Medicinal Chemistry I

II$^{nd}$ Module

Antiviral Agents
Classical antiviral agents

More than 50 years have elapsed since the discovery of the first antiviral agents, i.e. methisazone and 5-iodo-2′-deoxyuridine. In contrast to the evolution in other fields, the antiviral chemotherapy has evolved very slowly at the start. The reasons therefore are multiple:

• close association between the replicative cycle of the virus and the metabolism of the cell;
• the intracellular location of the virus;
• viruses possess considerably fewer virus-associated or -encoded enzymes than bacteria;
• effective vaccines have been developed for the prevention of some severe viral
• infections; and
• antiviral research is a high-risk enterprise for industry.

However, the interest in antiviral chemotherapy has been boosted considerably since the identification of HIV (human immuno deficiency virus) as the causative agent of the acquired immune deficiency syndrome (AIDS).
Ribavirin

(16.14) Ribavirin

AlCAR

GMP

AMP
5-Iodo-2′-deoxyuridine (IdUrd, IDU) (16.1) has a structure that is very similar to that of the natural nucleoside thymidine (16.2). The van der Waals radius of an iodo group is somewhat larger than that of a methyl group, and the pKa of 5-iodouracil, the 6-membered heterocyclic unit of 16.1, is about 1.5 units lower than that of thymine, the corresponding heterocyclic unit of 16.2. This results from the inductive effect of the iodo group in the 5-position. These slight differences are apparently sufficient for IdUrd to become a rather selective antiviral agent. W.H. Prusoff synthesized IdUrd first in 1959 by iodination of 2′-deoxyuridine with iodine/nitric acid. IdUrd is active against the multiplication of Herpes simplex virus type 1 (HSV-1), Herpes simplex virus type 2 (HSV-2) and vaccinia virus (VV) in vitro and has proven efficacious in the treatment of herpes eye infections (i.e. herpetic keratitis). Its toxicity, however, does not allow systemic use.
Classical antiviral agents

Also there is a great resemblance in the structures of thymidine and 3’-azido-3’-deoxythymidine (zidovudine, AZT) (16.3). Here, the 3’-position is substituted with an azido group. This compound was the first to be approved by the FDA for the treatment of AIDS patients. J.P. Horwitz synthesized it first in 1964 starting from thymidine. Also, the modes of action of IdUrd and zidovudine are quite similar. They have to be metabolized intracellularly to their 5’-triphosphate derivatives and these triphosphates then interact with (viral) DNA synthesis.

These two nucleoside analogs (IDU and AZT), which resemble very well their natural counterpart, have had a tremendous impact on antiviral research. IDU has long been a model compound for the design of new and more selective antiherpes agents. The advent of AIDS, the identification of a retro virus as the causative agent of the disease and the observation that its replication can be blocked by simple nucleosides, gave an important incentive to the search for new antiviral agents.

We will see how selectivity can be obtained by interference with virus specific targets. The identification of specific, virus-encoded, enzymes has proved the key step in the design of new antiviral compounds.

In the design of new nucleoside analogs, targeted at viral DNA synthesis, the discovery of HPMPC [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] and PMPA [(R)-9-(2-phosphonylmethoxypropyl)adenine] as potent inhibitors of CMV (cytomegalo virus) and HIV, respectively, could be considered as important progress.
Classical antiviral agents

Their antiviral activity clearly indicates that mimicking nucleoside metabolites, e.g. nucleoside monophosphates, can overcome at least the first step of intracellular phosphorylation. It also proves that such phosphonate analogs can be taken up by the cell sufficiently well to exhibit their antiviral action. This brings us one step nearer to the target (viral DNA) site.
Classical antiviral agents

Nucleosides are naturally occurring molecules, which play a crucial role in cell multiplication and function. As a consequence, cells contain a whole battery of enzymes for the anabolism and catabolism of nucleosides. All of these enzymes are potential targets for the action of the modified nucleosides, and this can lead to premature death of the cell. Especially the interaction of the inhibitor with normal cellular DNA may be hazardous in that it could lead to mutagenicity, carcinogenicity or teratogenicity. Moreover, good in vitro antiviral activity not necessarily predicts equivalent in vivo activity. These considerations make the design of new nucleoside antivirals both a difficult and challenging task.
Base-modified pyrimidine nucleosides as antiherpes agents

The intracellular metabolism and mode of action of IdUrd can be presented as follows:

I. IdUrd can be phosphorylated by both cellular and virus-encoded thymidine kinases.

II. However, IdUrd is phosphorylated more efficiently by the HSV-encoded thymidine kinase than by the cellular thymidine kinase, which explains its (modest) selectivity as an antiherpes agent. IdUrd 5′-monophosphate is then phosphorylated to the diphosphate.

III. and triphosphate.

IV. IdUrd can be incorporated in both cellular and viral DNA. This incorporation impairs the subsequent transcription and replication processes and is believed to be the major reason for the activity and toxicity of IdUrd. As also evident from the above reaction scheme (Scheme 16.1), IdUrd (16.1) is a substrate for thymidine phosphorylase (iv) and for thymidylate synthase.

V. Both processes lead to deactivation (of IdUrd). Together with the feedback inhibition of the phosphorylated products on the regulatory enzymes of nucleotide biosynthesis, the general biochemical reaction scheme as depicted for IdUrd also holds for most other pyrimidine nucleoside analogs, and could explain their antiviral activity and toxicity.
Base-modified pyrimidine nucleosides as antiherpes agents

A crucial enzyme in the anabolism of pyrimidine 2′-deoxynucleosides is the thymidine kinase, which phosphorylates the nucleoside to its 5′-monophosphate derivative. Some herpes viruses (i.e. HSV-1, HSV-2) and also Varicella-zoster virus (VZV) encode for their own thymidine kinase. Introduction of a substituent in the 5-position of the pyrimidine ring has led to compounds with higher affinity for the virusesencoded enzyme than for the cellular enzyme, and thus greater selectivity as antiviral agents. Pertinent examples of this ‘second’ generation of antiviral compounds are 5-ethyl-2′-deoxyuridine (EtdUrd) and, even more so, 5-(E) bromovinyl-2′-deoxyuridine (BVdUrd, BVDU).
There is a marked difference in the phosphorylation capacity of the thymidine kinases of different herpes viruses. While the **HSV-1-encoded** thymidine kinase is capable of converting BVdUrd to its 5’-monophosphate and further onto its 5’-diphosphate, the **HSV-2-encoded** thymidine kinase is unable to further phosphorylate BvdUrd monophosphate onto its diphosphate. This differential behavior in phosphorylation may explain the differences found in the activity of BVdUrd against HSV-1 and HSV-2. The mode of binding of BVdUrd to the HSV-1 thymidine kinase has been determined. The 3-NH and 4-CO groups of the pyrimidine moiety interact with Gln-125. The bulky 5-substituent occupies a pocket available in the neighborhood of residues Trp-88, Tyr-132, Arg-163 and Ala-167. A change in conformation of one residue (Tyr-132) is needed for accommodation of the bromovinyl group. The binding mode of the deoxyribose moiety of BVdUrd is similar to that of dThd.
The BVdUrd is superior in potency to any other antiherpes agent against both HSV-1 and VZV infections, i.e. it is 1000-fold more active in vitro against VZV than acyclovir, the most commonly used drug for the treatment of VZV infections. The VZV is responsible for primary (varicella or chickenpox) and recurrent (zoster and shingles) infections, following reactivation of the virus. BVdUrd (brivudin) is currently the most potent antiviral agent on the market for the treatment of VZV infections.
9-(β-D-Arabinofuranosyl)adenine (ara-A) is a naturally occurring nucleoside, which was synthesized 8 years before it was isolated. Ara-A is an antiviral agent with a multiple mode of action. Theoretically, drugs that have multiple modes of action are most likely to avoid drug-virus resistance but, they may also have the highest risk for toxic side effects. The relative role of the different actions in the overall antiviral activity of ara-A is not well known. Ara-A is phosphorylated to its monophosphate and further to its di- and triphosphate. This triphosphate inhibits DNA polymerases, which could explain the activity of ara-A against DNA viruses. Ara-A can also be incorporated into both host cell DNA and viral DNA. Furthermore, ara-A inhibits methyltransferase reactions presumably through inhibition of 5-adenosylhomocysteine hydrolase and accumulation of S-adenosylhomocysteine. The latter acts as a product inhibitor of transmethylation reactions such as those involved in the maturation of viral mRNA. Ara-A has been used for the treatment of HSV-1 encephalitis and Herpes zoster in immunocompromised patients, but is now surpassed by acyclovir for this purpose. A major disadvantage of ara-A is that it is promptly deaminated in vivo by adenosine deaminase, converting the amino group into an oxo group. The resulting hypoxanthine analog has markedly reduced antiviral activity as compared to ara-A.
Sugar-modified purine nucleosides

