Chapter 20
Antiviral Agents: Structural Basis of Action and Rational Design

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Abstract During the last 30 years, significant progress has been made in the development of novel antiviral drugs, mainly crystallizing in the establishment of potent antiretroviral therapies and the approval of drugs inhibiting hepatitis C virus replication. Although major targets of antiviral intervention involve intracellular processes required for the synthesis of viral proteins and nucleic acids, a number of inhibitors blocking virus assembly, budding, maturation, entry or uncoating act on virions or viral capsids. In this review, we focus on the drug discovery process while presenting the currently used methodologies to identify novel antiviral drugs by using a computer-based approach. We provide examples illustrating structure-based antiviral drug development, specifically neuraminidase inhibitors against influenza virus (e.g. oseltamivir and zanamivir) and human immunodeficiency virus type 1 protease inhibitors (i.e. the development of darunavir from early peptidomimetic compounds such as saquinavir). A number of drugs in preclinical development acting against picornaviruses, hepatitis B virus and human immunodeficiency virus and their mechanism of action are presented to show how viral capsids can be exploited as targets of antiviral therapy.

Keywords Antiretroviral drugs • Capsid proteins • DNA polymerases • Drug development • Fusion inhibitors • Hepatitis virus • Herpesviruses • Human
immunodeficiency virus • Influenza virus • Ligand docking • Neuraminidase
• Nucleoside analogues • Proteases • Viral assembly • Viral entry • Viral replication
• Virtual screening

Abbreviations

AIDS Acquired immune deficiency syndrome
CoMFA Comparative molecular field analysis
CTD C-terminal domain
HBV Hepatitis B virus
HCMV Human cytomegalovirus
HCV Hepatitis C virus
HIV Human immunodeficiency virus
HR Heptad repeat
HSV Herpes simplex virus
HTS High-throughput screening
LBVS Ligand-based virtual screening
mRNA Messenger RNA
Neu5Ac N-acetylneuraminic acid
NMR Nuclear magnetic resonance
NNRTIs Nonnucleoside RT inhibitors
NTD N-terminal domain
PDB Protein Data Bank
RSV Respiratory syncytial virus
RT Reverse transcriptase
SBVS Structure-based virtual screening
THF Tetrahydrofuran
VZV Varicella-zoster virus

20.1 Introduction

Antiviral drugs are compounds that stop the development and propagation of a virus without causing a relevant damage in the host cell. Despite landmark achievements (e.g. >30 new drugs approved during the last three decades to fight AIDS), the number of available antiviral compounds is still small and effective only against a limited group of pathogens. Examples are human immunodeficiency virus (HIV), herpes simplex virus (HSV), varicella-zoster virus (VZV), human cytomegalovirus (HCMV), influenza virus and hepatitis B and C viruses (HBV and HCV, respectively) [1].

There are several reasons that account for the difficulties in developing antiviral agents. First, viruses are obligatory intracellular parasites. Because every step in the
viral life cycle engages host functions, it is difficult to interfere with virus growth without having a negative impact on the host cell. Side effects are relatively common. Second, clinically important viruses are often dangerous and cannot be propagated or tested in model systems. Thus, viruses causing fatal diseases in humans (e.g. smallpox or several hemorrhagic diseases) have to be handled by well-trained and experienced scientists, and in facilities with strict containment requirements. Not surprisingly, these labs are expensive and difficult to maintain. Apart from the biological safety limitations, sometimes viral infections cannot be properly monitored for antiviral drug development due to the lack of appropriate animal models of human disease (e.g. smallpox or measles) or to difficulties in growing the virus in cell culture (e.g. HBV).

A third factor that limits the efficacy of antiviral drugs is their potency requirements. Ideally, an antiviral agent should be extremely potent. Partial inhibition is not acceptable for an antiviral drug. The reason is that limited viral replication under drug pressure allows for the generation of variants that can be selected under treatment. The emergence of resistance is a major drawback of many antiviral therapies. For example, in the case of HIV, therapies prescribed in the late 1980s or early 1990s were based on a single drug (mostly zidovudine) or combinations of two drugs (usually two inhibitors of the viral polymerase) [2]. However, those drugs were not potent enough to limit the emergence of drug-resistant variants [3] and therefore these viruses were almost impossible to combat successfully with the available drug armamentarium. In a patient with full blown AIDS, HIV production has been estimated at about $10^{12}$ virions per day. The high replication rate of HIV and the low fidelity of its DNA polymerase [4] trigger the appearance of drug resistance under suboptimal therapies.

