Annu. Rev. Med. 2002. 53:595–614

RATIONAL APPROACH TO AIDS DRUG DESIGN THROUGH STRUCTURAL BIOLOGY*

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■ **Abstract** The discovery and development of more than a dozen drugs in the past 15 years for the treatment of AIDS offer an excellent example of progress in the field of rational drug design. At this time, the principal targets are reverse transcriptase and protease, enzymes encoded by the human immunodeficiency virus. The introduction of protease inhibitors, in particular, has drastically decreased the mortality and morbidity associated with AIDS. This review presents the methods used to develop such drugs and discusses the remaining problems, such as the rapid emergence of drug resistance.

INTRODUCTION

The structure-assisted ("rational") drug design and discovery process (1–3) utilizes techniques such as protein crystallography, nuclear magnetic resonance (NMR), and computational biochemistry to guide synthesis of potential drugs. The structural information is used to help explain the basis of effective inhibition and to improve the potency and specificity of new lead compounds. The term "rational drug design" is, however, often misinterpreted. There is nothing irrational about traditional methods, such as screening of compound libraries, and indeed, screening and design are often used together. The complementary methods of computer-aided molecular design (4) and combinatorial chemistry (5) are now routinely employed in both the lead identification and the development phases of drug design. These different approaches have been successfully combined in many cases, although rational drug design is a much more recent phenomenon than screening or serendipity-based techniques.

Many structure-based techniques of drug discovery and development have evolved in the past 20 years during the search for therapeutically useful agents in the treatment of acquired immunodeficiency syndrome (AIDS). This major epidemic is caused by two variants of the human immunodeficiency virus, HIV-1 and HIV-2. The complete nucleotide sequence of HIV-1 (6) shows a relatively simple retrovirus whose genome consists of three open reading frames (ORF), *gag*, *pol*,

and *env*. The *gag* ORF contains structural proteins such as capsid, nucleocapsid, and matrix, whereas regulatory proteins are encoded in the multiply spliced *env* ORF. The HIV-1 genome encodes only three unique enzymes, all located within the *pol* ORF. These enzymes, reverse transcriptase (RT), integrase, and protease (PR), have all become targets for drug discovery.

The first AIDS drugs to be identified were nucleoside inhibitors of RT (Figure 1*a*), discovered and developed long before the structure of RT itself was solved (7, 8). However, the development of newer RT-targeted drugs, nonnucleoside inhibitors, is closely coupled to structural investigations of enzyme complexes. Even now, only fragmentary structural data have been described for integrase (9–12); and extensive efforts to discover integrase inhibitors (13) have as yet produced no clinical candidates. Retroviral protease, however, was identified as a potential target early on (14, 15), and the discovery and development of its inhibitors (reviewed in 16–23 and discussed below) exemplify an unqualified success of modern pharmacology and structural biology. Selected aspects of the development of RT-targeted drugs are also mentioned in this review.

DEVELOPMENT OF THE INHIBITORS OF HIV PROTEASE

HIV-1 PR (Figure 1*b*) shares considerable similarity (24) with the much-studied family of aspartic proteases, which includes such mammalian enzymes as pepsin, renin, and chymosin (25). Pepstatin, a signature inhibitor of aspartic proteases (15, 26), inhibits HIV-1 PR and other retroviral proteases; in addition, they are inactivated by mutation of the active-site aspartates (15, 27). Inactivation of HIV-1 PR by either mutation or chemical inhibition leads to the production of immature, noninfectious viral particles (15, 27); thus, the function of this enzyme was shown to be essential for proper virion assembly and maturation. For such reasons, HIV-1 PR became an important target for drug design, and between 1995 and 2001 the U.S. Food and Drug Administration (FDA) has approved six drugs targeting this enzyme, with more certain to come.

Crystallographic and NMR Studies of Retroviral Proteases

The availability of crystal structures of HIV-1 PR was an important reason for the rapid progress in drug development. The structure of the uninhibited enzyme was determined independently in several laboratories (28–30). Crystal structures of HIV-2 PR (31, 32) and simian immunodeficiency virus (SIV) PR (33, 34) also became available. The structures of many complexes of HIV and SIV PRs with inhibitors have been reported in a number of crystal forms at resolutions up to 1.55 Å (35). More than 180 structures of the complexes of HIV-1, HIV-2, and SIV PRs with inhibitors are publicly available in an Internet-accessible database (36). The structures of inhibitor complexes of HIV-1 PR have also been studied using NMR (37–40). NMR combined with computational methods confirmed that a combination of computational models and simulations, along with NMR data,

