New HIV protease inhibitors

'there is still much room for improvement in the next generation of HIV protease inhibitors'

Just over a year ago, the outlook for drug therapy in the treatment of HIV infection was grim. The various reverse transcriptase inhibitors such as AZT, 3TC, DDI and nevirapine all suffered from a fatal flaw: resistant viral strains emerged soon after the initiation of therapy. But now there is new hope. First, Hoffmann-LaRoche introduced the HIV protease inhibitor saquinavir in December of 1995. Then, in March 1996, the FDA approved the use of indinavir (Merck) and ritonavir (Abbott).

In July 1996, at the XI International Conference on AIDS, held in Vancouver, Canada, researchers reported that these new protease inhibitors, when given in combination with reverse transcriptase inhibitors, were remarkably effective in reducing the viral load in AIDS patients. In fact, for a significant number of patients the new drug regimen reduced the virus to a level where it could no longer be detected.

Renin inhibitors recycled

The success of the HIV protease inhibitors rides on the shoulders of another protease inhibitor program that ended in failure. Until the mid-to-late 1980s numerous pharmaceutical companies, including Hoffmann-LaRoche, Merck and Abbott, had major research programs to discover inhibitors of renin, a protease that plays a key role in regulating blood pressure. Potent peptide inhibitors for renin were found that initially contained eight amino acids. Considerable efforts by medicinal chemists to move from an eight-amino-acid peptide to a small organic molecule that might be used to control blood pressure ended in failure. In spite of great effort, the size of the renin inhibitor could not be reduced to less than about four amino acids, and this oligopeptide had extremely poor bioavailability. The blockbuster drug remained out of reach, and by the mid 1980s, most companies were giving up and eliminating or downsizing their renin programs.

Then the AIDS epidemic happened, and one of the obvious targets was the HIV protease. Because the HIV protease and renin both belong to the same family of aspartyl proteases, it was natural for pharmaceutical researchers to bring out their renin inhibitors and test them against the HIV protease. They quickly found that some of the compounds from the now defunct renin programs could be redesigned as potent inhibitors of HIV protease, saving them years of time that would otherwise be needed to find lead compounds. But of course the same old problem remained – would it be possible to go from a peptide inhibitor to an orally active drug with reasonable bioavailability?

Currently approved HIV protease inhibitors

Again, the medicinal chemists went to work to improve the adsorption, distribution and pharmacokinetic properties. The first protease inhibitor to come to market, saquinavir, has very poor oral bioavailability, but the subsequent compounds, indinavir and ritonavir, have a much improved bioavailability profile. These protease inhibitors are currently prescribed in combination with reverse transcriptase inhibitors. The patient takes a complex drug regimen at precise intervals to keep the concentration of the drugs at a sufficient level in the body to prevent the emergence of resistant strains of virus. However, the currently approved protease inhibitors are chemically complex molecules and are challenging to synthesize on a large scale. Thus, their supply and cost continue to be a problem.

Certainly there is still much room for improvement in the next generation of HIV protease inhibitors, but the amazing thing is that useful drugs acting as inhibitors of an aspartyl protease were obtained at all given past failure in identifying useful renin inhibitors. Moreover, the drugs already on the market are not necessarily the best – several companies are working on the next generation of protease inhibitors.

Evolution of the next generation

Dr Patrick Lam's team at Dupont Merck Pharmaceuticals (Wilmington, DE, USA) recently described the discovery of one of these next generation protease inhibitors [*Drug Discovery Today* (1997) 2, 6–18]. The drug, a cyclic molecule termed DMP450, is currently in clinical trials. Lam explained how the X-ray structural data of the original peptide inhibitors complexed to the HIV protease revealed the presence of a critical water

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molecule hydrogen bonded to the amide hydrogens of two isoleucine residues of the protease and to two carbonyl oxygens of the inhibitor peptide. His group, like many others with the same structural data, reasoned that the water molecule must be essential for protease function, and that a compound capable of displacing the water from the active site would likely be a potent inhibitor.