The search for inhibitors of the adenosine deamination reaction has led to the discovery at the Wellcome Research Laboratories of 9-(2-hydroxyethoxymethyl) guanine (acyclovir) as an antiviral agent. This compound, whose action is surprisingly similar to that of the aforementioned pyrimidine nucleoside analogs, has oriented research in the direction of the acyclic nucleoside analogs. This research has yielded a number of active congeners, i.e. 9-(1,3-dihydroxy2-propoxymethyl)guanine (ganciclovir) and penciclovir. As of today, acyclovir has remained the ‘gold’ standard for the treatment of HSV infections, whereas ganciclovir is used in the treatment of CMV infections, and both penciclovir (as its prodrug form, famciclovir) and acyclovir (as its prodrug form, valaciclovir) for the treatment of VZV infections.
Sugar-modified purine nucleosides

All of these compounds can be considered as analogs of 2′-deoxyguanosine or carbocyclic 2′-deoxyguanosine (vide infra) from which the 2′-carbon (ganciclovir, penciclovir) or both the 2′- and 3′-carbons (acyclovir) have been deleted. The antiviral activity of acyclovir was discovered by accident, whereas ganciclovir was the result of a structure-activity design starting from acyclovir as the model compound. From a structural viewpoint, ganciclovir is more closely related to 2′-deoxyguanosine than is acyclovir. The antiviral activity of acyclovir can be explained by the same biochemical reaction scheme as presented for IdUrd. There are, however, subtle differences that explain the greater selectivity of acyclovir. Acyclovir is phosphorylated to its monophosphate by a virus-specific thymidine/deoxycytidine kinase, which actually recognizes acyclovir as a deoxycytidine analog. Viruses, which encode for such an enzyme (HSV-1, HSV-2, VZV, but not CMV) are susceptible to the antiviral action of acyclovir. Although the natural substrates for this enzyme are pyrimidine nucleosides, it apparently accepts purine derivatives as substrates. The structure of the complex of HSV-1 thymidine kinase with acyclovir, ganciclovir and penciclovir has been determined by X-ray crystallography. The guanine moiety of all three compounds lay in a similar location, with hydrogen bond pairing being made with Gln-125 via the 1-NH and 6-CO groups. In uninfected cells, phosphorylation occurs to a limited extent. The monophosphate of acyclovir is phosphorylated to the diphosphate by GMP kinase and further to its triphosphate by various cellular enzymes.
Sugar-modified purine nucleosides

The triphosphate of acyclovir is a competitive inhibitor of dGTP for the viral DNA polymerase and can also function as a substrate resulting in the incorporation of acyclovir into DNA and chain termination. Acyclovir is given orally or intravenously in the treatment of HSV and VZV infections, and topically in the treatment of HSV infections (i.e. herpetic keratitis and herpes labialis). For oral use, acyclovir, because of its limited oral bioavailability is now substituted by its oral prodrug form, valaciclovir.

As compared to acyclovir, ganciclovir is more easily phosphorylated in CMV infected cells by the virus-encoded (UL 97) protein kinase and its triphosphate has a fivefold greater affinity than ACV (acyclovir) triphosphate for CMV DNA polymerase.

Ganciclovir can be incorporated both internally and at the 3′-terminal end of DNA. Ganciclovir is active against HSV-1, HSV-2, VZV, CMV and EBV. It is fairly toxic for the bone marrow (neutropenia). Its clinical use is restricted to the treatment of CMV infections in immunocompromised patients.

Penciclovir has the same antiviral spectrum as acyclovir. As compared to acyclovir, penciclovir leads to higher triphosphate concentrations in virus-infected cells and its antiviral activity persists for a longer time after removal of the compound. In fact, after removal of acyclovir, antiviral activity rapidly disappears. Not only penciclovir, but also BVdUrd and ganciclovir show persist-ent antiviral activity after the drugs have been removed from the medium. This is due to the greater stability of their triphosphates as compared to that of acyclovir triphosphate.
Sugar-modified purine nucleosides
(Synthesis of Acyclovir)

Acyclovir, 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one (36.1.5), is synthesized by alkylating guanine with 1-benzoyloxy-2-chloromethoxyethane in triethylamine. The hydroxyl and amino groups of guanine are previously protected with a trimethylsilyl group by being treated with hexamethyldisilazane. After hydrolysis the resulting product with water, 9-(2-benzoyloxymethoxymethyl)guanine (36.1.4) is isolated. Treating this with a methanol solution of ammonia removes the benzoyl protecting group from the hydroxyethoxymethyl fragment, giving acyclovir.
Anti-HCV - Ribavirin

In contrast with the preceding compounds, ribavirin has a broad spectrum activity against RNA and DNA viruses both in vitro and in vivo. R.K.Robins first synthesized ribavirin. The structural requirements for the broad-spectrum antiviral activity of ribavirin are very stringent. The compound shows its greatest potency against myxo (influenza) and paramyxo (respiratory syncytial) virus infections. Ribavirin also shows activity against some hemorrhagic fever viruses such as Lassa, Machupo, Pichinche, Rift Valley and Hantaviruses.

Therapeutic efficacy has been demonstrated with ribavirin, given as a small-particle aerosol, in infants suffering from respiratory syncytial virus (RSV) infection. The compound has been approved for the treatment of RSV infections, as well as human hepatitis C virus (HCV) infections, for the latter only in combination with interferons.

The mode of action of ribavirin is multipronged and may also vary from one virus to another. Ribavirin (5′-monophosphate) can be considered as an analog of AICAR which is a precursor of both AMP and GMP.

