Clinically effective HIV-1 protease inhibitors

Joseph P. Vacca and Jon H. Condra

For 10 years now, the HIV-1 protease has been recognized as an attractive target for antiviral therapy, and the discovery of potent inhibitors has been the focus of many research groups. There are currently four HIV protease inhibitors approved in the USA for use in treating AIDS, and several compounds are in various phases of clinical development. All of these compounds represent important advances and result from intensive research efforts. This review outlines the approaches taken by the research groups in discovering their clinical compounds, the problems encountered during this process and their subsequent solutions. Limited clinical data are also presented for each compound.

HIV, the causative agent of AIDS, is a member of the *Lentiviris* subfamily of retroviruses and, like other retroviruses, contains three major genes (*gag*, *pol*, and *env*)[1,2]. The *gag* and *pol* gene products are expressed as polyproteins, which are processed by an aspartic acid protease (HIV-1 protease) to produce essential structural proteins. Inactivation of the enzyme by site-directed mutagenesis leads to a non-infectious virus[3], thus making HIV-1 protease an attractive target for antiviral therapy. There are currently four HIV protease inhibitors approved for use in treating AIDS: Inverase® (saquinavir mesylate, Hoffmann-La Roche), Norvir® (ritonavir, Abbott Laboratories), Crixivan® (indinavir sulfate, Merck) and Viracept® (nelfinavir mesylate, Agouron Pharmaceuticals) (Figure 1). All four compounds represent important advances in the treatment of AIDS and are the result of an intensive research effort. In addition, several compounds are currently in various phases of clinical development,

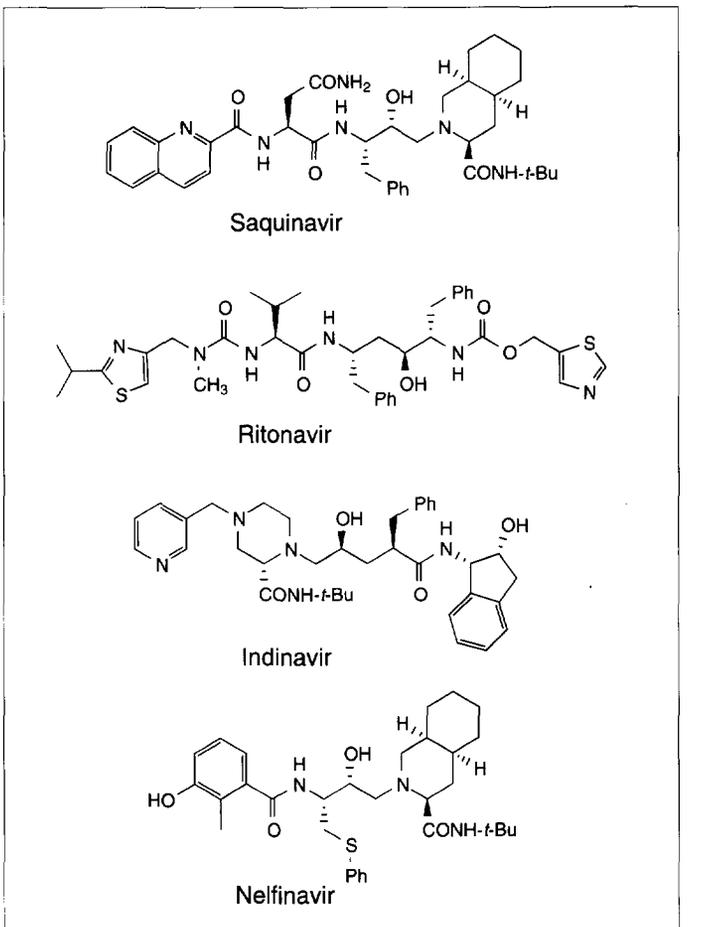

Figure 1. *Chemical structures of saquinavir, ritonavir, indinavir and nelfinavir.*

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including 141W94 (VX478, Glaxo Wellcome, licensed from Vertex, Phase II–III). This review will outline the approaches taken by the research groups in discovering their clinical compounds and will outline problems encountered during this process and their subsequent solutions. In addition, limited clinical data will be presented for each compound.

Inhibitor design: general considerations

The ideal HIV-1 protease inhibitor should be potent against various HIV clinical isolates, should be specific for HIV-1 protease compared with other mammalian aspartic acid proteases (to minimize possible adverse effects), should have good oral bioavailability and duration in humans, and must be safe and well tolerated. The last criterion is critical because the compounds represent a chronic therapy, and it is important to maintain the dosing regimen over a long period of time.

Factors that affect a compound's potency in cell culture include its affinity for the enzyme, its ability to penetrate cells, and how highly bound it is to physiological proteins. The latter property is an important one because high protein binding has been shown to result in lower cell culture potency in the presence of physiologically significant proteins such as human serum albumin or α_1-acid glycoprotein (AGP)[4,5]. These proteins severely diminish the cell culture potency of the compound when they are added to the culture medium, and reducing the binding of a compound to such proteins is now part of the total characterization of a proposed protease inhibitor. Binding to AGP has been speculated to be the cause of failure for at least one HIV protease inhibitor (SC52151) tested in human clinical trials. Factors that affect oral bioavailability include aqueous solubility (to enhance absorption), molecular weight (to lower liver clearance), and first-pass metabolism by the liver. Discovering a compound with these ideal characteristics has been the major focus of many research laboratories over the last nine years[6,7].

Lead identification

The first problem in any research program is to identify a lead structure for modification and testing in *in vitro* and *in vivo* assays. The usual approaches to identify a lead compound are:

- random screening of a sample collection (including combinatorial libraries),
- rational screening of inhibitors of related targets,
- modifying inhibitors known in the literature, and
- *de novo* design, utilizing structural knowledge of the target obtained either from X-ray crystal structures or from NMR analysis.