Another issue that deserves some attention is the short duration of many viral infections (e.g. flu, common cold, etc.). Very often, the symptoms appear when the virus is no longer replicating and are due to the immune response of the patient. In those cases, antiviral drugs should be administered early in infection or as a prophylactic measure in populations at risk. However, this could be potentially harmful for healthy populations.

### 20.2 Drug Discovery and Potential Targets of Antiviral Intervention

Early evidence of activity against vaccinia virus was reported for several thiosemicarbazone derivatives [5], and one of them (N-methylisatin-β-thiosemicarbazone) entered clinical studies for the prophylaxis of smallpox [6] at the time when vaccination against smallpox virus took over. The first antiviral drug licensed for clinical use was a thymidine analogue known as idoxuridine ($5'$-iodo-$2'$-deoxyuridine), whose synthesis was described in 1959 [7]. Idoxuridine has been used topically to treat eye and skin infections caused by herpes simplex virus. The drug acts on viral
replication by interfering with the normal function of thymidylate phosphorylase and viral DNA polymerases.

Encouraged by the success with antibiotics in the 60s and 70s, drug companies launched huge blind-screening programs that were not very fruitful. Today, recombinant DNA technology and sophisticated chemistry [8], as well as impressive advances in structural and functional genomics, have facilitated the identification and analysis of particular proteins or mechanisms. Essential viral genes can be cloned and expressed in appropriate organisms so that the encoded proteins can be purified and analyzed in molecular and atomic detail.

Drug discovery programs start with the identification of suitable drug targets (Fig. 20.1). A drug target can be defined as a biomolecule (usually a single protein or a protein complex) linked to a disease and containing a suitable binding site that can be exploited to modulate its function. These targets need to be validated to demonstrate that they are critically involved in a disease and that their modulation is likely to have a therapeutic effect. In virology, many drug targets are viral proteins (e.g. enzymes), nucleic acids or other biological macromolecules required in the virus life cycle. Infection and viral propagation can be blocked by small compounds binding to relevant targets. Once the target has been validated in vitro and/or in animal models, lead identification starts with the design and development of a suitable assay to monitor the biological function under study. Active compounds that demonstrate dose-dependent target modulation and some degree of selectivity for the target under study are called lead compounds. These molecules are optimized in terms of potency and selectivity to become drug candidates.

In order to become a marketable drug, the candidate undergoes additional preclinical evaluation, including pharmacokinetic and toxicity studies in animal models. Clinical trials involving new drugs are commonly classified into four phases. Approval is usually granted if the drug advances successfully through phases I, II and III. The main objective of phase I is to assess drug safety and pharmacokinetics in a relatively small number of healthy individuals who receive small doses of the compound. In phase II, the testing protocol is established. These are trials trying to find out
appropriate doses, prescription regimens, etc. . . and are carried out with patients. Phase III constitutes the final testing before approval and these trials try to show whether the drug has a measurable benefit or advantage over other treatments with a relatively large number of patients (usually >100 patients for antiviral drugs). Phase IV includes all studies carried out after approval of the drug and may address many different issues, from side-effects to efficacy in comparison with other drugs or regimens, and usually involve a large number of patients worldwide.

Recent years have witnessed a remarkable progress in the discovery and development of antiviral agents, fueled by advances in the understanding of viral life cycles which in turn have provided new opportunities for therapeutic intervention. Although the mechanisms of replication and propagation can show significant variations between different viruses, a prototypic life cycle is presented in

**Fig. 20.2** Basic steps of viral replication as potential targets of antiviral therapy
Fig. 20.2 (see also Chap. 1). Typically, a virion (i.e. an infectious viral particle) first attaches to the surface of the host cell. This interaction can be specific and involve the participation of one or more different types of proteins. For example, in HIV-1, the main receptor is CD4, but other proteins (e.g. chemokine receptors CCR5, CXCR4, etc.) can facilitate viral entry acting as coreceptors (see Chap. 15).

Viral uptake occurs through different mechanisms such as endocytosis (as for example, in the case of influenza virus) or the fusion of the cellular membrane with the viral envelope, as demonstrated in the case of retroviruses (see Chap. 16). These events allow the internalization of the capsid containing the viral genome. Uncoating or disassembly is a still poorly understood process that releases the viral genome from its protein shell (see Chaps. 15 and 16). Disassembly may occur rapidly after fusion, as occurs in HIV, or be triggered by pH variations, as observed in viruses entering the cell by an endocytic pathway (e.g. influenza virus). The replication of the viral genome occurs by different mechanisms depending on whether the viral genome is a single- or double-stranded DNA or RNA. In general, DNA viruses use cellular pathways to replicate their genomes, but RNA viruses (and in general, those replicating in the cytoplasm) provide their own enzymes to complete virus replication. Cellular factors, together with specific proteins encoded within the viral genome, contribute to transcription and post-transcriptional modification of viral messenger RNAs (mRNAs). In some viruses (for example, in retroviruses), viral mRNAs are generated in the nucleus by the action of the cellular RNA polymerase. This is due to the integration of the viral genome (as a double-stranded DNA) into the genome of the host cell.