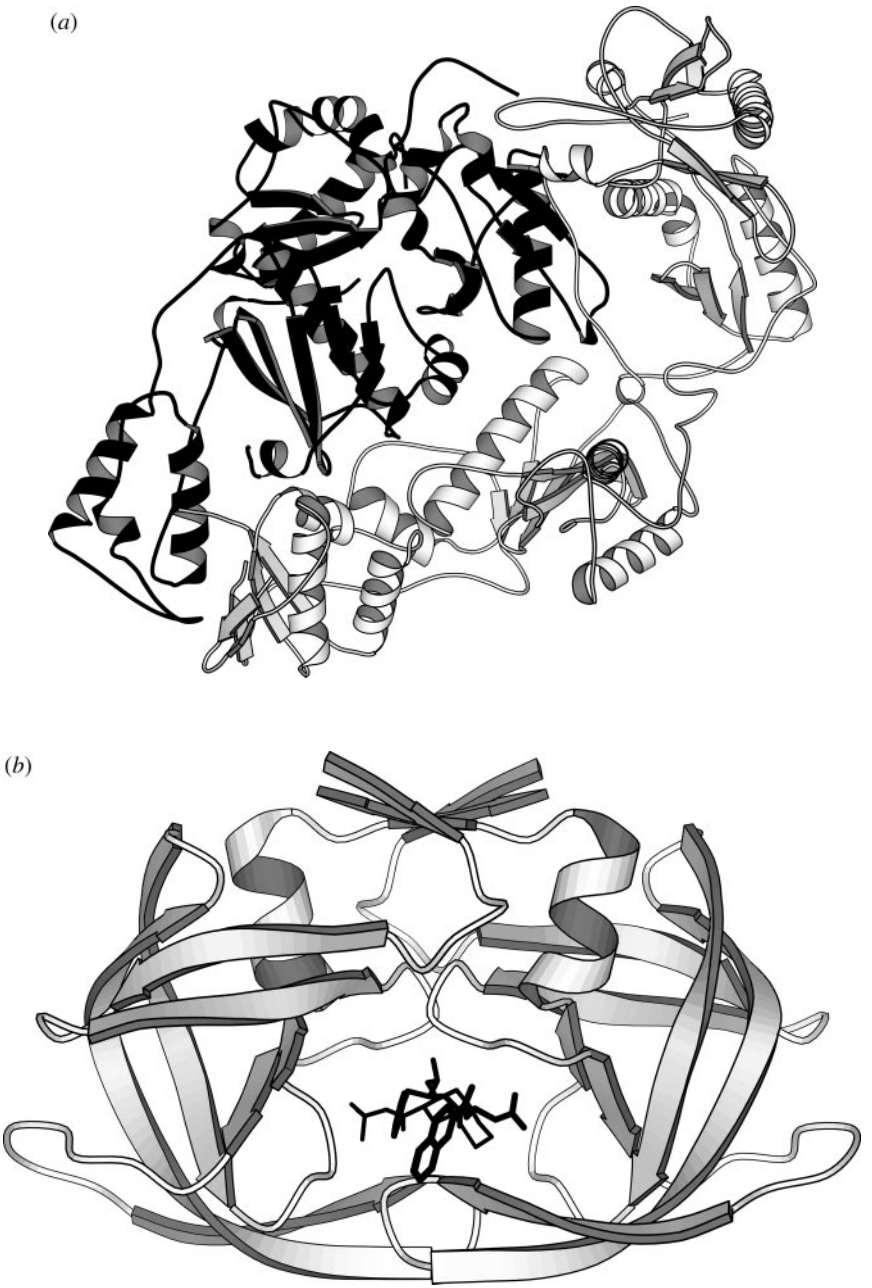

Figure 1 Ribbon diagrams of the enzymes that are targets for the approved AIDS drugs. (*a*) HIV reverse transcriptase (RT), with its active p66 domain shown in grey and the smaller p51 domain in black. (*b*) HIV protease (PR) complexed with the drug saquinavir (in black stick representation). The loops on the right side that cover the inhibitor are the flaps (see text).

can provide a basis for further modification and design (41). The ionization state of the catalytic residues was studied by NMR, using chemically synthesized HIV-1 PR in which the Asp-25 in each monomer was specifically labeled with ^{13}C (42).

The Structure of HIV-1 PR and Enzyme-Inhibitor Interactions

A molecule of HIV-1 PR is composed of two identical protein chains, each containing 99 amino acids (Figure 1*b*). Each monomer contains nine β strands and a single helix, arranged with internal pseudosymmetry (29). Four antiparallel β strands, two from each molecule, form the principal dimer interface. Another two β strands lead to the active-site triplet (Asp25-Thr26-Gly27). Four of the β strands in the molecular core are organized into a Ψ-shaped sheet characteristic of all aspartic proteases. The active-site triplet is located in a loop whose structure is stabilized by a network of hydrogen bonds. The carboxylate groups of the active-site residues, Asp25 from both chains, are nearly coplanar and in close contact. The active site is covered by two symmetric flaps that change their conformation between the free and inhibited enzymes. This structural feature is different from the single flap present in pepsins. The area adjacent to the active site is the most rigid and most highly conserved in the whole molecule (16, 35), whereas the flaps are the most dynamic. The areas leading to the flaps have been implicated in facilitating motions necessary to allow substrate entry and release (43).