There were no known inhibitors available when HIV-1 protease was first identified as a good target for antiviral chemotherapy. The enzyme itself, however, provided a good clue as to what type of compound might be a starting point. HIV-1 protease is an aspartic acid protease like renin and endothiopepsin and cleaves peptides in a similar manner. It is a dimer made up of two 99-amino acid monomers, each contributing an aspartic acid to form the catalytic site[8,9]. HIV-1 protease functions as a molecular pair of scissors and hydrolyzes the viral gag-pol precursor proteins to produce viral structural proteins. Figure 2 depicts a simplified scheme of how HIV-1 protease cleaves a protein substrate. A water molecule is delivered by the two aspartic acids to the substrate cleavage site, creating initially what is referred to as a tetrahedral transition-state intermediate[10] (residues to the left of the cleavage site are referred to as

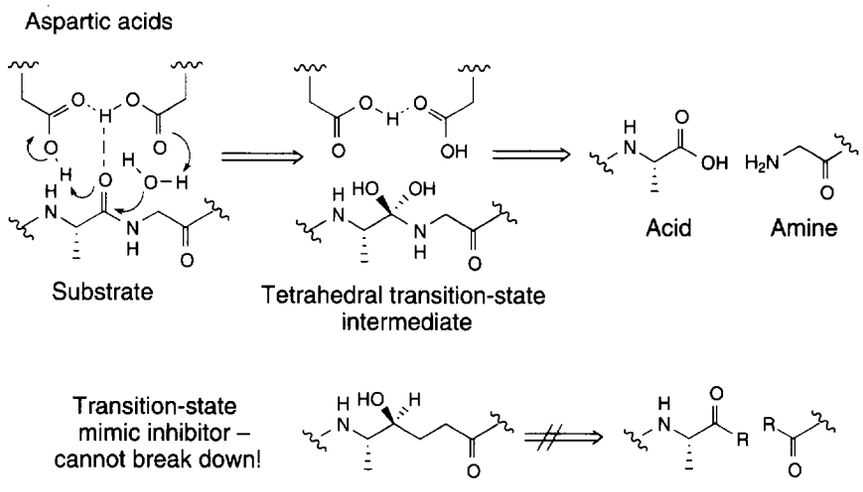


Figure 2. *Proposed mechanism by which aspartic acid protease cleaves substrates.*

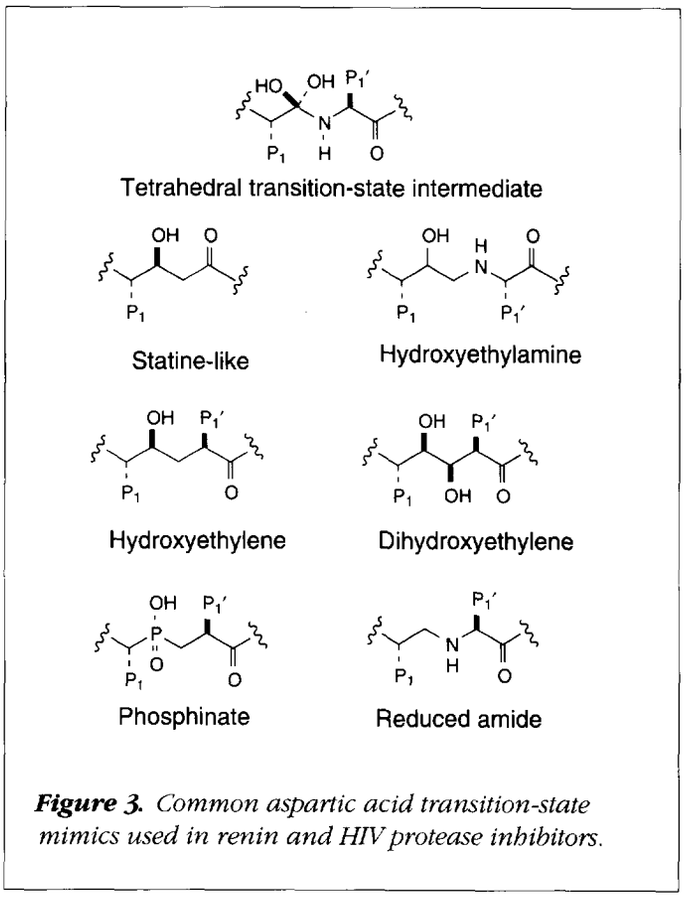


Figure 3. *Common aspartic acid transition-state mimics used in renin and HIV protease inhibitors.*

the P1, P2, P3, etc. position, and those to the right are given the designation P1′, P2′, P3′, etc.). This intermediate then rapidly breaks apart to give a C-terminal acid and an N-terminal amine, which are subsequently used by the virus to build its structural core. Numerous inhibitors of the aspartic acid proteinase renin have been developed by incorporating a non-hydrolyzable P1/P1′ cleavage site mimic[11] [a transition state analog (TSA); see Figure 3] into a substrate sequence. The compound binds to the enzyme, cannot be hydrolyzed and causes inhibition of the enzyme. This approach towards the design of HIV-1 protease inhibitors was first reported by Dreyer *et al*[12].

Saquinavir

Inverase® (saquinavir, **4**) contains a hydroxyethylamine (HEA) P1/P1′ transition state isostere in its core (Figure 4). Researchers at Hoffman-La Roche[13,14] initially began to explore substrate-based inhibitors that contained an asparagine-phenylalanine/proline transition-state mimic in place of the pol cleavage site Leu165-Ile169. They chose the HEA isostere over a dihydroxyethylene or phosphinate transition-state analog because they felt that the HEA core would infer specificity at inhibiting HIV-1 protease compared with other common mammalian proteases (such as cathepsins D and E, pepsin and renin) and was not usually found in potent renin inhibitors developed at that time. Additionally, they felt that, because this isostere contained a proline moiety it was similar to proline-containing peptides, which are more stable towards protease degradation, and this should help the compound's oral bioavailability and *in vivo* duration. One of their initial lead compounds (**1**; Figure 4) had an IC_{50} of 750 nM against HIV-1 protease. Each residue was then systematically changed to explore the structure–activity relationships of this series. It was found that an asparagine residue was preferred at the P2 position and that a 2-quinoline carboxylic acid was considered optimal at P3. A phenylalanyl mimic was optimum in P1 of the TSA, while larger amines such as piperidine (**3**) and decahydroisoquinoline (**4**) were preferred at P1′. The best P2′ group was a simple *t*-butylcarboxamide