As has been elucidated by X-ray crystallography studies, there is a nice resemblance between ribavirin and guanosine; by rotating the amide group, also good resemblance is found between ribavirin and adenosine.
Ribavirin

(16.14) Ribavirin

AICAR

GMP

AMP
Synthesis of Ribavirin

Ribavirin, 1-β-D-ribofuranosyl-1H-1,2,4-triazol-3-carboxamide (36.1.28), is synthesized by reacting methyl ester of 1,2,4-triazol-3-carboxylic acid with O-1,2,3,5-tetraacetyl-β-D-ribofuranose to make methyl ester of 1-O-2,3,5-tetraacetyl-β-D-ribofuranosyl-1,2,4-triazol-3-carboxylic acid (36.1.27), which is treated with an ammonia solution of methanol to simultaneously dezacylate the carbohydrate part and amidation of the carboxyl part of the product to give ribavirin [29–37].
Compounds which inhibit the replication of the human immunodeficiency virus (HIV)

Nearly 40 million people are infected with the human immunodeficiency virus (HIV). Over half of those infected reside in sub-Saharan Africa. Worldwide during 2004, it is estimated that nearly 14,000 people a day were infected. Human immunodeficiency virus type 1 is the primary etiological source for the acquired immunodeficiency syndrome (AIDS). Fortunately, people infected with HIV are leading longer and more productive lives due to the availability of more effective therapies. Better medicines have evolved due to the efforts of scientists worldwide who find targets and compounds that inhibit the virus life-cycle. The current treatment for HIV infection is via a drug cocktail that usually includes a protease inhibitor (PI), a nucleoside reverse transcriptase inhibitor (NRTI), and a non-nucleoside reverse transcriptase inhibitor (NNRTI).
Compounds which inhibit the replication of HIV (Viral life cycle and targets)
Compounds which inhibit the replication of HIV
(nucleoside reverse transcriptase inhibitor NRTI)

Human immunodeficiency virus is a retrovirus, which means that, once it has infected the cell, its genomic RNA is transcribed to proviral DNA by a virus-specific enzyme (the RT). This enzyme has a broader substrate specificity than cellular DNA polymerases, and has since long been recognized as a target for antiviral chemotherapy. Here, the task to design specific antiviral agents is somewhat more difficult than for herpes viruses, since HIV does not encode for a virus-specific kinase which could confine the metabolism of the nucleoside analogs to the virus-infected cells.

Fifteen compounds have now been approved for the treatment of HIV infection from which zidovudine (16.3) was the first one. Nine of them are targeted at the viral RT and the other six are targeted at the viral protease. Among the reverse transcriptase inhibitors, six are nucleoside analogs (zidovudine) didanosine, zalcitabine, stavudine, lamivudine, abacavir. These dideoxynucleoside analogs must be phosphorylated through three consecutive kinase reactions to the triphosphate form before they can interact as competitive inhibitors with respect to the natural substrates at the RT. The intracellular metabolism of dideoxynucleosides is dependent on the type of compound, the type of cell and the anabolic state of the cells. incorporated into DNA and functions as a chain terminator.
Compounds which inhibit the replication of HIV (nucleoside reverse transcriptase inhibitor NRTI)

Zidovudine is phosphorylated by the cellular thymidine kinase to its 5’-monophosphate. This 5’-monophosphate is then phosphorylated to the di- and triphosphate by thymidylate kinase and, subsequently, nucleoside-5’-diphosphate kinase, respectively. As the efficiency of conversion of zidovudine 5’-monophosphate to its diphosphate by thymidylate kinase is much lower than the efficiency of phosphorylation of the natural substrate, zidovudine 5’-mono-phosphate accumulates in the cells. In its triphosphate form, AZT is a more efficient inhibitor of the RT than of the cellular DNA polymerases. The AZT is incorporated into DNA and functions as a chain terminator.

[Chemical structures of compounds]
Compounds which inhibit the replication of HIV
(nucleoside reverse transcriptase inhibitor NRTI)
Compounds which inhibit the replication of HIV
(nucleoside reverse transcriptase inhibitor NRTI)
Compounds which inhibit the replication of HIV (nucleoside reverse transcriptase inhibitor NRTI)

Figure 7  X-ray crystal structure of AZT bound to reverse transcriptase. The dsDNA is represented by the dark blue coiled structures and zidovudine is represented by the spheres color-coded by atom type: green, C; blue, N; red, O; purple, P.
Compounds which inhibit the replication of HIV (nucleoside reverse transcriptase inhibitor NRTI)

Figure 8 Detail of x-ray crystal structure of AZT bound to reverse transcriptase. The active site magnesium atom is shown as the large orange sphere. The locations of the common NRTI-mutated amino acids are shown in pink.
Compounds which inhibit the replication of HIV (nucleoside reverse transcriptase inhibitor NRTI)

Table 1 Dosing regimens for NRTIs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage (mg)</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td>300</td>
<td>bid</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>tid</td>
</tr>
<tr>
<td>Stavudine</td>
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<td>Zalcitabine</td>
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<td>tid</td>
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<td>Didanosine</td>
<td>250–400</td>
<td>qd</td>
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<tr>
<td></td>
<td>125–200</td>
<td>bid</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>300</td>
<td>qd</td>
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<tr>
<td></td>
<td>150</td>
<td>bid</td>
</tr>
<tr>
<td>Emtricitabine</td>
<td>200</td>
<td>qd</td>
</tr>
<tr>
<td>Abacavir</td>
<td>600</td>
<td>qd</td>
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<tr>
<td></td>
<td>300</td>
<td>bid</td>
</tr>
<tr>
<td>Tenofovir DF</td>
<td>300</td>
<td>qd</td>
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*a qd, once daily; bid, twice daily; tid, three times daily.
Compounds which inhibit the replication of HIV
(non-nucleoside reverse transcriptase inhibitor NNRTI)

The second major class of drugs to be used in the battle against HIV are the NNRTIs. Although these compounds are also targeted at RT, their mechanism of action is substantially different than the NRTI class. The NNRTIs nevirapine, delavirdine and efavirenz block the HIV-1 RT reaction through interaction with an allosterically located, nonsubstrate binding ‘pocket’ site. This binding pocket is located at about 10 Å distance from the substrate-binding site. The binding of an NNRTI and NRTI (nucleoside reverse transcriptase inhibitor) to the RT is a co-operative process, and this might explain the synergistic effect of both types of inhibitors when used in combination.
Compounds which inhibit the replication of HIV
(non-nucleoside reverse transcriptase inhibitor NNRTI)

Several studies have revealed a common mode of binding for the different NNRTIs with their target site at the HIV-1 RT. The NNRTIs cause a repositioning of the three stranded β-sheet in the p66 subunit (containing the catalytic aspartic acid residues 110, 185 and 186). This suggests that the NNRTIs inhibit HIV-1 RT by locking the active catalytic site in an inactive conformation. As an example, the binding of delavirdine to HIV-1 RT is described. Delavirdine is hydrogen-bonded to the main chain of Lys-103 and extensively interact with Pro-236 by hydrophobic contacts. Part of delavirdine protrudes into the solvent creating a channel between Pro-236 and the polypeptide segments 225–226 and 105–106.
Compounds which inhibit the replication of HIV (non-nucleoside reverse transcriptase inhibitor NNRTI)

Before the advent of high-throughput screening, it was not practical to test very large numbers of compounds in a sample collection. Using a technique called computational dissimilarity analysis, a subset consisting of 1500 compounds was selected for testing against HIV-1 RT. This resulted in the selection of 100 compounds for further testing in cell culture for antiviral activity and toxicity. The lead compound that emerged from this approach was the arylpiperazine U-80493E. Optimization of this lead structure lead to the first clinical candidate from this class, atevirdine (U-87201E). Unfortunately, no significant antiviral effect was seen in HIV-infected patients at doses of 600 mg three times daily, which may have been due to inadequate drug trough concentrations. Further optimization of substituents on the indole portion of the molecule were guided by potency and metabolic concerns, leading to the identification of U-90152S and advancement of delavirdine (Rescriptor) into clinical trials and regulatory approval.
Compounds which inhibit the replication of HIV (non-nucleoside reverse transcriptase inhibitor NNRTI)