Most of the inhibitors cocrystallized with HIV PR, including all petidomimetic inhibitors, are bound in the protease active site in an extended conformation (16). The hydrogen bonds are made mostly between the main chain atoms of both the enzyme and the inhibitor and follow a similar pattern. The nonhydrolyzable scissile bond analogs of each inhibitor align with the active-site aspartate carboxyl groups Asp25/Asp25′. The hydroxyl group at the nonscissile junction, present in many inhibitors, is positioned between the PR aspartate carboxyl groups, within hydrogen bonding distance of at least one carboxylate oxygen of each aspartate. A feature common to almost all complexes of HIV-1 PR is a buried water molecule that bridges the P2 and P1′ CO groups of the inhibitor and the Ile50 and Ile50′ NH groups of the flaps. This water is approximately tetrahedrally coordinated and is completely inaccessible to solvent (44). The functional substitution of this water led to the design of a number of inhibitors (see below).

Several distinct subsites (45) that accommodate side chains of the inhibitors can be identified in HIV PR. Owing to the symmetry of the enzyme, subsites on both sides of the peptide bond replacement are practically the same, with only three (S1–S3) well-defined. The protease side chains comprising the pockets S1 and S1′, with the exception of the active-site aspartates, are mostly hydrophobic; thus, these subsites are usually occupied by hydrophobic residues, often large. Although the S2 and S2′ pockets are hydrophobic, both hydrophilic and hydrophobic residues can occupy these sites. The subsites S3 and S3′ can accommodate a variety of residues, with much less specificity, while the parts of the inhibitors extending further are binding, if at all, largely on the surface of the protein.

Computational Studies of the Inhibitor Complexes of HIV-1 PR

Whereas structural studies of HIV-1 PR and its inhibitor complexes have played a very important role in drug design, computational studies were necessary to understand the mode of binding and to optimize inhibitor design. Studies that involved energy minimization using molecular mechanics (46, 47) have shown that the calculated contribution of the main-chain atoms to the total interaction energy ranged from 56% to 68%, providing a substantial contribution to the total binding energy. A study aimed at predicting the activity of HIV-1 PR inhibitors resulted in a high correlation between the interaction energy and the experimentally determined IC_{50} constants for almost 50 inhibitor-enzyme complexes (48). Free energy perturbation calculations coupled with molecular dynamics have been relatively reliable in predicting the free energy of binding by computational methods (49, 50). Such studies were also performed to explain the differences between the binding constants of similar inhibitors (51, 52), to analyze HIV-1 PR mutants and their affinity to different inhibitors (53), or to aid molecular modeling and drug design (54).

A number of docking procedures based on the crystal structures of the inhibitor complexes of HIV-1 PR have been reported. Docking methods and algorithms were tested using the structural data and experimental characteristics by Monte Carlo docking (55) or by comparison with de novo constructed inhibitors using the fragment-based method (56). Other studies examined empirical free energy as a target function in docking and design, showing the advantages of this approach over the calculation of interaction energy (57). These and similar studies have played major roles in the design of a variety of inhibitors, some of which became clinical candidates.

PROTEASE INHIBITORS IN CLINICAL AND PRECLINICAL USE

Several inhibitors of HIV-1 PR (Figure 2) are already in use or in clinical trials as anti-HIV drugs; more are under way (58). Indeed, the availability of these inhibitors has drastically changed the course of the disease and significantly decreased its associated mortality and morbidity (59). Chemical structures of all current clinical inhibitors of HIV-1 PR, and of selected inhibitors that are in advanced clinical trials, are shown in Figure 2.

Saquinavir

Saquinavir (Ro 31-8959, Invirase, Fortavase), from Hoffmann-La Roche, was designed in a rational drug design program initiated with peptide derivatives that were transition-state mimics (60). The basic design criterion relied on the observation that HIV-1 PR cleaves the sequences containing dipeptides Tyr-Pro or Phe-Pro.