The first pharmacophore model the group considered consisted of three linked phenolic rings with appropriate hydrophobic groups attached to facilitate binding to the aspartates in the active site of the enzyme. A ketone oxygen moiety was included in the central ring in anticipation that it would mimic the essential water and hydrogen bond to the two isoleucine residues of the protease. Intricate chemical reasoning led to ever more complex structures. The critical ketone oxygen remained, but the central ring was changed from six to seven members, nitrogen atoms were incorporated to produce a urea-like central structure, and additional groups were attached to optimize binding of the three-ring complex to the active site of the protease. The result, DMP450, is a highly potent HIV protease inhibitor that is orally active and has good bioavailability. What has to be most satisfying to the chemists who designed DMP450, however, is the recent X-ray crystal structure and NMR data showing the central ketone oxygen hydrogen bonded to the two critical isoleucine residues, displacing the water molecule from the active site of the protease. It was just as they predicted – find a nonpeptide compound that will kick out the water, the HIV protease will be inactivated, and a new type of aspartyl protease inhibitor will be born.

Other HIV protease inhibitors in the pipeline

Vertex/Glaxo Wellcome (Cambridge, MA, USA and Research Triangle Park, NC, USA) are also seeking approval for what they believe to be an improved HIV protease inhibitor. The drug, VX478, was also designed on the basis of the crystal structure of the protease–peptide inhibitor complex. It is a sulfonamide compound with a mean IC_{50} of 0.012 μM against six HIV clinical isolates. According to Lynn Brum, Director of Corporate Communications at Vertex Pharmaceuticals, VX478 has approximately 70% bioavailability, is very inexpensive to manufacture, and has a distinct resistance profile from the currently used protease inhibitors, which may serve as a therapeutic advantage. It is currently in Phase II clinical trials.

Agouron Pharmaceuticals, Inc. (La Jolla, CA, USA) also has a protease inhibitor, viracept, which has just successfully completed all three phases of clinical trials. The company applied to the FDA for US marketing approval at the end of 1996. Equivalent regulatory filings are being made in Canada and Europe. According to Dr Richard Ogden, Agouron's Director of Scientific Development, viracept is a unique nonpeptide inhibitor that was designed and optimized through iterative X-ray structure analysis of the HIV protease complexed with various inhibitors, including the classical mechanism-based drugs, such as those discovered in the renin program.

Viracept was the best drug candidate to be derived from five or six different chemical series leading to candidate compounds; all were designed over a period of 9–12 months. The Agouron approach was to use computer-based design exclusively in discovering new antiprotease compounds. 'All of our candidate compounds were designed on the computer utilizing crystal structures and then discussed extensively in project meetings before any compound was synthesized,' said Ogden. Viracept has an oral availability of 20–80% depending upon the test animal, a plasma life-time of 1–2 h, and few side effects with the exception of mild diarrhoea. Other companies with pipeline protease inhibitors include Nikko Kyoto and Pharmacia & Upjohn.

Prospects for inhibitors of renin and other aspartyl proteases

Almost all of the programs that are now vying for drug approval originated from the now-defunct renin programs or from crystal structures of the renin-derived inhibitors complexed to the HIV protease. So why did the renin programs fail where the HIV protease programs have succeeded? Ogden believes this is because renin has a large peptide recognition site, distinct from the catalytic site, that requires a high degree of structural constraint for renin to recognize a peptide as a substrate. This large recognition domain is not present on the HIV protease, which is much more forgiving to slight variations in the sequence of the substrate. The decrease in specificity and size of the substrate recognition site in the HIV protease made it easier to design drugs to successfully disrupt the enzyme–substrate interaction.

'The HIV protease story clearly demonstrates that small aspartyl protease inhibitors can be found, and that the larger inhibitors can be made to be orally active,' says Dan Rich, Professor of Medicinal Chemistry and Organic Chemistry at the University of Wisconsin (Madison, WI, USA). Will the success of the HIV protease inhibitors revive the renin inhibitor programs? Rich thinks not, 'The ACE inhibitors will soon be going generic and the angiotensin II antagonists look good. With drugs available for targets both up- and down-stream from renin, it is unlikely the market will support a renin inhibitor.' However, Rich believes that the success of the HIV protease programs bodes well for other physiological targets that happen to be aspartyl proteases. In his view, pharmaceutical management may now look favorably upon such targets, which until recently they would have dismissed immediately because of the renin experience. Ogden expresses the same view and suggests that cathepsin D, a possible target for new therapeutics to treat cancer and immune disorders, and some very interesting antifungal targets, may be the next aspartyl proteases to be tackled.

So, the development of drugs targeted to aspartyl proteases may be just beginning. Because of the recent success of HIV protease inhibitors, Rich believes it has been 'converted from a dead field to one that is thriving'.

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