Viral polyproteins are synthesized by the cellular translational machinery. After post-translational modification, viral proteins and their genomes are transported to assembly sites within the host cell (see Chap. 14). Viral factors may also participate in these processes. The sites of assembly are frequently located at the plasma membrane or in intracellular factories (often associated with membranes). Virion assembly is a complex process which involves multiple molecular recognition steps and conformational transitions. The viral capsid is assembled in a multimerization reaction, with or without the help of scaffolding proteins or viral nucleic acids (see Chaps. 10, 11 and 19). The viral nucleic acids are packaged into the capsid during or after its assembly (see Chap. 12). Finally, the viral particle can be transformed into an infectious virion through a maturation process that involves changes in the structure and properties of the capsid (see Chap. 13).

In enveloped viruses, a membrane with embedded viral proteins is incorporated into the virion (see Chap. 11), and the resulting viral particles are released after budding. In contrast, cell lysis mediates the release of non-enveloped viruses. In some cases (e.g. in retroviruses), maturation occurs once the immature virion has been released and involves the proteolytic processing of precursors containing the viral proteins, including those that form the capsid.

All of those steps of the viral life cycle constitute potential targets of antiviral intervention. At present, drugs inhibiting viral enzymes involved in the replication or expression of the viral genome are commonly used in antiviral treatments. However, recent developments including the determination of structures, properties and functions of capsids and virions, as well as the elucidation of events involving
interactions between components of the viral particle or between them and host cell molecules (the subjects of this book) have opened novel avenues for the design of drugs acting directly on the viral particle. This chapter provides examples of approved or pre-clinical antiviral strategies directed at inhibiting viral nucleic acid metabolism, as well as others aimed at interfering with cell recognition, entry, uncoating, assembly, or maturation of virus particles.

20.3 Antiviral Drugs and Mechanisms of Action

Licensed compounds used in the treatment of viral infections target HIV, HBV, HCV, influenza virus, HSV, and other herpesviruses such as VZV and HCMV. A number of drugs act on steps that lead to the formation of the viral capsid or the mature virion (i.e. assembly, budding and maturation) while others, whose target is the assembled capsid or the virion, interfere with processes affecting viral entry and uncoating [1]. Nonetheless, most of the approved drugs block intracellular events affecting the synthesis and dynamics of viral proteins and nucleic acids (Table 20.1). Within this group, viral polymerases constitute the major target for many antiviral drugs.