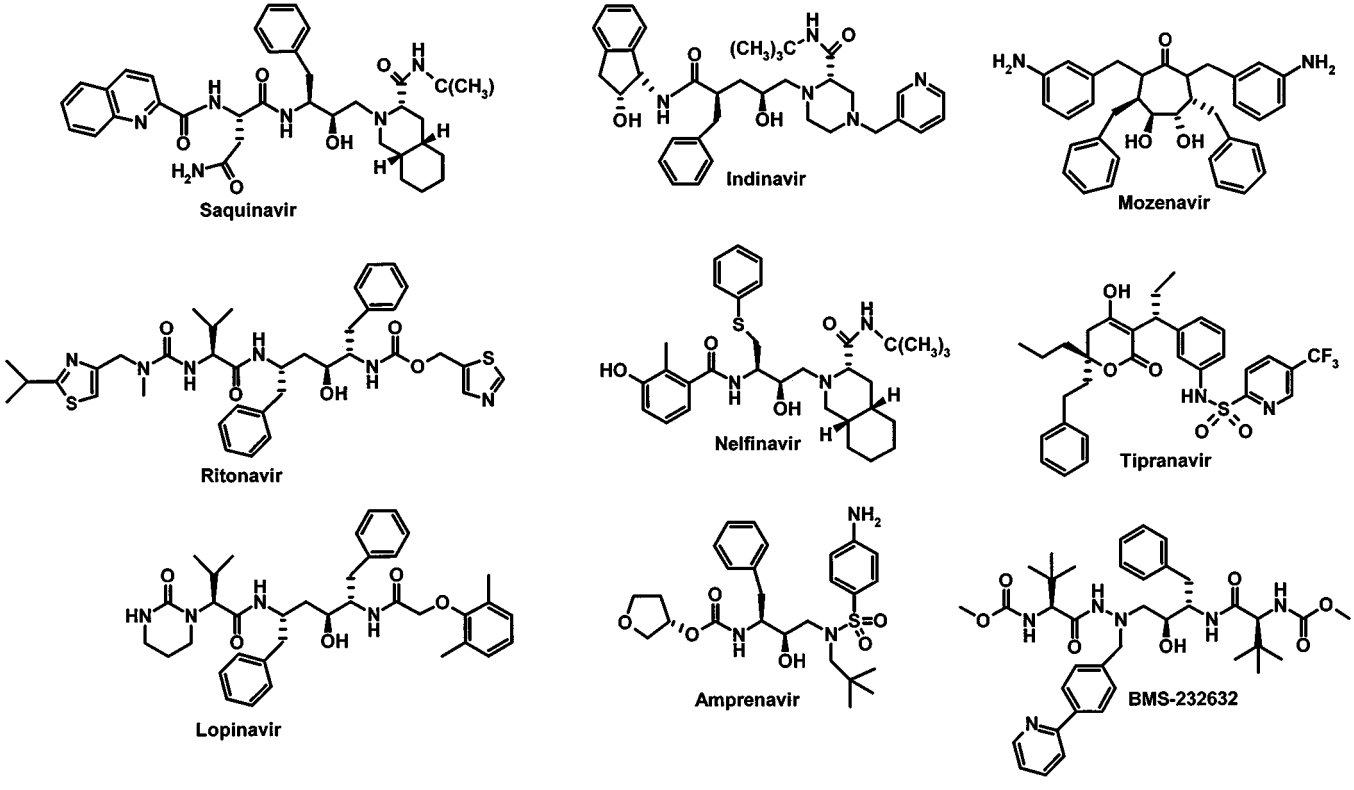

Figure 2 Chemical formulas of the six FDA-approved inhibitors of HIV-1 PR and of other selected inhibitors that are currently in advanced clinical trials.

Mammalian proteases do not cleave peptide bonds followed by a proline; thus, this target promised selectivity. Because reduced amides and hydroxyethylamine isosteres most readily accommodate the imino acid moiety, they were chosen for further studies.

A peptidic inhibitor, Ro 31-8558, was studied crystallographically in complex with HIV-1 PR, showed the expected mode of binding and suggested possible future modifications (61). Replacement of a proline at the P1′ subsite by (S,S,S)-decahydro-isoquinoline-3-carbonyl (DIQ) significantly improved the potency of the inhibitors. A compound that included such a modification (Ro 31-8959, saquinavir) (60) exhibited K_i of 0.12 nM at pH 5.5 against HIV-1 PR and was even better against HIV-2 PR ($K_i < 0.1$ nM). It was shown to be highly selective, causing only minor inhibition of human aspartic proteases. Crystallographic study of saquinavir has shown that the carbonyl of the DIQ group is able to maintain the hydrogen bond between the water molecule connecting the inhibitor with the flap regions (Wat301) (62). In 1995, saquinavir became the first protease inhibitor approved by the FDA. It is available in two forms: as Invirase in hard-gel formulation and as Fortovase in soft-gel capsules. The latter formulation considerably increased the bioavailability of the drug.

Ritonavir

The initial basis of the design and development efforts that led to the discovery of Abbott's ritonavir (ABT-538, Norvir) was the symmetry of HIV-1 PR, and the first Abbott inhibitors were also symmetric (63, 64). These inhibitors showed good kinetic profiles but poor bioavailability. In order to enhance the aqueous solubility, the terminal phenyl residues were modified to pyridyl groups (65). A lead compound, A-77003, revealed broad-spectrum activity against both HIV-1 and HIV-2 in a variety of transformed and primary human cell lines. However, crystallographic studies of inhibitor complexes of HIV-1 PR have shown that even symmetric inhibitors may bind to HIV-1 PR in an asymmetric fashion (66). In addition, imposition of symmetry made it difficult to improve bioavailability, which is often influenced by the termini of the inhibitor. For these reasons, a development process that started from symmetric or pseudosymmetric inhibitors led to compounds with significant asymmetry. The knowledge of the superior potency of the deshydroxy diols finally led to creation of a series of compounds, one of which was ABT-538 (ritonavir) (67), ultimately approved by the FDA as Norvir.