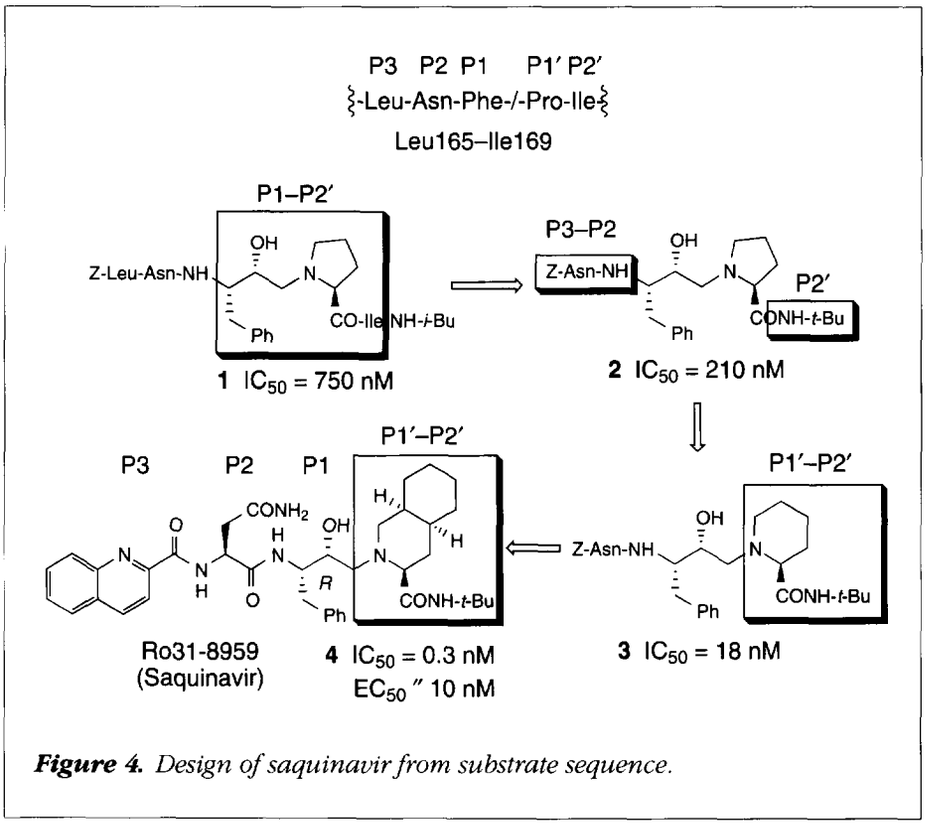


Figure 4. *Design of saquinavir from substrate sequence.*

moiety, which was equipotent to the *t*-butyl ester but was more stable in animal studies. Finally, it was found that the *R*-stereochemistry of the transition-state hydroxyl group was essential. Inversion of this center to the *S*-configuration led to complete loss of activity. All of these findings together led to the identification of Ro31-8959 (saquinavir, Inverase®) as the best candidate. This compound is very potent against both HIV-1 (IC_{50} < 0.37 nM) and HIV-2 (IC_{50} < 0.8 nM) protease *in vitro*, does not inhibit other mammalian aspartic acid proteinases at concentrations less than 10 μM, and is effective at inhibiting the spread of virus in many HIV producing cell lines at an EC_{50} < 10 nM. The compound is highly bound to human plasma proteins (98%), which diminishes the antiviral activity by two- to fourfold upon the addition of 50% human serum or AGP (2 mg/ml) to the cell culture medium (E. Emini and W.M. Schleif, pers. commun.). Saquinavir is only 4% orally bioavailable in animals. This is caused primarily by poor absorption and a large first-pass effect, both of which may be a result of the number of amide bonds in the molecule and its high molecular weight (671 g/mol).

Ritonavir

Researchers at Abbott Laboratories had extensive prior experience with renin inhibitors and realized the limitations, such as short duration of action and poor oral bioavailability in animals, that these compounds had. For this reason they decided to work on inhibitors that were based on the symmetrical nature of the protease[15–17]. Their reasoning was that most other mammalian proteases were not symmetric, so that this inhibitor design could lead to novel compounds that were specific for HIV-1 protease. Symmetric compounds might also be less susceptible to peptidases, which normally degrade peptide-containing compounds, thus limiting their oral bioavailability.

The initial design procedure is visualized in Figure 5. An axis of symmetry is imposed upon a typical hydroxyethylene isostere-based inhibitor (which fills the P1-P2 etc., pockets) at, or next to, the TSA hydroxyl group. The group on either side of the axis is eliminated, and the remaining group is then added to the other side of the axis to produce a symmetric inhibitor. When doing this symmetry operation, inhibitors can be prepared based on either the C-terminal portion or N-terminal groups. The Abbott group decided to pursue inhibitors based on an N-terminal group symmetrization strategy. The initial structure (**5**) prepared in this new series (Table 1) was a weak inhibitor with an IC_{50} > 1 μM. However, by adding other amino acid residues to the P2/P2′ positions, potency could be regained (**6**). Further manipulation of the end groups to reach out into the P3 positions led to their first-generation clinical candidate A77003 (**7**). This compound had a K_i = 0.2 nM versus HIV-1 protease, did not inhibit other mammalian proteases at concentrations < 10 μM, and was active in a cell culture assay at concentrations between 70 and 200 nM. Unfortunately, the compound was not orally bioavailable in animals and required testing in humans as an intravenous agent. Clinical studies showed that the compound had a very short half-life in man (< 30 min) and amounts adequate to inhibit viral replication could not be maintained[13].

Additional work was carried out in this series in order to find a more potent, orally bioavailable inhibitor. Removal of one hydroxyl group from A77003 led to increased potency (**8**) but the compound still had poor oral bioavailability. Deletion of a valine residue at the P2 position adjacent to R = H to give a carbamate, and modification of the terminal pyridine groups to increase aqueous solubility gave their second clinical candidate A80987 (**9**). This compound is potent (K_i = 0.25 nM; EC_{50} = 0.13–0.25 μM) and has good oral bioavailability in three species (> 20%). It entered into clinical trials and was well absorbed, but, like their earlier compound, it also suffered from a short plasma half-life. In addition, the high protein binding of this compound reduced its potency in a cell-based assay by greater than 10-fold, and efficacious plasma levels could not be safely maintained in patients[4,18,19].

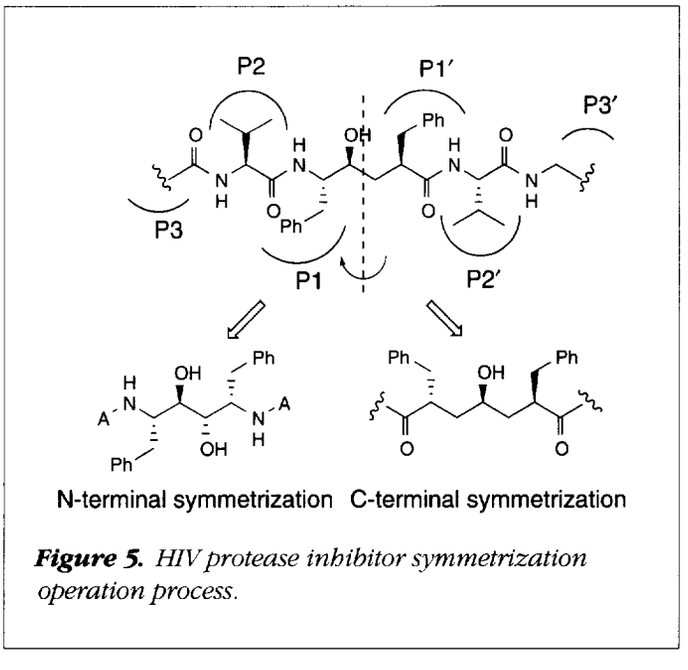
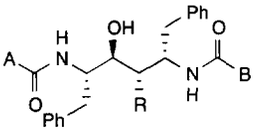
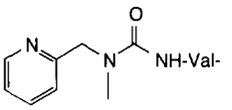
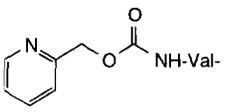
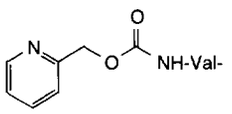
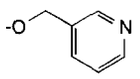
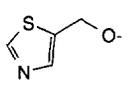
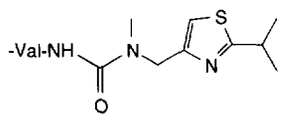