Because of the relatively rapid selection for resistant viral populations in patients undergoing therapy with NNRTIs, a search for second-generation inhibitors with improved pharmacokinetics and a higher barrier to resistance was under way in several laboratories. Beginning from a second structural class of dihydroquinzolinethiones identified from directed screening, L-608,788 became the lead compound. Optimization of this series was initially driven by chemical and metabolic stability issues, as well as potency in cell culture. In particular, oxidative metabolism of either the alkyl group on the nitrogen atom at the 3-position or substituents at the 4-position were thought to be responsible for rapid clearance in animals. Introduction of an acetylene substituent and cyclopropyl group at the 4-position allowed the 3-position to go unsubstituted, thus circumventing the metabolism issues. This led to the identification of L-738,372, which possessed an excellent pharmacokinetic profile in animals and good potency in cell culture. Detailed enzymatic studies demonstrated that the compound was a slow-binding, noncompetitive inhibitor that was synergistic with NRTIs. A second approach entailed the substitution of an oxygen atom at the 3-position to give the benzoxazine ring system. Further optimization was guided by relative activity versus a panel of single and double mutants previously identified as conferring resistance to a variety of NNRTIs. The optimized compound L-743,726 (DMP-266) displayed an attractive combination of good pharmacokinetics and an improved resistance profile. Clinical studies confirmed the preclinical profile, and efavirenz (Sustiva, Stocrin) received regulatory approval as one element of combination therapy for HIV, and the first HIV drug indicated for oncedaily dosing.
Compounds which inhibit the replication of HIV (non-nucleoside reverse transcriptase inhibitor NNRTI)

Figure 13 Evolution of Efavirenz (EFV).
Compounds which inhibit the replication of HIV (non-nucleoside reverse transcriptase inhibitor NNRTI)

Figure 14  X-ray crystal structure of efavirenz bound to reverse transcriptase. Efavirenz is shown as spheres color-coded by atom type: green, C; red, O; blue, N; and purple, F or Cl. This NNRTI allosteric binding site is approximately 65 angstroms from the polymerase active site.
Compounds which inhibit the replication of HIV (non-nucleoside reverse transcriptase inhibitor NNRTI)

Figure 15  Detail of x-ray crystal structure of efavirenz bound to reverse transcriptase. Locations of the K103 and Y181 mutations are shown in red.
Compounds which inhibit the replication of HIV
(non-nucleoside reverse transcriptase inhibitor NNRTI)

**Table 2 Dosing regimens for NNRTIs**

<table>
<thead>
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<th>Drug</th>
<th>Dosage (mg)</th>
<th>Frequency</th>
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<td>Nevirapine</td>
<td>200</td>
<td>qd 14 days</td>
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<tr>
<td></td>
<td>200</td>
<td>bid after</td>
</tr>
<tr>
<td>Delavirdine</td>
<td>400</td>
<td>tid</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>600</td>
<td>qd</td>
</tr>
</tbody>
</table>

*a qd, once daily; bid, twice daily; tid, three times daily.*
Compounds which inhibit the replication of HIV (protease inhibitors PI)

The third viral enzyme is HIV protease, and is responsible for the maturation of the newly created viruses during or shortly after the budding process. Because the products from transcription and translation of the proviral DNA are expressed as long polyproteins, the protease enzyme is required to cleave at nine distinct sites to provide the individual functional and structural proteins of the virus. This enzyme belongs to the family of aspartic acid proteases (renin, pepsin, etc.), and exists as a homodimer composed of two 99 amino acid proteins. From a number of studies, it became apparent that this enzyme would be an attractive biochemical target for HIV therapeutics. A number of drugs have been developed that target this enzyme, and represent the second major mechanism for anti-HIV drugs. A common approach to inhibitor design was implemented by several laboratories in the late 1980s. The strategy evolved from the identification of HIV protease as a member of the aspartic acid proteases and built on the large body of knowledge related to the structure and inhibition of members of this enzyme class. The proper folding of the two identical proteins results in a C2 symmetric tertiary structure that has been solved by x-ray crystallography and that has been a major focus of structure-based drug design in research laboratories. Before the structure of the enzyme was determined experimentally, molecular modeling techniques were used to predict the three-dimensional structure based on sequence homology.
Compounds which inhibit the replication of HIV (protease inhibitors PI)

The results of this prediction were borne out when the three-dimensional structure of the native enzyme was determined by x-ray crystallography. When potent inhibitors were later identified, a number of inhibitors were cocrystallized with the enzyme and the structure of the enzyme–inhibitor complex determined crystallographically. When compared with the native structure the indinavir-containing enzyme clearly shows the movement of the flap regions toward the inhibitor. Mechanistic studies of this family of aspartyl proteases indicated that two aspartates in the active site catalyze the addition of a water molecule to the amide bond at the cleavage site, leading to a tetrahedral transition state. Breakdown of this intermediate leads to the formation of the two smaller peptide fragments. The protease enzyme is responsible for cleavage of the gag and gag-pol polyproteins at multiple sites, and the specific amino acids on either side of each cleavage site were determined. From this information, it then became possible to design PIs based on the principle of the transition state isostere model. This approach entails replacement of the scissile amide bond within a peptide substrate with a nonhydrolyzable replacement, termed an isostere. Because the structure of the isostere mimics the geometry and structure of the transition state of amide bond hydrolysis, tight binding to the enzyme results in potent inhibition. This concept had been previously applied to the related aspartyl protease renin.146 A wide variety of functional groups can fulfill the role of transition state isostere, and a number of the known structures have been incorporated into substrates of HIV protease to make inhibitors.
Compounds which inhibit the replication of HIV (protease inhibitors PI)

Left. X-ray crystal structure of native HIV protease. One of the monomers is shown in green and the other in blue. The ‘floor’ of the active site is near the two catalytic aspartate residues D25A and D25B, and the ‘ceiling’ includes the mobile flap regions that close down on the substrate or inhibitor upon binding. (right) X-ray crystal structure of indinavir bound HIV protease
Compounds which inhibit the replication of HIV (protease inhibitors PI)

Left. X-ray crystal structure of native HIV protease. One of the monomers is shown in green and the other in blue. The ‘floor’ of the active site is near the two catalytic aspartate residues D25A and D25B, and the ‘ceiling’ includes the mobile flap regions that close down on the substrate or inhibitor upon binding. (right) X-ray crystal structure of indinavir bound HIV protease
Compounds which inhibit the replication of HIV (protease inhibitors PI)

The HIV protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir) prevent the cleavage of the gag and gag-pol precursor polyproteins to the functional proteins (p17, p24, p7, p6, p2, p1, protease, RT, integrase), thus arresting maturation and thereby blocking infectivity of the nascent virions. The HIV protease inhibitors have been tailored after the target peptidic linkage in the gag and gag-pol polyproteins that need to be cleaved by the viral protease, i.e. the phenylalanine-proline sequence at positions 167 and 168 of the gag-pol polyprotein.
Compounds which inhibit the replication of HIV (protease inhibitors PI)

All protease inhibitors that are currently licensed for the treatment of HIV infection, are peptidomimetics where the carbonyl group of the peptide bond is substituted by an hydroxyethylene group.
Compounds which inhibit the replication of HIV (protease inhibitors PI)
Compounds which inhibit the replication of HIV (protease inhibitors PI)