It was later shown that ritonavir potentiates the activity of other protease inhibitors, most likely by inhibiting the cytochrome P450 (CYP)-mediated metabolism (68). Because of this property, ritonavir was included in the formulation of a second-generation protease drug, Kaletra (see below).

Indinavir

The development of indinavir (MK-639, L-735,524, Crixivan) by Merck was based on a transition-state mimetic concept (69), previously utilized in design of renin

inhibitors (70). A series of peptidomimetic inhibitors of different lengths, containing a hydroxyethylene isostere in an *S* configuration, was examined, with a number of substitutions of the side chains. Another approach to the design of nanomolar inhibitors of HIV PR led to a series of analogs of L-682,679 in which the carboxyl terminus had been shortened and modified (71). Inclusion of (aminomethyl) benzimidazole provided the most potent compounds in that series, as the imidazole portion appeared to be mimicking a carboxamide, while the phenyl portion was probably contributing additional hydrophobic binding. Some of the inhibitors with excellent IC_{50} values were considerably less potent in cell culture, presumably because of their inability to penetrate the hydrophobic cell membrane. The important conclusion was that as the terminal amide increased in size or polarity, the intrinsic potency improved but not the minimum inhibitory concentration.

The design of indinavir was guided by molecular modeling and X-ray crystal structure of the inhibited enzyme complex (72, 73). This potent inhibitor was highly active against HIV-1 and HIV-2 PRs, both in enzymatic and in tissue culture assays. Indinavir (Crixivan) was orally bioavailable in three animal models and gained FDA approval at the beginning of 1996.

Nelfinavir

Agouron (now part of Pfizer) was one of the first companies established in the 1980s for the explicit purpose of creating drugs through rational design, and nelfinavir (AG-1343, Viracept) is one of the first products of such companies to reach the stage of FDA approval. Nelfinavir was also the first PR drug that was not a peptidomimetic, although peptidomimetics were created along its development pathway. Two early Agouron inhibitors, AG-1002 and AG-1004, had statine isosteres instead of normal peptide bonds in their central parts (74, 75). The best inhibitor in that series had an inhibition constant of about 30 nM.

Iterative protein cocrystal structure analysis of peptidic inhibitors and the replacement of parts of the inhibitors by nonpeptidic substituents (76) were used to design orally bioavailable, nonpeptidic inhibitors. A Monte Carlo program that could generate ligands was used to fill the S1 subsite (77), resulting in the placement of a large cyclopenthylethyl group in this position. Combining the 5-chloro with the dimethylbenzyl and cyclopentyl amides resulted in the best compound in this series, with K_i of about 2 nM, comparable with saquinavir in efficiency.

The final steps in the design of nelfinavir have been described in some detail (78). Nelfinavir contains a novel 2-methyl-3-hydroxybenzamide group, whereas its carboxyl terminus contains the same DIQ group as saquinavir (see above). However, nefinavir is a mesylate salt of a basic amine of DIQ. The P1 subsite is occupied by the S-phenyl group, increasing the potency by an order of magnitude. Nelfinavir's mode of binding to HIV-1 PR was verified by determination of the crystal structure of the complex (78). Nelfinavir (Viracept) was approved by the FDA in 1997 and was the first protease inhibitor to be indicated for pediatric AIDS.

Amprenavir

Amprenavir (VX-478, 141W94, Agenerase), from Vertex Pharmaceuticals and GlaxoSmithKline, shares some structural features with the other successful protease inhibitors described above. Its central core is identical to that of saquinavir, although both ends are quite different. The P2 group consists of tetrahydrofuran carbamate, whereas the P1′–P2′ moieties consist of an isobutylphenyl sulfonamide with an added amide. This design gives amprenavir fewer chiral centers than saquinavir, facilitating synthesis and increasing water solubility to allow oral bioavailability as high as 40%–70% (79). The crystal structure of HIV-1 PR with bound amprenavir has been reported (80). Amprenavir is quite potent against HIV-1 PR ($K_i = 0.6$ nM), whereas its inhibition constant against human aspartic proteases is low. It was also shown to be very potent in vitro against a variety of clinical isolates of HIV-1 (79). Early clinical results indicated that amprenavir was very potent and well-tolerated (81). Amprenavir in a combination with two nucleoside RT inhibitors was more potent than these two inhibitors alone, although less effective than indinavir (82). The necessary dose of amprenavir could be reduced by coadministration with ritonavir (also see below). Prodrug formulations of amprenavir (GW433908) are being developed.

Kaletra

Kaletra, developed at Abbott, was approved by the FDA in the second half of 2000; it is the first second-generation protease inhibitor to reach drug status. It is a mixture of two protease inhibitors: a novel compound, lopinavir (ABT-378), and a smaller amount of ritonavir (a typical pill combination is 133 mg of the former and 33 mg of the latter).

