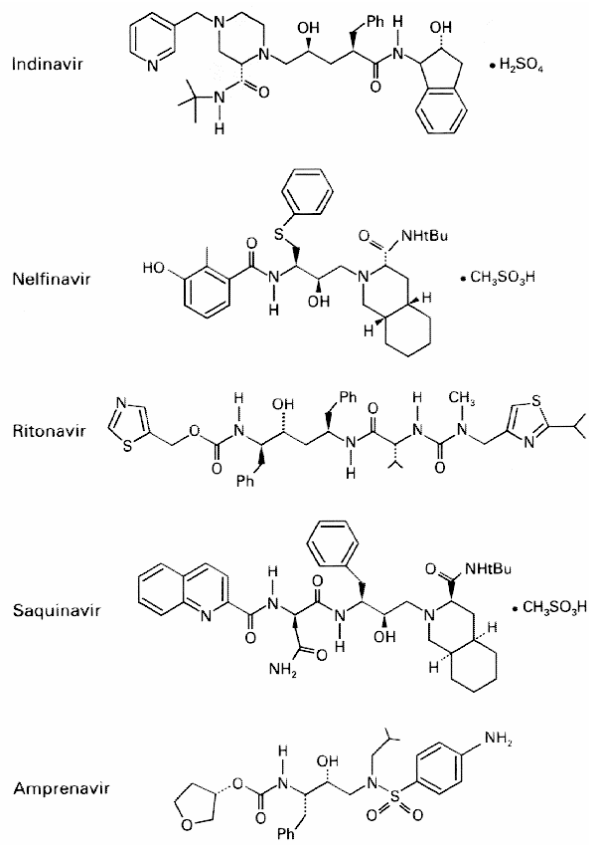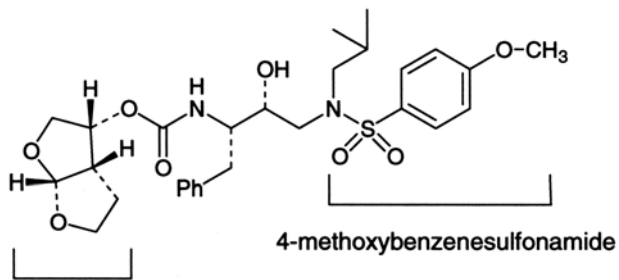**Figure 1**. Structure and Function of HIV-1 Protease (4).

Figure 2. Structures of Five HIV-Protease Inhibitors (4).

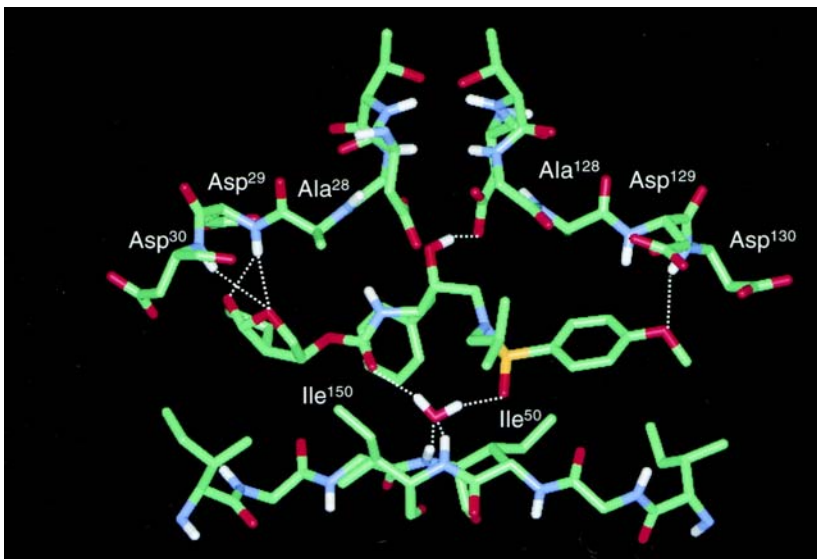**Figure 3**. Structure of UIC-94003 (9).

Figure 4. Modeling of UIC-94003 bound in the active site of HIV-1 protease (9).

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