Discovery of saquinavir  The first PI to be approved for the treatment of HIV infection in the USA was saquinavir (Invirase, Fortovase). The medicinal chemistry approach taken for this compound began from one of the key substrates for the enzyme. Although HIV protease is required for cleavage at a variety of amino acid pairs within the viral proteome, one of the more unusual cleavages is between either tyrosine (Tyr) or phenylalanine (Phe) and proline (Pro). Because most mammalian proteases are unable to cleave between these amino acid residues, inhibitors based on the corresponding transition state isosteres were synthesized. Based on the sequence of amino acids 165–169 in the pol polyprotein (Leu-Asn-Phe-Pro-Ile) around the Phe-Pro cleavage site, a series of inhibitors were synthesized that included a stable hydroxyl-containing isostere to replace the amide bond and mimic the presumed tetrahedral intermediate resulting from enzymatic hydrolysis. For a series of compounds containing the transition state isostere, it was noted that the stereochemistry of the key hydroxyl group was critical for potency against the enzyme. Optimization of this series included an effort to determine the smallest sequence that preserved tight binding to the enzyme, followed by optimization of the N and C termini, as well as the amino acid side chains. This led to the identification of saquinavir as a development candidate. Regulatory approval of the first HIV PI, saquinavir, by the FDA was granted in December 1995 as part of a combination with at least one other antiviral agent approved for the treatment of HIV. A few months later, in February 1996, the HIV PIs ritonavir (Norvir) and indinavir (Crixivan) also received regulatory approval.
Compounds which inhibit the replication of HIV (protease inhibitors PI)

Figure 20  Evolution of saquinavir.
Compounds which inhibit the replication of HIV
(protease inhibitors PI)

Discovery of indinavir The design path to indinavir began from the knowledge that HIV protease was also related to the aspartyl protease renin. Renin is a key enzyme in the renin–angiotensin pathway and is intimately involved in the control of blood pressure. A relatively large collection of synthetic renin inhibitors were available for counterscreening to identify compounds that would also inhibit HIV protease. However, in order to provide enough HIV protease to conduct such a screen, a chemical synthesis of the enzyme was undertaken. This effort provided sufficient quantities of the enzyme that were crucial for the timely identification of PIs from the renin collection. A hexapeptide-based inhibitor (L-364,505) containing a hydroxyethylene isostere with relatively weak activity against human renin was soon identified as a potent inhibitor of HIV protease. This isostere was a replacement for a Phe-Phe dipeptide, and only one of the many possible diastereomers gave potent inhibitors. Deletion of the two N-terminal Phe residues and replacement of the C-terminal dipeptide amide with an aminooindanol amide gave a series of compounds with equivalent potency and significantly diminished molecular weight. Although L-685,434 was of manageable size and lacked any traditional amino acid residues, it had very poor oral bioavailability and limited water solubility. This prompted a new strategy that involved a hybrid design using the published structure of saquinavir. As noted previously, the stereochemistry of the hydroxyl group in the transition state isosteres was critical for both series of inhibitors. This observation led to the hypothesis that the bound conformation of the decahydroisoquinoline (DIQ) portion of saquinavir might occupy the same space as the N-terminal portion of L-685,434. To test this hypothesis, a hybrid structure was synthesized that replaced the N-terminus of the current inhibitors. Importantly, this design also introduced a basic nitrogen atom into the backbone of the inhibitor that proved the solubility characteristics of the compounds. Optimization of this new hybrid inhibitor led to the replacement of the DIQ with a substituted piperidine, and ultimately to the synthesis of indinavir
Compounds which inhibit the replication of HIV (protease inhibitors PI)

[Diagram showing chemical structures and IC50 values for L-364,505 (IC50 = 1 nM), L-685,434 (IC50 = 0.3 nM), Indinavir (IDV) (IC50 = 0.6 nM), and L-704,486 (IC50 = 8 nM).]

Figure 22 Evolution of indinavir. N-terminus of L-685,434 shown in blue; DIQ region of saquinavir shown in pink.
Compounds which inhibit the replication of HIV (protease inhibitors PI)

Figure 28  X-ray crystal structure of indinavir-bound HIV protease with mutants; locations of commonly mutated amino acids shown in orange.
Compounds which inhibit the replication of HIV (protease inhibitors PI)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage (mg)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saquinavir (sgc)</td>
<td>1200</td>
<td>tid</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>600</td>
<td>bid</td>
</tr>
<tr>
<td>Indinavir</td>
<td>800</td>
<td>tid</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>1250</td>
<td>bid</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>tid</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>1400</td>
<td>bid</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>400</td>
<td>qd</td>
</tr>
<tr>
<td>Fosamprenavir</td>
<td>1400</td>
<td>bid</td>
</tr>
</tbody>
</table>

*a qd, once daily; bid, twice daily; tid, three times daily.

*b sgc, soft gel capsule, marketed as Fortovase.
Compounds which inhibit the replication of the human immunodeficiency virus (HIV)

Synergistic anti-HIV activity is observed when NRTIs are combined with other NRTIs or NNRTIs or with protease inhibitors (PIs). This treatment regimen allows the individual compounds to be used at lower doses and, in preventing virus breakthrough, they also prevent the virus from becoming resistant to the compounds. Combination of NRTIs, NNRTIs and PIs have been found to decrease HIV viral load, to increase CD4 count and to decrease mortality and delay disease progression, particularly in AIDS patients with advanced immune suppression. When initiated during early asymptomatic HIV infection, highly active antiretroviral therapy (HAART) initiates rapid reversal of disease-induced T-cell activation, while preserving pretherapy levels of immune function, suggesting that therapeutic benefit may be gained from early aggressive anti-HIV chemotherapy.
Compounds which inhibit the replication of HIV  
(Synthesis of AZT)

The synthesis of the first anti-HIV drug to reach the clinic, zidovudine, more familiarly known as AZT, starts with the reaction of thymidine, shown as the enol tautomer, with the powerful dehydrating agent diethylaminosulfur tertafuoride (DAST). The stereochemistry of the resulting cyclic ether strongly suggests that the initial step involves the transformation of the sugar hydroxyl to a good leaving group. This is then displaced by the pyrimidine enol with consequent inversion. Treatment of the intermediate with sodium azide leads to a ring opening and a second inversion step; there is thus obtained the reverse transcriptase inhibitor zidovudine.
Compounds which inhibit the replication of HIV (Synthesis of AZT)

Zidovudine is 3-azido-3-deoxythymidine (36.1.26), is synthesized from 1-(2-deoxy-5-O-trityl-β-D-lyxosyl)thymine, which is treated with methansulfonyl chloride in pyridine to make the corresponding mesylate 36.1.24. Replacing the methyl group with an azide group using lithium azide in dimethylformamide makes the product 36.1.25 with inverted configuration at C3 of the furanosyl ring. Heating this in 80% acetic acid removes the trityl protection, giving zidovudine.
Compounds which inhibit the replication of HIV
(Synthesis of Nevirapine)

The structures of the non-nucleoside reverse transcriptase inhibitors (NNRTI) that comprise an ingredient in the three-part drug cocktails for treating HIV have little in common. The bispyridodiazepinone nevirapine was the first NNRTI approved for treating HIV and still finds extensive use. The first step in the syntheses comprises the acylation of the aminopyridine with the chlorinate nicotoyl chloride to afford amide. Treatment of the product with cyclopropylamine leads to the selective displacement of the halogen adjacent to the activating carbonyl. The anion from the reaction of that intermediate then displaces the corresponding halogen on the adjacent pyridyl function to form the diazepinone ring. This then affords the NNRTI nevirapine.
Compounds which inhibit the replication of HIV
(Synthesis of Nevirapine)