Lopinavir was originally designed to diminish the interactions of the inhibitor with Val82 of HIV-1 PR, a residue that is often mutated in the drug-resistant strains of the virus (83). The core of lopinavir is identical to that of ritonavir. The 5-thiazolyl end group in ritonavir was replaced by the phenoxyacetyl group, and the 2-isopropylthiazolyl group in rotonavir was replaced by a modified valine in which the amino terminus had a six-membered cyclic urea attached. The chemical (84) and therapeutic (85) properties of lopinavir have been reviewed elsewhere. Lopinavir is a very powerful competitive inhibitor of HIV-1 PR ($K_i = 1.3$ pM) against wild-type enzymes, and still excellent against a number of mutants (83). As expected based on the design criteria, lopinavir exhibited much higher potency against a number of drug-resistant mutants of HIV-1 PR than did the competing inhibitors, although new mutants with increased resistance to that compound were observed early on (86). The plasma level of lopinavir decreased very quickly in rats and even faster in humans, but in the presence of subclinical amounts (50 mg) of ritonavir, lopinavir concentration exceeding EC_{50} could be maintained for over 24 h after a single 400 mg dose (84). Kaletra is now considered an important salvage drug, administered after the primary therapy with protease inhibitors has failed.

Other Inhibitors in Clinical Trials

One of the unexpected structural features of complexes between HIV-1 PR and the inhibitors is a conserved water molecule that mediates the contacts between the P2/P1′ carbonyl oxygen atoms of the peptidic inhibitors and the amide groups of Ile50/Ile50′ of the enzyme. Replacement of the tetrahedrally coordinated Wat301 was proposed early on as a possible way of making highly specific protease inhibitors (87), and several groups implemented that suggestion. Scientists from DuPont Merck and from the University of Uppsala designed molecules with a seven-membered cyclic urea ring as the starting pharmacophore (88, 89). Some such inhibitors were symmetric and others were not (90). One of these inhibitors, DMP-450 (mozenavir), with excellent inhibitory properties ($K_i = 0.3$ nM) and high potency against the virus in cell culture, was shown to be orally available in humans (91). As of spring 2001, this inhibitor was in phase I/II clinical trials conducted by Triangle Pharmaceuticals.

A very different inhibitor of HIV-1 PR was created at Pharmacia and is being further developed by Boehringer-Ingelheim. Tipranavir (PNU-140690) is an entirely nonpeptidic compound with $K_i = 5$ pM and $IC_{90} = 100$ nM in antiviral cell culture (92). The discovery of tiprinavir followed a broad screening program that identified a small molecule, phenprocoumon, as a possible template for designing inhibitors of HIV PR. Several potential clinical candidates, each more potent than the last, originated from this combination of screening and rational drug design. Crystal structure analysis of tipranavir has shown that despite its structural differences from the peptidic inhibitors, its mode of interaction with HIV PR is similar in many respects. The lactone oxygen atom of the dihydropyrone ring forms hydrogen bonds with the amide nitrogen atoms of the flap residues, in a manner analogous to Wat301. The 4-hydroxy group is bonded in a pseudosymmetric manner to the two active-site aspartates. This drug is soluble and highly bioavailable (93), and clinical trials are under way. It appears to exhibit significant activity against HIV-1 isolated from patients with multidrug resistance to other protease inhibitors (94).

An azapeptide inhibitor of HIV-1 PR is being developed by Bristol-Myers Squibb under the name of BMS-232632 (95). The principal advantage of this potential drug is its once-daily dosing and lower toxicity. Its most likely future use is in multidrug combination therapy.

DRUGS TARGETING REVERSE TRANSCRIPTASE

Reverse transcriptase is a heterodimer consisting of two chains, p66 and p51 (Figure 1*a*), encoded by the same gene belonging to the *pol* ORF. The larger chain contains an additional RNase H domain; the rest of the sequence (but not the three-dimensional structure) is the same. The active sites for both primary activities of the enzyme (RNA- or DNA-dependent DNA polymerase, and RNase H) are contained in the p66 domain. The structures of RT have been solved (*a*) in the presence of nonnucleoside inhibitors (see below) (7); (*b*) in a ternary complex

with a bound template:primer and Fab (8); (*c*) for the apoenzyme (96); and (*d*) as a covalently trapped catalytic complex with a DNA template:primer and a deoxynucleoside triophosphate (97), among others.

RT inhibitors can be divided into two general classes. The first to be discovered were compounds that act as terminators of chain elongation. These analogs of the nucleoside substrates bind in the substrate-binding site and can inhibit both HIV-1 and HIV-2 RT. Another class of RT inhibitors, nonnucleoside inhibitors (NNIs), are specific to a pocket that is found in the vicinity of the active site in HIV-1 RT but does not exist in HIV-2 RT. These noncompetitive inhibitors were initially identified largely by serendipity, and their practicality as AIDS drugs was initially met with considerable skepticism. However, some NNIs have been found to be excellent drugs when used in combination with other antiretroviral drugs, even though they are largely useless in monotherapy. However, a single dose of NNIs offers considerable protection against mother-to-child transmission of HIV during birth.