Figure 5. *HIV protease inhibitor symmetrization operation process.*

Table 1. Development of ritonavir from Abbott's first-generation symmetrical compounds



A	B	R	Compound	K_i (nM)[b]	EC_{50} (μM)
$OC(CH_3)_3$	B = A	OH	**5**	40	> 3
-Val-Z[a]	B = A	OH	**6**	0.07	0.016
	B = A	OH	**7** (A77003)	0.15	0.07–0.2
	B = A	H	**8**	< 0.1	0.12
		H	**9** (A80987)	0.25	0.13–0.25
		H	**10** (ABT538)	0.015	0.025

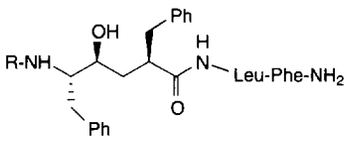
[a] Z = benzyloxycarbonyl.

[b] Inhibition of HIV-1 protease.

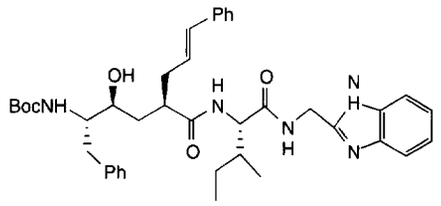
In an attempt to understand the reason for the short duration of **9**, a more in-depth study of its metabolism with liver microsomes indicated that in all species the pyridyl groups underwent extensive oxidation. To circumvent this problem, many compounds were prepared in which the pyridyl groups were replaced with other heterocyclic groups either separately or together. The more potent compounds were then tested *in vitro* and *in vivo* to assess their metabolic profile. This study led to the identification of ABT538 (**10**; ritonavir; Norvir®)[20]. Ritonavir is active against both HIV-1 protease (K_i = 0.015 nM) and HIV-2 protease (K_i = 19 nM) *in vitro*. It is inactive against human pepsin (> 10 μM) and human renin (> 10 μM), but inhibits human cathepsin D (K_i = 20 nM) and human cathepsin E (K_i = 8 nM). It is active against a variety of clinical HIV isolates with EC_{50} in the 0.03–0.12 μM range. Like A80987, ritonavir is highly protein-bound (99%), and the addition of either 50% human serum (HS) or the plasma protein AGP reduces its potency in cell culture by 10–20-fold (apparent EC_{90} = 1.5 μM). Despite the compound having a high molecular weight (721 g/mol) and many amide bonds, ritonavir is highly bioavailable in animals and man. The main reason for this is explained in a recent report[21] that shows that ritonavir is a potent inhibitor of the CYP3A4 enzyme system and shuts down its own metabolism. Although this leads to favorable pharmacokinetics in man, it also causes extensive interactions with more than 23 other drugs that are metabolized by the same system, and requires dose adjustments or drug substitutions in patients taking concomitant therapy.

Indinavir

Merck's initial approach to discovering a lead structure involved screening compounds that had previously been synthesized as inhibitors of the related aspartic acid protease renin. This resulted in the identification of L364505 (**11**, Figure 6 and Table 2; IC_{50}(renin) = 73 nM; IC_{50}(HIVP) = 1 nM) as a promising lead[22]. The compound is considered a heptapeptide and contains a hydroxyethylene isostere as its transition-state mimic. Other stereoisomers tested showed that all stereocenters had to be fixed as drawn or else intrinsic activity was lost. Although it was potent in the enzyme assay, the compound only completely stopped the spread of



11 R = Boc-Phe-Phe (L364505)
12 R = Boc- (L682679)



13 (L687908)

Figure 6. *Discovery of L687908 from renin lead hydroxyethylene isosteres. (See Table 2 for compound details.)*

HIV-1 infection in our cell-based assay (referred to as CIC_{95}) at concentrations > 50 μM (Table 2).

The first goal of the medicinal chemistry program was to diminish the renin activity of this and related compounds without compromising HIV protease activity. It was found that renin activity was abolished by removing the two N-terminal phenylalanine groups. L682679 (**12**) maintained activity towards HIV-1 protease (IC_{50} = 0.6 nM), was devoid of renin activity (IC_{50} > 10 μM) and completely stopped the spread of infection in cell culture with CIC_{95} = 6 μM. Many analogs based on this structure were prepared, and the culmination of this exercise was the discovery of the potent compound L687908 (**13**; IC_{50} < 0.03 nM, CIC_{95} = 12 nM). The compound was not developed further, however, because of low oral bioavailability in animals (< 5%), which was probably a result of too low aqueous solubility and instability of an isoleucine group in the P2 position towards intestinal and liver enzymes.

Table 2. Development of L687908 from renin lead hydroxyethylene isosteres

Compound[a]	IC_{50} (nM)		Inhibition of viral spread (CIC_{95})[b]
	Renin	HIV-1 protease	
11 (L364505)	73	1.0	> 50 μM
12 (L682679)	> 10,000	0.6	6 μM
13 (L687908)	> 10,000	< 0.03	12 nM

[a]See Figure 6 for structures.
[b]Concentration at which the spread of HIV-1 infection is stopped completely (measured by Merck in-house cell-based assay).

A concurrent approach to resolve the latter problem is depicted in Figure 7. A series of compounds related to L682679 was developed which contain a simple benzylamide in the C-terminal portion of the molecule (**14**, R = H). Constraining the benzyl group of **14** as an indane amide to reinforce a bioactive conformation (**15**) led to a fivefold increase in potency. Alternatively, addition of a hydroxymethyl group (which mimics a carbonyl group found in L687908) to the benzyl group of **15** (**16**, R = CH_2OH) resulted in a 2.5-fold enhancement in potency. The combination of a cyclic constraint and *trans*-hydroxy group (**17t**) gave a poorly active compound (IC_{50} = 250 nM). However, a large increase in activity was realized when the hydroxyl group was inverted to afford **17c** (L685434; IC_{50} = 0.3 nM, CIC_{95} = 200 nM). This was the first example of *cis*-1-amino-2-hydroxyindane as a P2′ peptide surrogate[23]. Modeling studies of L685434 docked into the active site of HIV-1 protease indicated that the indane group fitted into a lipophilic P2 pocket, the now *trans*-hydroxyl group formed a hydrogen bond with the Asp29 of the enzyme and the indane amide group interacted with the Gly27 of the enzyme.