Nevirapine was invented and synthesized by scientists at Boehringer Ingelheim Pharmaceuticals, Inc. Nevirapine is a tricyclic dipyriddiazepinone and a specific noncompetitive inhibitor of reverse transcriptase (Hargrave et al., 1991; Merluzzi et al., 1990). Nevirapine does not inhibit the reverse transcriptase from simian immunodeficiency virus or feline leukemia virus, nor does it inhibit calf thymus DNA polymerase a or human DNA polymerase a, b, g, or d. This compound also has no effect on HIV-1 protease, human plasmin renin, and HSV-1 ribonucleotide reductase enzymes. With regard to inhibiting HIV-1 replication, nevirapine inhibits HIV-1 replication in CD4+ T-cell cultures (c8166) (Salahuddin, 1983). The IC50 against HIV-1 (IIIb) is 40 nM, with a maximum inhibition of 100% as determined by inhibition of p24 production. Viability of c8166 cells is determined by means of a tetrazolium salt (MTT) metabolic assay (Denizot and Lang, 1986). This assay shows 50% toxicity of nevirapine at 321,000 nM, resulting in an 8025 in vitro selectivity ratio (ED50/IC50). In addition, nevirapine shows no cytotoxic effects on human bone marrow colonies including erythroid burst-forming units and colony-forming units of granulocyte, erythroid, macrophage megakarocyte, and granulocyte macrophage at concentrations up to 37,500 nM. The NRTI inhibitor, AZT, is a more potent inhibitor (IC50 ¼ 6 nM); however, AZT is more cytotoxic (CC50 ¼ 66,000 nM) in the same assays. In in vivo assays in cynomolgus monkeys and chimpanzees, the plasma levels remained between 35 and 600 times the IC50 during an 8-h period after a single oral dose of 20 mg/kg of body weight. Nevirapine can also treat HIV, which may reside in the central nervous system. In rodent and primate models, the ratio of nevirapine in plasma versus that in the brain is 0.8 to 1.0, which is observed using tissue distribution studies following oral distribution.
Compounds which inhibit the replication of HIV
(Synthesis of Nevirapine)

Most of the viral resistance of RT to nevirapine is based on the mutation found in position 181. In this mutation, cysteine is substituted for tyrosine (Y181C) (Richman et al., 1991, 1994). The Y181C mutant is less sensitive to nevirapine than the wild-type enzyme (IC50 ¼ 2.6 mM vs. IC50 ¼ 0.08 mM). This mutation is also less sensitive to other NNRTIs (Dueweke et al., 1993; Nunberg et al., 1991). Other mutations known to occur from nevirapine treatment are K103N, V106A, G190A, and Y188L. Nevirapine is given orally at doses of one 200-mg tablet per day for 14 days followed by two 200-mg tablets for one day. Nevirapine is always given as a part of the AIDS cocktail, which may contain one or two of the following protease inhibitors, Reyataz (atazanavir), Invirase (saquinavir), Kaletra (lopinavir and ritonavir), Crixivan (indinavir), Agenerase (amprenavir), or Lexiva (fosamprenavir), and sometimes with AZT or other NRTIs. In terms of pharmacokinetics, nevirapine has a half-life of 56 h. Women and patients with higher CD4 counts are at increased risk of liver problems while taking nevirapine.

A pharmacokinetic study shows that CYP3A4 is responsible for the oxidative metabolism of nevirapine, resulting in five major metabolites. The major metabolite of nevirapine from the liver microsomes of humans, rats, monkeys, and dogs results from the hydroxylation of the 4-methyl substituent (compound 8) (Cheeseman et al., 1993; Grozinger et al., 2000).
Compounds which inhibit the replication of HIV
(Synthesis of Efavirenz)

Efavirenz was discovered by Merck scientists for the treatment of HIV infection (Young et al., 1995, 1996). The IC95 of efavirenz against the wild-type laboratory adapted strains and clinical isolates ranges from 1.7 to 25 nM in lymphoblastoid cell lines, peripheral blood mononuclear cells, and macrophage/monocyte cultures. Efavirenz was tested for its ability to inhibit the spread of single and double mutants. Based on the results observed in their RT assay, substitutions at A98G, K101E, V106A, V108I, V179E, and Y181C did not show any resistance as much as substitutions at positions L100I and K103N (efavirenz was approximately six times less effective in relation to the wild-type virus). The double mutants K103N-Y181C and L100I-K103N are eight to twenty times less sensitive to efavirenz. In the cell culture assay, mutants V108I, V179E, and Y181C have comparable sensitivity to the wild-type virus (IIIb, MN, and RFH). However, mutants A98G, K101E, and V106A are four to fifteen times less sensitive. Single mutants L100I and K103N have significantly less sensitivity (30–60 times less sensitive). Double mutant L100I–K103N is greater than 8000 times less sensitive. In clinical trials, 90% of the virus from patients whose viral load rebounded after the initial response of load reduction had the K103N mutation. This mutation is observed in both the monotherapy, in vitro selection experiments (Bacheler, 1999) and in clinical trials (Bacheler et al., 1998). Approximately four months following the appearance of the K103N mutation, double mutations K103N–V108I or K103N–P225H are observed in a large number of samples. Results from testing against a variety of polymerase enzymes show that efavirenz is inactive up to 300 mM for a 50% inhibition (Young et al., 1995). The polymerase enzymes studied were Moloney murine leukemia virus RT, human DNA polymerases a, b, and g, Escherichia coli RNA polymerase, and the Klenow fragment. Cytotoxicity studies in their primary cells and in a T-cell line reveal that efavirenz has a selectivity index of 80,000.
The establishment of the stereocenter in efavirenz provides a challenging goal for the synthetic chemist (Pierce et al., 1998; Thompson et al., 1995). The synthesis starts by treating 4-chloroaniline with pivaloyl chloride under biphasic conditions to provide the desired amide 10 (Scheme 6.2). Ortho metallation as directed by the amide is accomplished with two equivalents of n-butyllithium (or n-hexyllithium) in tetramethylethylene diamine (TMEDA) and MTBE. The resulting dianion is quenched with ethyl trifluoroacetate to provide pivaloylamide ketone 11 (Fuhrer and Gschwend, 1979). The amide is hydrolyzed in situ to provide the trifluoroketone hydrate hydrochloride 12, which crystallizes from the reaction mixture (98% pure). The free base 13 is obtained by stirring with sodium acetate in MTBE. Benzylation by treatment with a mild acid and p-methoxybenzyl alcohol provides 14 (Emert et al., 1977; Henneus et al., 1996).
Compounds which inhibit the replication of HIV
(Synthesis of Efavirenz)

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Compounds which inhibit the replication of HIV (Synthesis of Efavirenz)

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Compounds which inhibit the replication of HIV
(Synthesis of Efavirenz)

Removal of the p-methoxybenzyl group is accomplished by treatment with dichlorodicyanoquinone (DDQ), which forms quantitatively aminal 17 in an 11.5:1 diastereomeric ratio (Yu and Levy, 1984). The solution is treated with sodium methoxide in methanol, which decomposes the aminal into the desired amine 18 and p-methoxybenzaldehyde. Due to the difficulties in separating the p-methoxybenzaldehyde from amino alcohol 18, the aldehyde is reduced in situ with sodium borohydride. The amino alcohol 18 is crystallized from the reaction mixture after neutralization with acetic acid. Additional recrystallization provides the desired amino alcohol 18 in 94% yield. The final step of the synthesis is to complete the benzoxazinone ring of efavirenz. The most direct and economically desirable route utilizes phosgene (THF–heptane, 0–258°C). After aqueous sodium bicarbonate work-up, efavirenz was crystallized in excellent yields (93–95%) with excellent chemical and optical purities (.99.5%, .99.5% ee). When methyl chloroformate is used, the two-step process does not perform as well as phosgene because the residual intermediate carbamate is not easily removed from the desired product and the yield (83%) is therefore lower than the phosgene method. The procedure utilizing the p-nitrophenylcarbamate intermediate is conducted in one pot (However, the reaction does not proceed to completion if run at initially high pH (pH . 11) so KHCO3 followed by KOH was used. In this case, ring closure is rapid and K-nitrophenylate competes with 18 for nitrophenyl chloroformate (forming nitrophenyl carbonate as a by-product). The use of KHCO3 ensures pH , 8.5 and complete formation of the carbamate before addition of KOH to effect ring closure.) The work-up is quite simple. The organic phase is washed with brine and the solvent is switched to isopropanol. The product is crystallized by the addition of water, which provides the desired efavirenz in 94% yield (.99.5% purity), with no trace of p-nitrophenylcarbamate.
Compounds which inhibit the replication of HIV
(Synthesis of Efavirenz)
Compounds which inhibit the replication of HIV  
(Synthesis of Efavirenz)