Nucleoside Analogs

The inhibitory properties of nucleoside analogs against RT are due either to the lack of 2′ or 3′ hydroxyl groups, or to their replacement by other functional groups. In the case of AZT (azidothymidine, zidovudine), for example, the presence of the 3′-azido group prevents subsequent creation of a 3′-5′ phosphodiester bond and thus terminates the chain. The introduction of nucleoside analogs as potential AIDS drugs was based on the understanding of RT's mechanism of action rather than on the knowledge of its structure, which was unknown when these compounds were introduced. Five nucleoside analogs (NRTIs) have so far been approved by the FDA (Figure 3). Zidovudine (AZT, Retrovir, GlaxoSmithKline) was approved for monotherapy in 1987 as the first generally available AIDS drug, although its efficacy in that mode was shown to be only transitory (98). The drug is delivered orally with very high bioavailability. Another nucleoside analog AIDS drug is didanosine (dideoxyinosine, ddI, Videx), a product of Bristol-Myers Squibb. It is an analog of inosine, lacking both the 2′- and 3′-hydroxyl groups on its ribose moiety. In common with zidovudine, its active form is a triphosphate produced by a cellular enzyme. Its intracellular half-life is 8–24 h, much longer than that of zidovudine, thus allowing once-a-day dosing.

Another drug related to didanosine is zalcitabine (dideoxycytidine; ddC, Hivid), a product of Hoffmann–La Roche. This pyrimidine analog is active against HIV in vitro at very low concentrations, although its plasma half-life is rather short, requiring several daily doses of the drug. The FDA approved Stavudine (d4T, 3′-deoxy-2′-thymidinene, Zerit), a modification of thymidine manufactured by Bristol-Myers Squibb, for the treatment of HIV-infected adults who have received prolonged zidovudine therapy (99). The drug is generally well-tolerated with minimal side effects. Lamivudine (3TC, 3′-thia-2′,3′-dideoxcytidine, Epivir), discovered by BioChem Pharma and developed by GlaxoSmithKline, is also potent against another viral disease, chronic hepatitis B. After it was found that the

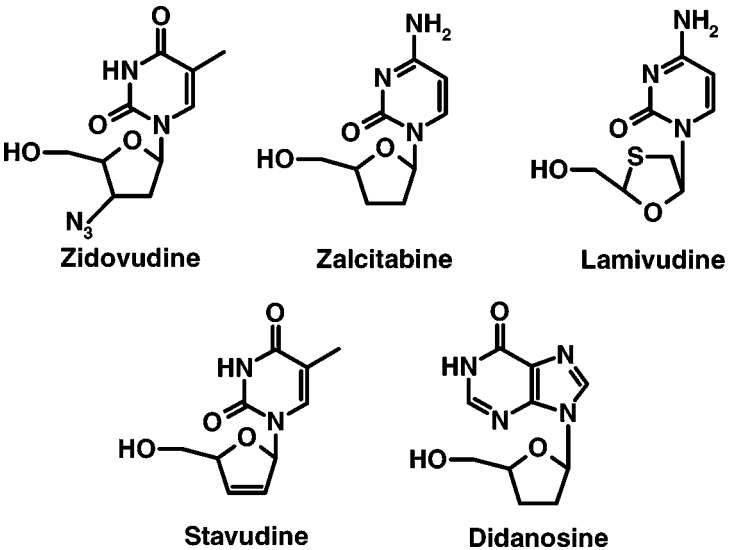

Figure 3 Chemical formulas of FDA-approved nucleoside inhibitors of HIV-1 RT.

principal mutation in RT that occurs in individuals receiving lamivudine, M184V, prevents resistance to zidovudine, a combination of both drugs (Combivir, which consists of 150 mg of lamivudine combined with 300 mg of zidovudine) was developed. Ziagen (Abacavir) is another nucleoside inhibitor recently developed by GlaxoSmithKline, and Triangle Pharmaceuticals is currently developing emtricitabine (Coviracil). It is clear that the usefulness of the nucleoside AIDS drugs will continue in the foreseeable future.

Nonnucleoside Analogs

Nonnucleoside analogs are potent inhibitors of HIV-1 RT but not of HIV-2 RT. The first such compounds, HEPT (100) and TIBO (101), were discovered to be active in cell culture before their target was identified. Several other members of this heterogeneous class, including nevirapine, were identified in screening programs specifically targeting HIV-1 RT (102). The NNIs bind in a pocket located about 10 Å from the substrate binding site. When bound into that pocket in HIV-1 RT, most NNIs maintain a similar, butterfly-like shape. The mode of action of NNIs is not completely clear, although it has been suggested that their proximity to the active site might alter the conformation of this domain, or that they restrict the motions of the p66 thumb domain (7).