Although L685434 no longer contained any natural amino acids, it was not developed further because of its low oral bioavailability in animals. One of the main problems was its poor solubility in aqueous solvents. Upon further analysis of models of L685434 docked into the enzyme active site, it was determined that groups added to the P1 and P1′ phenyl rings would reside outside of the active site in the solvent and therefore should not affect potency. This hypothesis was confirmed by the synthesis of many P1/P1′ analogs containing a solubilizing group, the best being L689502 (**18**; IC_{50} = 0.45 nM, CIC_{95} = 12 nM, oral bioavailability 5% in dogs)[24]. It was entered into safety studies but was not developed further because of liver hepatotoxicity[25]. Despite this setback, the compound represented an important milestone in the program because it showed that oral bioavailability could be achieved in the L685434 series by increasing the acid solubility of compounds. In addition, further studies in this series showed that the toxicity was compound specific and was not a general phenomenon of the series. Finally, this compound was one of the first inhibitors to be cocrystallized with HIV protease and gave us an insight into how this series actually bound into the active site[24]. This structure was

utilized many times to guide us in the design of other potent and novel structures. At that point, the program focused on incorporating acid-solubilizing groups into the series without compromising potency or safety.

A solution to the problem was evident by studying the similarities between saquinavir and L685434. Figure 8 shows a view of how the compounds might actually be similar. Two facts were considered: first, that the active site was symmetric and, second, that compounds could fit in the active site in both directions. It seemed probable that the decahydroisoquinoline (boxed in Figure 8) 'fits' over the Boc-Phe portion of our inhibitors; that is, it occupies the same position in the enzyme, and subsequent modeling studies confirmed that this was possible[26]. It was anticipated that combining the solubility enhancing group from saquinavir with the nonpeptide inhibitor L685434 would afford a novel inhibitor series with different properties. The initial hybrid target, compound **19** (L704486), was synthesized and found to be a good inhibitor (IC_{50} = 7.8 nM, CIC_{95} = 200 nM) and was 15% orally bioavailable in dogs. Further modification of this series was carried out to optimize potency and physical properties. The best compound to emerge from this exploration was L735524 (**20**, indinavir, Crixivan®)[27,28]. It is potent against HIV-1 protease (IC_{50} = 0.4 nM), does not inhibit other mammalian proteases at < 20 μM and is active in cell culture against several clinical isolates (CIC_{95} = 25–100 nM). The compound is not highly bound to plasma proteins (40% free) and its activity is not diminished in cell culture upon the addition of 50% HS or AGP. The bioavailability of the compound in rats, dogs and rhesus monkeys after oral administration as a citric solution is 22%, 70% and 13%, respectively, and is 30–50% in dogs when administered as a free base suspension in 0.5% methocel or as a capsule of the sulfate salt[29].

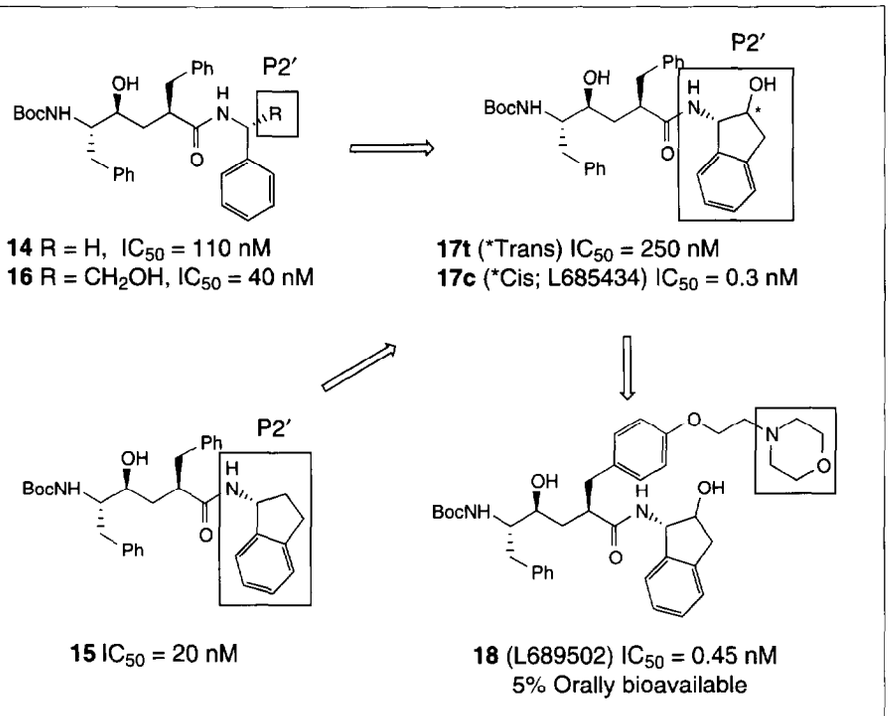


Figure 7. *Design and discovery of Merck's first safety assessment candidate, L689502.*

