levels. Crespi described a high-throughput P450-inhibition screen, using fluorometric substrates, which has been developed by researchers at Gentest. It is only necessary to use a single substrate probe for each enzyme, because if one substrate of a particular enzyme is inhibited, all substrates of that enzyme will be.

Under trial

Konrad Tomaszewski (Pfizer Central Research, Sandwich, UK) related pharmacokinetic and toxicity screening to clinical development by describing the factors affecting the choice of dose range for the first clinical trial of a potential drug: the so-called first-in-man (FIM) study. Generally, in such studies, single doses of escalating concentration are given to healthy male volunteers. Animal toxicity studies using high drug concentrations are used to determine the maximum dose given in this trial. In a typical paradigm, the dose is escalated until 10–20% of the highest tested concentration that showed no adverse effects in animals (the NOAEL) is reached. Tomaszewski suggested that, in some cases, it should be possible to progress to much higher concentrations in an FIM trial. Suitable candidates for this could be 'me-too' drugs of known mechanism, and cases where the adverse effects observed in animals are very minor or specific to the animal species used. He recommended a pragmatic approach to clinical trial design: 'FIM design should be based on science, not a calculator'.

This conclusion echoed a point made by Nigel Brown (Pheonix International Life Sciences, Montreal, Canada) in his keynote address. He said that, especially now that screening techniques can be almost fully automated, scientists working in this area need a broad understanding of the complete drug discovery process. Researchers are often too specialized. It would be useful to apply the 'horizontal' team based approach, which works well in small biotechnology companies, throughout the industry.

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Overcoming HIV resistance

A novel protease inhibitor developed by US chemists could be effective against mutant strains of HIV that have developed resistance to currently used drugs, such as retrovir, and even combination therapies.

AIDS drugs disable HIV by inhibiting viral replication enzymes, such as protease. But, HIV mutates quickly and resistance to individual inhibitors can evolve within weeks. Combination therapy using reverse transcriptase and protease inhibitors together has so far remained a reasonably effective approach, although doubts about its long-term efficacy have being raised.

Glimmer of hope

Chi-Huey Wong at The Scripps Research Institute (La Jolla, CA, USA) believes he now understands the mechanism behind HIV's rapid adaptation to current treatments. More than 45 distinct drug-resistant variants of HIV have been found in the past three years, according to Wong. 'We have studied the mutation patterns of HIV protease from patients who take the existing drugs and found that the enzyme often rejects the drug by reducing the size of the drug binding site', he explains. The mutant enzymes often exhibit cross-resistance to structurally distinct inhibitors, which means an alternative broad-ranging inhibitor is needed.

To exploit their findings the team looked at the corresponding binding site on current HIV protease inhibitors. They found that most of the drugs have a bulky group at the P3 position, which corresponds to the side chain of the third amino acid from the scissile bond of the substrate. This interacts with the constricted areas in drug-resistant proteases. They reasoned that reducing the size of the P3 group might lead to a new class of inhibitors that could still latch on to the HIV aspartyl protease even if a restricted region had evolved to block access of other drugs.

The team used molecular modelling techniques to look at the enzyme fit of alternative P3 substituents. They found that by modifying various known antivirals to have a methyl group at P3 instead of the usual bulky substituents endows them with a remarkably different pattern of inhibition, in the test tube at least. The team observed a 120–1000-fold improved inhibitory activity against HIV aspartyl protease and at least three orders of magnitude higher potency for feline immunodeficiency virus (FIV) aspartyl protease compared with the activity of the parent drugs. Modification of two existing drugs showed a similar effect, says Wong. The researchers also found that if there was no group at all at P3 (i.e. the group was replaced with

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a hydrogen atom) then the drugs had only marginal activity [*J. Am. Chem. Soc.* (1999) 121, 1145–1155].

Lack of emergent resistance

More important than the baseline efficacy in the laboratory tests is the fact that the new class of inhibitors did not lead to emerging resistance. 'No resistant mutants were detected in cell culture after one year', explains Wong, 'the new drug may last longer as the chance for development of drug resistance is lower.'

One particular compound an α-keto amide was found to be a slow-binding inhibitor, effective at low concentration against HIV and FIV. This level of potency against FIV, in particular, in spite of the molecular mass of the compound being a mere 649 is 'truly remarkable' according to Wong. After all, the smallest efficient substrate for the enzyme is an eight-residue peptide, Acetyl-Pro–Gln–Ala–Tyr–Pro–Ile–Gln–Thr. Wong points out that their modifications to the antiviral backbone do not reduce their efficacy against wild-type HIV.

The studies have also provided evidence for the similarity of the feline virus to HIV in some aspects. In particular, the aspartyl protease of resistant HIV is more closely matched to the FIV enzyme, which has a smaller binding region for the P3 group. This bodes well for developing a feline model for studying new inhibitors. 'The current results represent only an initial step toward development of therapeutic agents efficacious against both native and mutant HIV proteases,' cautions Wong, 'but using FIV aspartyl protease as a general model for the drug-resistant mutant HIV enzymes is clearly an effective strategy.'

Trojan horse strategy

An alternative approach to overcoming HIV resistance using gene therapy has been developed by Steven Dowdy and his colleagues at Washington University School of Medicine (St Louis, MO, USA) using gene therapy. They say that while highly active antiretroviral therapies are still the gold standard in HIV treatment, the emergence of resistance is problematic.

Dowdy and his team have engineered a fusion protein, TAT–Casp3, containing the proteolytic enzyme caspase 3, which is activated only in the presence of the HIV protease enzyme. Thus, TAT–Casp3 is triggered to induce apoptosis in infected cells [*Nat. Med.* (1999) 5, 29–33]. When they applied the method to HIV-infected cells that had been treated with the commonly used protease inhibitor ritonavir, they found that they could not induce apoptosis. This they say implies that TAT–Casp3 is selective for cells that contain HIV protease.

HIV-infected cells can only produce infectious virions if HIV protease is present to cleave and mature the polyproteins Gag and Gag-Pol. Without this function, infection cannot be transmitted and it is this property that forms the basis of the protease-inhibitor approach to the disease. Aside from the development of resistance to these drugs, the cells that respond ironically survive longer than untreated infected cells. Dowdy believes that his alternative approach involving cell death and not merely inhibiting enzymes could limit or eliminate the virus without damaging uninfected cells. This, he says, would offer a considerable advantage over treatments currently available.

The team further suggests that their 'Trojan Horse' strategy could be applied to other pathogens encoding specific proteases, such as hepatitis C virus, cytomegalovirus and malaria, as these diseases are dependent on the proteases encoded by the pathogen.

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