Merck scientists made changes to this process, resulting in fewer equivalents of acetylide and chiral ligand without the use of N-protection (Chen et al., 1998). In a general procedure, a solution of the chiral ligand (1.5 equivalents) is treated with either dimethyl or diethylzinc at 0°C to room temperature (Scheme 6.4). This mixture is stirred for 1 h followed by the addition of an additive. Most of the additives studied are either carboxylic acids or alcohols. The range of ee values from the different additives is 71.6% (2,2-dimethylpropanoic acid) to 96% (2,2,2-trichloroethanol). This solution is mixed with a solution of the chloromagnesium acetylide (1.2 equivalents). The mixture is stirred for about 30 min and then cooled to 210°C. A solution of trifluoroketone 13 is added and the mixture is allowed to stir for 7 h. The optimized conditions are employed on a kilogram scale to provide 95.2% yield of efavirenz that is 99.8% pure and 99.3% ee.
Compounds which inhibit the replication of HIV  
(Synthesis of Efavirenz)

Efavirenz is given orally at a dose of one 600-mg tablet per day. As an important part of the AIDS cocktail, efavirenz is administered with the same protease inhibitors and NRTIs as described in the nevirapine section. The half-life of efavirenz after a single dose is 52–76 h, and multiple doses lower the half-life to 40–55 h. Treatment with efavirenz has been associated with the development of serious psychiatric side-effects, including severe depression, suicidal thoughts, aggressive behavior, and paranoid and manic reactions. The major isozymes that metabolize efavirenz are CYP3A4 and CYP2B6. In fact, after the administration of efavirenz, the CYP3A4 isozyme is upregulated, which is the same isozyme responsible for the metabolism of protease inhibitors. As a result, the amount of protease inhibitor may need to be increased. The major primary metabolite is a result of hydroxylation at C-7 (compound 19).
Compounds which inhibit the replication of HIV
(Synthesis of Delavirdine)

Delavirdine mesylate is a member of the bis(heteroaryl)piperazine (BHAP) class of nonnucleoside HIV-1 reverse transcriptase inhibitors (Adams et al., 1998; Romero et al., 1993; Romero, 1994). This class of compounds was discovered by Upjohn scientists from a computer-directed dissimilarity analysis of the Pharmacia & Upjohn chemical library to select compounds for screening against HIV-1 RT. The result of the in vitro assay (Deibel et al., 1990) is an IC50 of 0.260 uM, which is comparable to AZT. In accordance with the previous NNRTIs, delavirdine is a noncompetitive inhibitor of reverse transcriptase, and has a synergistic effect with nucleoside transcriptase and protease inhibitors (Chong et al., 1994).

The antiviral effect of delavirdine against multiple laboratory adapted strains and clinical isolates of HIV-1 is measured in cell culture using various cell lines [MT-2 cells and peripheral blood mononuclear cells (PBMC)] (Dueweke et al., 1993a). In the MT-2 cells infected with HIV-1 (IIIb) with a MOI (multiplicity of infection) of 0.001 (as determined by measuring levels of syncytia formation), the ED50 values for delavirdine and AZT are 10 nM and 100 nM, respectively.

The CC50 for MT-2 cells is >10 uM for delavirdine and 10 uM for AZT. Under the same MOI with HIV (IIIb), comparable results are observed in the PBMC cell line. Using this assay, the ED50 of delavirdine is 0.1–1 uM, and the ED50 for AZT is 1 uM.
Compounds which inhibit the replication of HIV
(Synthesis of Delarvidine)

As the NNRTIs are structurally diverse and yet bind to RT at a common site, the similar occurrence of resistance-conferring mutations is not surprising. As a consequence, the effectiveness of other NNRTIs may be compromised by the emergence of HIV-1 variants caused by a previous NNRTI therapy (Sardana et al., 1992). Experiments were performed in which HIV-1 strains (JR-CSF or MF) are cultured in human lymphocytes in the presence of partially inhibitory concentrations of delavirdine (Dueweke et al., 1993b). These conditions yield mutants that are 100-fold resistant. In order to determine what mutation(s) occurs, PCR (polymerase chain reaction) amplification and DNA sequence analysis of the RT coding region were applied and indicated that mutation P236L had occurred. Mutations at amino acids 181 or 183, which have been associated with resistance to other NNRTIs, are not detected. In order to measure the effect of other mutated HIV (IIIb) RTs, oligonucleotidedirected mutagenesis is conducted (Sheehan et al., 1961). Both the Y181C and K103N mutations possess resistance to delavirdine and nevirapine. However, delavirdine has a greater inhibitory effect than nevirapine. As an example, the IC50 for delavirdine against the K103N mutant was 8.3 mM. The other NNRTIs studied fail to achieve 50% inhibition at 60 mM (the highest concentration examined). The role of position 181 appears to be more important in the binding of nevirapine and the other NNRTIs tested than in the case of delavirdine. Additional cell culture experiments are conducted to confirm the sensitization observed with the mutant RTs. Lower concentrations of nevirapine (5–20-fold) are required to completely inhibit viral replication of delavirdine-resistant HIV-1 variants as compared to wild-type HIV-1, substantiating the above results (Sheehan et al., 1961).
Compounds which inhibit the replication of HIV (Synthesis of Delavirdine)

Synthesis of delavirdine begins with the addition of piperazine (20) to chloropyridine 21 (Scheme 6.5). The nitro group is reduced and the resultant amine undergoes reductive amination with acetone to provide pyridyipiperazine 23. Coupling of 23 with 6-nitroindole-2-carboxylic acid (24) is accomplished using either 1-ethyl-3-(dimethylamino) propylcarbodiimide (EDC) (Desai and Straniello, 1993; Sheehan et al., 1961) or 1,10-carbonyldiimidazole (CDI) (Morton et al., 1988; Romero et al., 1994) to give amide 25. The nitro group is reduced under standard palladium on carbon hydrogenation conditions. The resulting amine is then sulfonylated with methanesulfonfyl chloride to provide delavirdine, which is then transformed to delavirdine mesylate (3).
Compounds which inhibit the replication of HIV
(Synthesis of Delavirdine)