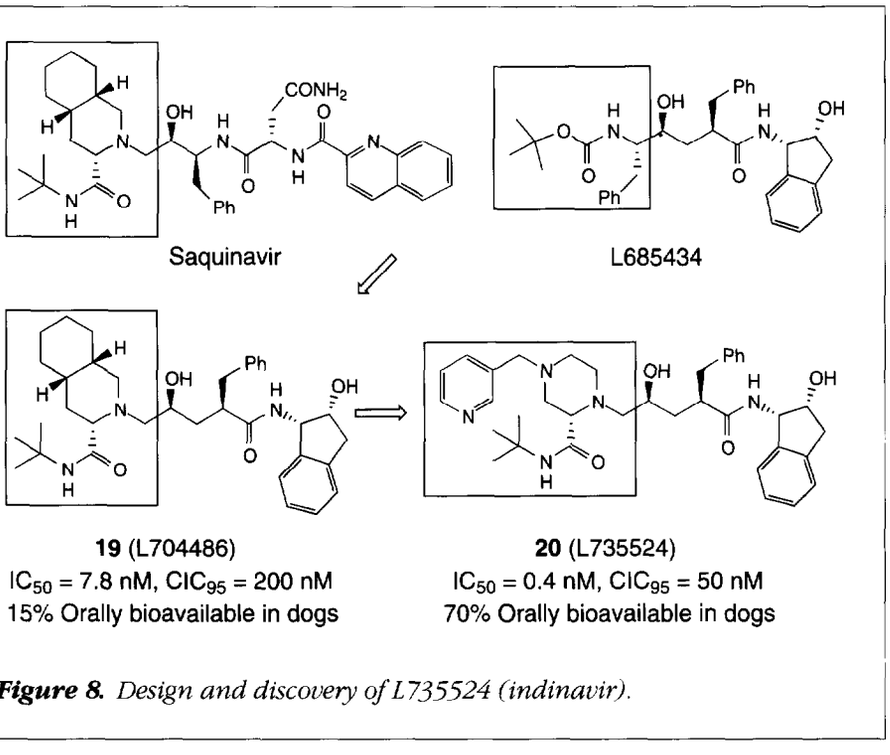


Figure 8. *Design and discovery of L735524 (indinavir).*

Nelfinavir

Nelfinavir (Figure 9) resulted from a collaborative effort between Agouron Pharmaceuticals and Lilly Research Laboratories[30]. The compound's design is based upon the saquinavir structure and contains two key changes. First, the enzymatically labile P3-P2 fragments found in saquinavir were replaced with a simple hydroxytoluene amide group (**21**). Second, to increase the potency lost with this change, the P1 phenyl group was replaced with a phenylthio group, which more efficiently fills a hydrophobic pocket of HIV protease (**22**). These two modifications lead to a dramatic increase in oral bioavailability in animals[31] and humans. Nelfinavir is a potent HIV protease inhibitor with IC_{50} = 2 nM, and is active against a variety of laboratory and clinical HIV isolates with EC_{95} in the 0.007–0.196 μM range. The compound is greater than 98% bound to plasma proteins.

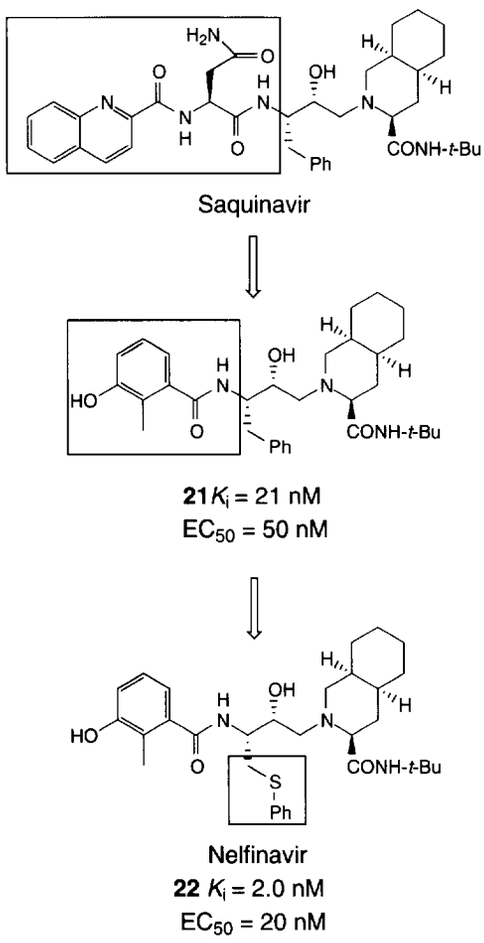


Figure 9. *Design of nelfinavir.*

Summary of clinical results

Saquinavir

The recommended dose for saquinavir (SQV) is 600 mg three times daily (t.i.d.) in combination with one or more nucleoside analogs. The absolute oral bioavailability in human subjects is low, ranging from 1% to 9%, and the compound must be given within 2 h of food for maximum absorption. Saquinavir is generally well tolerated, with gastrointestinal disturbances being the most frequently encountered adverse event. Saquinavir is metabolized by CYP3A4; caution is therefore advised when coadministering saquinavir with other drugs metabolized by this system[32].

In a large clinical study in zidovudine (ZDV) experienced (> 73 weeks) patients, the triple combination of SQV, ZDV and zalcitabine (ddC) had a statistically significant effect on surrogate markers with an increase of 24 CD4 cells/mm^3 and a 0.5 $\log_{10}$ drop in viral RNA from baseline at 24 weeks of therapy. There were no significant differences in CD4 cell counts in either of the ZDV/ddC or ZDV/SQV arms of the study. A 16-week interim analysis of surrogate markers in a clinical endpoint trial in ZDV experienced patients (≥ 16 weeks) showed that the combination of SQV/ddC was associated with greater CD4 cell increases (+40) and drop in viral load (–0.6 $\log_{10}$) compared with SQV (CD4: 0 cells; –0.1 $\log_{10}$) or ddC (CD4: –5 cells; –0.4 $\log_{10}$) alone. A recent analysis of this same trial showed that the combination of SQV and ddC delayed the time to an AIDS-defining event or death by 46%, and the number of deaths by 68% compared with a ddC or SQV monotherapy group[33,34].

Ritonavir

The recommended final daily dose for ritonavir (RTV) is 600 mg twice a day (b.i.d.) once the patient completes a gradual dose escalation schedule from 300 mg b.i.d. The dose escalation is recommended because ritonavir induces its own metabolism after a few days of dosing. Patients who initiate dosing at 600 mg b.i.d. have very high initial ritonavir plasma levels and experience a significant amount of side effects, which subside somewhat once steady-state levels are reached. By initiating dosing at 300 mg and gradually escalating to 600 mg over 7–10 days, steady-state levels can be maintained with a reduction in side effects. The absolute bioavailability of ritonavir in humans has not been determined, but is estimated to be > 90% when taken with food. Adverse events associated with ritonavir therapy include nausea, paresthesia, vomiting and diarrhea[35]. Ritonavir is a potent cytochrome P450 3A4 inhibitor and undergoes many

more drug–drug interactions than either saquinavir or indinavir.

Ritonavir is considered to be a more potent protease inhibitor than saquinavir because of its favorable human pharmacokinetics, and has been approved for use as both monotherapy and in combination with other antiretroviral agents[36]. Study 245 compared RTV monotherapy (600 mg b.i.d.) versus the combination of RTV/ZDV (200 mg t.i.d.) versus ZDV alone (200 mg t.i.d.) in antiretroviral naive patients. An interim analysis carried out at 16 weeks showed mean CD4 cell increases for RTV alone, RTV/ZDV and ZDV alone of 62, 35 and 11 cells/mm^3, respectively. The mean viral RNA load decreases from baseline were: RTV, –1.03 $\log_{10}$; RTV/ZDV, –0.80 $\log_{10}$; ZDV, –0.42 $\log_{10}$. In another study, the addition of ZDV and ddC to 14 days of RTV monotherapy produced median increases in CD4 levels of 83–106 cells/mm^3 and decreases of viral RNA of 1.63–1.92 $\log_{10}$ after 20 weeks of therapy. Finally, in a clinical endpoint study, RTV or placebo was added to existing therapy taken by advanced AIDS patients with prior antiretroviral experience. At 6 months' follow-up, the incidence of clinical event or death was reduced by 50% in the RTV group compared with the placebo group. There was a 40% reduction in mortality for patients taking RTV compared with those randomized to placebo.