So far, three NNIs have gained FDA approval for use against HIV (103). Their chemical formulas are shown in Figure 4. Nevirapine (Viramune, Boehringer-Ingelheim) was the first to gain approval (in 1996). Nevirapine is highly bioavailable in oral form and induces its own metabolism by activating the hepatic

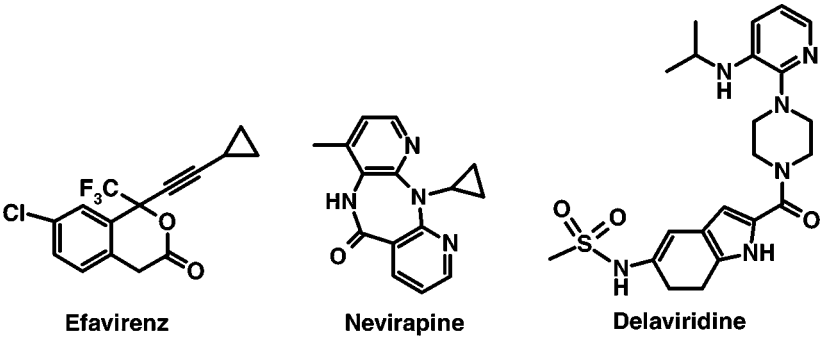

Figure 4 Chemical formulas of FDA-approved nonnucleoside inhbitors of HIV-1 RT.

cytochrome P450 pathways. Another FDA-approved NNI is delaviridine (Rescriptor), developed by Pharmacia and currently marketed by Pfizer. This compound has a relatively short plasma half-life, requiring several daily doses to maintain its concentration. The third NNI in current clinical use is efavirenz (Sustiva) from DuPont Pharmaceuticals. NNIs are not recommended in monotherapy because of rapid development of resistance, but they are very useful in multidrug combinations.

DEVELOPMENT OF DRUG RESISTANCE

Because retroviral RT has no editing function, transcription errors during nucleic acid replication are very common, and the viral pool contains species with all conceivable mutations. The presence of drugs provides a powerful selection pressure for virus modifications that produce lower susceptibility to such compounds. Development of resistance is often observed only a week or two after initiation of therapy. Rapid appearance of drug-resistant HIV species was considered a major obstacle in the development of newer therapies, such as PR inhibitors or NNIs.

Although nucleoside inhibitors of RT have been in clinical use for almost 15 years, the mechanism of resistance to them has been elucidated only recently (97). The structure of a trapped catalytic complex of RT provided data on the exact location of the incoming deoxynucleoside triphosphate and, by extension, of the nucleoside drugs. Not surprisingly, residues mutated in drug-resistant RT are located in the vicinity of the active site of the enzyme. An analysis of the steric nature of these mutations can also explain why resistance to one class of inhibitors may sensitize the enzyme to another class, forming the basis of sequential therapy. The emergence of resistance to NNIs is particularly rapid, since the binding site for these compounds is not a direct part of the active site of RT (103, 104). As a rule, mutations leading to resistance to NNIs involve residues lining the binding site of these inhibitors. However, it is now clear that resistance can be minimized, both by combining NNIs with other inhibitors and by starting therapy with high

concentrations of the drugs. NNIs are generally prescribed in combination with drugs from other classes (105).

A study of mutations resulting from the use of protease inhibitors found that about one third of the residues in HIV-1 PR were mutated in samples obtained after application of 21 drugs (104). Although some of these mutations were in pockets directly adjacent to the inhibitors, other mutations are observed throughout the protein. The appearance of the mutations is usually sequential, and remote mutations usually develop subsequent to the primary ones. The discovery of a pattern of multiple resistance mutations in patients subjected to indinavir monotherapy (106), as well as cross-resistance with six other PR inhibitors, raised serious questions about the efficacy of that category of drugs. However, this initial pessimism was unwarranted, since the use of sufficiently high doses and combination therapies have been quite successful in delaying or overcoming resistance. Drug-resistant mutations of HIV PR are considered so important that resistance studies now precede any attempts to introduce such compounds into clinical practice, as was recently described for lopinavir (86).

Combination therapies (highly active antiretroviral therapy, HAART) using different inhibitors promise the best clinical outcome. However, it is not clear whether it is better to use combinations of different drugs from the same family or drugs belonging to different classes. On one hand, combinations such as ritonavir-saquinavir, nelfinavir-saquinavir, or ritonavir-indinavir combine two similar drugs with distinct resistance patterns and, especially in the case of ritonavir, with different metabolisms. On the other hand, combinations of indinavir or nelfinavir with nevirapine, or indinavir plus efavirenz, assure that the development of resistance will require mutations in two different enzymes, making resistance less likely.

In Western countries, drug treatment is reducing AIDS to a manageable and treatable long-term disease. However, even with all the drugs already on the market, it is clear that the serious nature of the AIDS pandemic and the limitations of the therapies will make it necessary to continue drug development. Until a safe, effective vaccine against HIV has been found, it will be necessary to introduce new therapies and combinations of drugs to counteract the development of resistant variants. The understanding of drug-target interactions on the molecular level, coupled with extensive studies using the techniques of molecular biology, are of great help in achieving rapid success.

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