Delavirdine mesylate is given orally at doses of two 200-mg tablets, three times a day. The half-life of delavirdine mesylate is approximately 6 hs. Severe, life-threatening skin reactions have been associated with the use of delavirdine. Skin rashes appear in about 25% of patients. Delavirdine may cause serious and/or life-threatening side-effects if taken with certain other medications, including astemizole (Hismanal), terfenadine (Seldane), midazolam (Versed), triazolam (Halcion), ergotamine (Ergostat, Ergomar, and others), dihydroergotamine (D.H.E. 45), nifedipine (Procardiaw, Adalatw), sildenafil (Viagra), quinidine (Cardioquin, Quinaglute, Quinidex, and others), and others. Delavirdine mesylate is administered as part of the AIDS cocktail with protease inhibitors and NRTIs. Investigation of the in vitro metabolism of delavirdine is accomplished using mouse, rat, dog, monkey, rabbit, and human liver microsomes. The primary metabolite observed is the N-dealkylated delavirdine 26. Another primary metabolite observed is the hydroxylation of the pyridine ring at C-60 (compound 27). The primary metabolism is by CYP3A4 and also CYP2D6. Delavirdine reduces the activity of CYP3A4, thereby inhibiting its own metabolism.
Compounds which inhibit the replication of HIV
(Synthesis of Saquinavir)

The discovery that protease inhibitors were effective against HIV sparked intensive work in many laboratories on preparing proprietary compounds. Some nine discrete anti-HIV protease inhibitors have been approved by the Food and Drug Administration (FDA) as of this writing. The account that follows describes only a few from that large group. The statine-like moiety in one of the first drugs, saquinavir, comprises a transition state mimic for the cleavage of phenylalanylprolyl and tyrosylprolyl sequences.
Compounds which inhibit the replication of HIV (Synthesis of Saquinavir)

Construction of Saquinavir starts with the protection of the amino group of phenylalanine as its phthaloyl derivative (Phth) by reaction with phthalic anhydride; this is then converted to acid chloride. The chain is then extended by one carbon using a Friedel–Crafts-like reaction. The required reagent (21-2) is prepared by reaction of the enolate obtained from the bis-silyl ether (21-3) of glyoxylic acid and lithio hexamethyldisilazane (LiHMDS) with trimethylsilyl chloride [26]. The uncatalyzed reaction of acid chloride (21-1) with (21-2) gives the chain extended product (21-5) directly on acidification; the first formed $\beta$-carbonyl compound (21-4) apparently decarboxylates spontaneously. The terminal alcohol is then protected as a tetrahydropyranyl ether by adding it to dihydropyran; reduction of the ketone with sodium borohydride occurs enantioselectively due to the presence of the adjacent chiral center. Reaction with methanesulfonyl chloride then gives intermediate mesylate (21-6), which is not isolated. The pyranyl ether is then removed by acid catalyzed exchange with ethanol to give (21-7). The alkoxide formed from the terminal hydroxyl in this last compound on treatment with potassium tert-butoxide internally displaces the adjacent mesylate to form epoxide (21-8), in which the configuration of the former alcohol carbon is inverted.
Compounds which inhibit the replication of HIV (Synthesis of Saquinavir)

\[
\begin{align*}
21-1 & \quad \text{PhthN-COCl} \\
21-4 & \quad \text{PhthN-COCH_2CO_2H} \\
21-3 & \quad \text{TMSO-C=CHCH_2OTMS} \\
21-2 & \quad \text{TMSO-COCH_2OTMS} \\
21-5 & \quad \text{PhthN-COCH_2CH_2OH} \\
21-6 & \quad \text{PhthN-COCH_2CH_2OMs} \\
21-7 & \quad \text{PhthN-COCH_2CH_2OMs} \\
21-8 & \quad \text{PhthN-COCH_2CH_2} \\
\end{align*}
\]
Compounds which inhibit the replication of HIV  
(Synthesis of Saquinavir)

The other major fragment consists of a decahydroisoquinoline that may be viewed as a rigid analogue of an amino acid. Methanolysis of the adduct (22-1) from butadiene and maleic anhydride in basic methanol gives the half-ester (22-2); the obligate cis stereochemistry of the adduct determines that of the future perhydroisoquinoline ring fusion. The half-acid is then resolved as its salt with l-ephedrine. The desired enantiomer is next converted to the acid chloride (22-3); hydrogenation under Rosenmund conditions, and palladium in charcoal in the presence of quinoline, lead to the aldehyde (22-4). The next step involves essentially adding methyl glycinate to the aldehyde group. Conversion of that compound to its benzal derivative (22-5) serves to remove the more acidic amino protons and at the same time activates the protons on the methylene group. Condensation of the lithium salt from that compound with aldehyde (22-4) may be envisaged as first forming an adduct such as (22-6). The acidic workup serves to dehydrate the β-hydroxyester, to hydrolyze the Schiff base, and to cyclize the ester with the newly revealed amine, though not necessarily in that order. The first product isolated is in fact the lactam (22-7). Reaction with diborane in the presence of propylamine serves to reduce both the lactam and the olefin conjugated with the ester to afford (22-8). Displacement of the ester methoxyl by means of dibutylaluminum-tert-butylamide gives the decahydroquinoline (22-9)
Compounds which inhibit the replication of HIV
(Synthesis of Saquinavir)
Compounds which inhibit the replication of HIV
(Synthesis of Saquinavir)

The last stage in this convergent synthesis comprises the connection of the individual units. The ring opening of epoxide (21-8) by the secondary amino group on perhydroisoquinoline (22-9) gives the alcohol (23-1). The phthaloyl protecting group is then removed by traditional treatment with hydrazine or, alternatively, with methylamine, the latter being more suitable to large scale work (23-2). The free amino group is then condensed with the Cbz derivative (23-3) of the monoamide from aspartic acid to give amide (23-4). Hydrogenation over palladium on charcoal reductively removes the benzyl group from the Cbz derivative; the unstable carbamic amide that remains decarboxylates to afford the amine (23-5). Condensation with quinoline-2-carboxylic acid (23-6) catalyzed by DCC forms the last amide bond [28]. There is thus obtained the HIV protease inhibitor saquinavir (23-7).
Compounds which inhibit the replication of HIV (Synthesis of Saquinavir)
Compounds which inhibit the replication of HIV
(Synthesis of Indinavir)

The HIV protease inhibitor indinavir (24-11) differs markedly in its structural components and is notable for the fact that it does not include a single natural a-amino acid [29]. Construction of this compound starts with reaction of resolved 1-amino-2-indanol with acetone to afford the cyclic carbinolamine derivative (24-2) that will act as a protecting group for both the amine and the alcohol. Acylation of this intermediate with hydrocinnamyl chloride (24-1) gives the amide (24-3). One of the key transformations in the sequence involves the alkylation of the carbanion obtained on treatment of (24-3) with LiHMDS with the toluenesulfonate derivative (24-4) from chiral glycidol. The enantioselective course of the alkylation reaction leading to (24-5) can be attributed to the proximity of the two chiral centers on the indan. In the other arm of the converging scheme, the catalytic reduction of the tert-butylamide (24-6) of pyrazine carboxylic acid gives the corresponding piperazine (24-7). This is then resolved as its camphorsulfonate salt. The amine at the 4 position is next selectively protected as its tert-butoxycarbonyl derivative (24-8) using BOC anhydride. The lesser steric bulk about that amino group as well as the possible hydrogen bonding of the amine at 1 with the adjacent carbonyl group contribute to the selectivity of this acylation step. Condensation of intermediate (24-8) with the large fragment (24-5) leads to an attack of the free amino group of the piperazine on the epoxide with consequent ring opening and formation of the alcohol (24-9); this reaction proceeds with the expected retention of configuration of the chiral center bearing the hydroxyl group. The tert-butoxycarbonyl protecting group is then removed by exposure of the intermediate to acid; the carbinolamine hydrolyzes under reaction conditions. Alkylation of the newly revealed piperazine nitrogen with 3-chloromethylpyridine (24-10) affords the protease inhibitor indinavir
Compounds which inhibit the replication of HIV  
(Synthesis of Indinavir)