Indinavir

The daily recommended dose for indinavir (IDV) is 800 mg every 8 h on an empty stomach or with a light, low-fat meal. The absolute oral bioavailability of indinavir is 60%. Nephrolithiasis has been reported in approximately 4% of patients receiving indinavir in clinical trials. In general, these events were not associated with renal dysfunction and resolved with temporary stoppage of treatment. Patients are advised to maintain adequate hydration to minimize the occurrence of nephrolithiasis. Indirect hyperbilirubinemia has occurred in approximately 10% of patients taking indinavir; however, there has been no clinical significance or evidence of hepatic involvement or failure associated with the incidence of hyperbilirubinemia. Indinavir is a good substrate for the CYP3A4 isozyme; it has the ability to affect plasma levels of other drugs metabolized by this system but has few clinically significant drug interactions[37,38].

Indinavir, like ritonavir, is considered to be a much more potent HIV protease inhibitor than saquinavir, again resulting from a better pharmacokinetic profile. Indinavir has been approved for use as both monotherapy and in combination with nucleoside analogs. Protocol 035 studied the combinations of AZT/3TC (lamivudine) versus IDV/AZT/3TC and IDV monotherapy in 3TC and IDV naive patients who had extensive ZDV experience. At 24 weeks, patients in both IDV-containing treatment arms had a mean 100 CD4 cell increase from baseline compared with the ZDV/3TC arm, which had a 33 cell increase. The triple combination produced a mean 1.77 $\log_{10}$ decrease in viral RNA from baseline, while IDV monotherapy gave a 1 $\log_{10}$ drop and ZDV/3TC patients experienced a 0.75 $\log_{10}$ drop in viral load. Of those patients on the triple combination, 90% had a reduction of virus levels below the level of detection (≤ 500 copies/ml) compared with 35% of the patients on IDV alone and 0/19 (0%) on ZDV/3TC.

Nelfinavir

Nelfinavir (NFV) is the most recent HIV protease inhibitor to gain FDA approval. The recommended dose is 750 mg t.i.d. with food. Under this regimen, steady-state peak plasma concentrations of 5.3–7 μM and trough concentrations of 1.76–5.3 μM have been achieved. The adverse events reported are mainly nausea and diarrhea. Nelfinavir is an inhibitor of CYP3A4 at concentrations in the therapeutic range and has the ability to affect plasma levels of other drugs metabolized by this system[39].

Nelfinavir, like indinavir and ritonavir, is considered to be a more potent HIV protease inhibitor than saquinavir, again because of a better pharmacokinetic profile. Nelfinavir has been approved for use as both monotherapy and in combination with nucleoside analogs in both adults and children. Early dose-ranging clinical trials were encouraging[40,41] and supported large-scale pivotal studies. Study 511 studied the combinations of AZT/3TC (lamivudine) versus two doses (500 or 750 mg t.i.d.) of NFV in combination with AZT/3TC in antiretroviral naive patients. A branched DNA signaling assay was used to estimate the levels of circulating viral RNA and the lower limit of detection was 1,200 copies/ml. At 24 weeks, patients in both triple combination arms had a 1.7 $\log_{10}$ drop in viral RNA compared to a 1.3 $\log_{10}$ drop for those patients in the double nucleoside arm of the study. Plasma viral RNA levels of 1,200 copies/ml were assigned to the following proportions of patients in the indicated groups: 59/79 (74%) in 500 mg NFV/AZT/3TC; 73/83 (88%) in 750 mg NFV/AZT/3TC; and 30/84 (36%) in AZT/3TC. Study 506 compared the combination of NFV (500 or 750 mg t.i.d.) in combination with stavudine (d4T) versus d4T alone in a patient population in which 20% of the patients were

antiretroviral naive. At 24 weeks, 27% of the patients in either combination arm had plasma RNA levels assigned a value of 1,200 copies/ml versus 14% in the d4T monotherapy group.

141W94 (VX478)

One other HIV protease inhibitor currently undergoing extensive clinical investigation is 141W94 (VX478, Glaxo Wellcome/Vertex[42,43]; Figure 10), which was discovered by scientists at Vertex Pharmaceuticals and is licensed to Glaxo Wellcome for development. The compound was derived by first replacing the decahydroisoquinoline group in saquinavir with an isobutylphenyl sulfonamide group (**23**). This design reduced the number of chiral centers in saquinavir from six to three and enabled the rapid synthesis of many analogs. To further reduce the compound's complexity and peptide character, the P3-P2 groups were replaced with a tetrahydrofuran carbamate which was first discovered by chemists at Merck[44] as a P2 replacement in a series of saquinavir analogs. Finally, an amino group was introduced into the phenylsulfonamide group to increase water solubility and enhance oral absorption. The final compound, 141W94 (VX478), is potent against HIV-1 protease (K_i = 0.6 nM) and has reasonable specificity against other mammalian aspartic proteases (pepsin, K_i = 3,200 nM; cathepsin D, K_i >10,000 nM; renin, K_i = 1,750 nM), and is also active in cell culture against a number of cell-based lines (IC_{50} 0.01–0.08 μM). 141W94 is about 90% protein bound, mostly to AGP. Despite the high protein binding, researchers at Vertex have reported that the compound's activity in cell culture is only diminished fourfold[45]. Finally, the compound has good oral bioavailability in animals (40–70%) and this has been confirmed in man[46]. A recent report[47] from a dose-ranging clinical trial has shown that the compound had an antiviral effect at 4 weeks (1.55 $\log_{10}$ drop in viral load) when given at a dose of 1,200 mg b.i.d. Larger trials are in progress to assess the compound's long-term effects on surrogate markers and clinical outcomes.

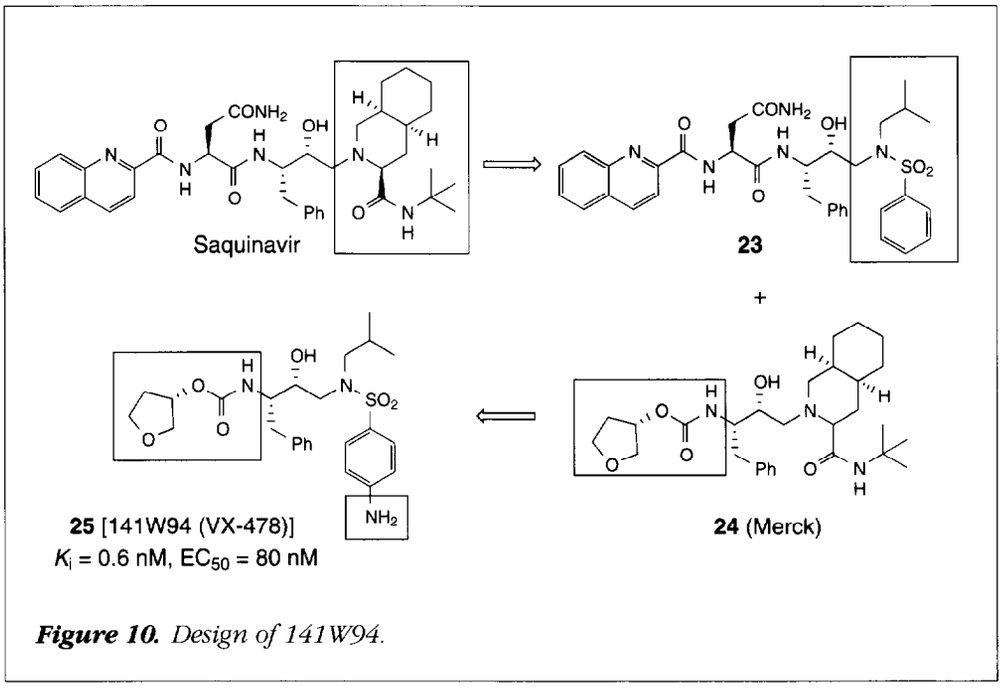


Figure 10. *Design of 141W94.*

Resistance

HIV-1 replication in the presence of protease inhibitors has been shown to select for viral variants with varying degrees of resistance to those inhibitors[48]. This development of resistance has been observed primarily when plasma drug levels are insufficient to block viral replication effectively. In contrast, potent inhibition of replication has been shown to suppress dramatically the emergence of drug resistance, thereby prolonging the benefits of therapy.

As would be expected from the structural differences between these inhibitors, different drugs select for differing, although highly overlapping, constellations of amino acid substitution mutations affecting the substrate binding sites. These include substitutions of V82, I84, or L90 (indinavir or ritonavir), G48, I84, or L90 (saquinavir), D30, I84, or L90 (nelfinavir).

In addition to these sites, all protease inhibitors that have been extensively studied have been shown to select for overlapping sets of multiple amino acid substitutions elsewhere in the enzyme. Although the mechanisms by which they contribute to resistance are not well understood, the involvement of many of these sites in phenotypic resistance has been shown by site-directed mutagenesis studies. These sites include L10, M46, L63, A71, and N88, among others. A summary, to date, of the amino acid residues implicated in resistance to the five major HIV-1 protease inhibitors is shown in Figure 11, together with the relevant references.

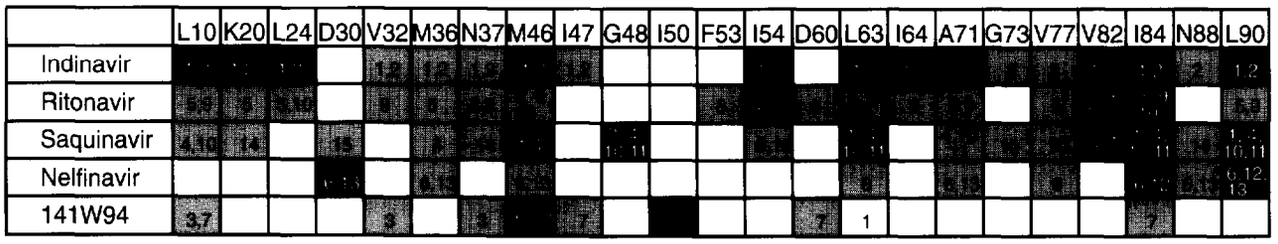


Figure 11. *Genetic overlap between determinants of resistance to protease inhibitors. Heavy shade, verified phenotypically; light shade, selected for but not verified phenotypically; no shade, not (yet?) implicated. The numbers in the chart refer to the references listed below.*

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11 Vaillancourt, M. *et al.* (1995) *J. AIDS* 10, 74
12 Patick, A. *et al.* (1996) *Antimicrob. Agents Chemother.* 40, 292–297
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14 Race, E. *et al.* (1997) *4th Conference on Retroviruses and Opportunistic Infections*, p. 175 (Poster 600)
15 Craig, C. *et al.* (1996) *XI International Conference on AIDS* (unscheduled poster and handout)

Given the extensive overlap among the mutations selected by the various protease inhibitors, it is not surprising that each drug has been shown to select for variants cross-resistant to others. Among the three most widely used (and most extensively studied) protease inhibitors – indinavir, ritonavir, and saquinavir – each has been shown to select, in some patients, viral variants that are cross-resistant to the other two. Although only limited clinical data for nelfinavir and 141W94 are available so far, each has also been reported to select for cross-resistant viruses in cell culture.

Although the selection for cross-resistant viruses appears to be a general property of HIV-1 protease inhibitors, the clinical significance of this observation is unknown. In particular, it is not known whether the development of resistance to one protease inhibitor will compromise the later clinical benefit of another. Accordingly, until such information is available, caution is warranted if sequential use of any of these inhibitors is contemplated.

Conclusion

In summary, all of the research groups have approached the discovery of potent and orally bioavailable HIV protease inhibitors in different ways. All have dealt with similar problems and eventually overcome them using a combination of classical medicinal chemistry in conjunction with molecular modeling. Unfortunately, this review is but a snapshot of the overall struggle to overcome these problems. In reality, thousands of people-hours went into the basic research programs that eventually discovered these compounds. Judging by the patent literature, over 20 pharmaceutical companies have had active research programs in this area at one time or another and more than 15 agents are known to have entered clinical trials. Two-thirds have failed because of toxicity, poor pharmacokinetic properties, or lack of efficacy. The compounds that did succeed are some of the most complex molecules ever synthesized on a tonne scale. Finally, none of this would have been possible without the cooperation of the patients and healthcare professionals. The new class of protease inhibitors represents a significant advance in the treatment of AIDS and expanding the arsenal of drugs for patients and physicians to choose from. The main challenge facing everyone is to determine the best way to use these agents in combination in order to maintain viral loads at a minimum and hold off the possibility of resistance.

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