20.3.1 Drugs Blocking Intracellular Processes Required for the Synthesis of Viral Components

Viral Genome Replication Inhibitors

Compounds inhibiting the replication of HSV, VZV and HCMV include prodrugs of nucleoside analogues (e.g. valacyclovir, valganciclovir and famciclovir) that need to be phosphorylated in order to become substrates of the viral DNA polymerase (Fig. 20.3). Viral enzymes (e.g. thymidine kinases in HSV and VZV, and a protein kinase in HCMV) are responsible for the transformation of acyclovir, ganciclovir and penciclovir into their monophosphate derivatives. Further phosphorylation steps are carried out by host cell kinases. The triphosphate derivatives of acyclovir, ganciclovir and penciclovir that mimic the natural substrates of the viral DNA polymerase, are incorporated into the growing DNA chain and often terminate viral replication, because they lack a 3'-OH in their ribose ring. Cidofovir is a phosphonate-containing acyclic cytosine analogue that, unlike the inhibitors described above, does not depend on viral enzymes for its conversion to the triphosphorylated form that competes with the dNTP substrates [9]. Foscarnet is an analogue of pyrophosphate, a product of the nucleotide incorporation reaction, and therefore behaves as an inhibitor of DNA polymerization. Unfortunately, our understanding of the mechanisms involved in resistance to acyclovir and other related inhibitors is limited by the absence of crystal structures of herpesvirus DNA polymerases.
<table>
<thead>
<tr>
<th>Target</th>
<th>Step of the viral life cycle or cellular function inhibited</th>
<th>Virus</th>
<th>Drug type and name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral</td>
<td>Entry</td>
<td>HIV</td>
<td>Fusion inhibitors: Enfuvirtide</td>
</tr>
<tr>
<td></td>
<td>Disassembly/uncoating</td>
<td></td>
<td>Drugs binding to the viral protein M2 (an ion channel): Amantadine and rimantadine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Influenza virus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genome replication</td>
<td>HIV</td>
<td>Nucleoside/nucleotide RT inhibitors: Zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), emtricitabine (FTC) and tenofovir (tenofovir disoproxil fumarate)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Nonnucleoside RT inhibitors: Nevirapine, delavirdine, efavirenz, etravirine and rilpivirine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HBV</td>
<td>Nucleoside/nucleotide analogues: Lamivudine, emtricitabine, entecavir, telbivudine, adefovir (adefovir dipivoxil) and tenofovir (tenofovir disoproxil fumarate)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV and VZV</td>
<td>Nucleoside/nucleotide analogues: Acyclovir (valaciclovir), penciclovir (famciclovir), idoxuridine, trifluridine and brivudine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HCMV</td>
<td>Nucleoside/nucleotide analogues: Ganciclovir (valganciclovir) and cidofovir</td>
</tr>
<tr>
<td></td>
<td>Integration into the host genome</td>
<td>HIV</td>
<td>HIV integrase inhibitors: Raltegravir and elvitegravir</td>
</tr>
<tr>
<td></td>
<td>Synthesis of viral mRNAs</td>
<td>HCMV</td>
<td>Antisense (phosphorothioate) oligonucleotide: Fomivirsen</td>
</tr>
<tr>
<td></td>
<td>Cleavage of viral polyproteins</td>
<td>HIV</td>
<td>HIV protease inhibitors: Saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and its prodrug fosamprenavir, lopinavir, atazanavir, tipranavir and darunavir</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HCV</td>
<td>HCV protease inhibitors: Telaprevir and boceprevir</td>
</tr>
<tr>
<td>Budding</td>
<td></td>
<td>Influenza virus</td>
<td>Viral neuraminidase inhibitors: Oseltamivir and zanamivir</td>
</tr>
<tr>
<td>Cellular</td>
<td>Viral entry</td>
<td>HIV</td>
<td>Viral coreceptor inhibitors: Maraviroc</td>
</tr>
<tr>
<td></td>
<td>Innate immunity</td>
<td>HCV and HBV</td>
<td>Interferons: Pegylated interferons α-2a and α-2b, and interferons α-2a and α-2b</td>
</tr>
<tr>
<td></td>
<td>mRNA capping enzymes and viral mutagenesis</td>
<td>HCV and influenza virus</td>
<td>Ribonucleoside analogue: Ribavirin</td>
</tr>
</tbody>
</table>

*aCompound approved as a pro-drug, whose name is indicated between parentheses*
HIV is a retrovirus that replicates through a proviral double-stranded DNA intermediate. Its polymerase, known as reverse transcriptase (RT), is able to synthesize DNA by using either RNA or DNA as templates. Nucleoside/nucleotide analogues and nonnucleoside RT inhibitors (NNRTIs) constitute the backbone of current antiretroviral therapies [2, 3]. Nucleoside/nucleotide RT inhibitors are prodrugs that need to be converted to active triphosphate analogues in order to be incorporated into the DNA during reverse transcription. They act as chain terminators thereby blocking DNA synthesis [10]. The HIV-1 RT is a heterodimer composed of subunits of 560 and 440 amino acids (known as p66 and p51, respectively). Both polypeptide chains have the same amino acid sequence, but the large subunit contains an RNase H domain that includes the 120 amino acids of its C-terminal region. The DNA polymerase domain is formed by residues 1–315 and aspartic acid residues 110, 185 and 186 in p66 constitute the catalytic triad.

There are many crystal structures available for HIV-1 RT, including relevant complexes such as the ternary complex of HIV-1 RT/double-stranded DNA/dTTP, or RT bound to RNA/DNA or to DNA/DNA template-primers. Additional structures containing DNA primers blocked with nucleoside analogues have also been described. A large number of NNRTIs have been crystallized in complex with the viral polymerase (for a review, see [11]). NNRTIs bind to a hydrophobic pocket

![Fig. 20.3](image_url)
located about 8–10 Å away from the DNA polymerase active site. The first NNRTIs were identified by using an antiviral screening approach, but structure-based drug design has played a prominent role in the design and development of next-generation NNRTIs such as etravirine or rilpivirine [12, 13].

Reverse transcription is also involved in the replication of HBV. These viruses form an immature RNA-containing nucleocapsid that, inside the cell, is converted into a mature nucleocapsid containing a relaxed circular DNA [14]. The DNA found in extracellular virions is synthesized by viral RT using the pregenomic RNA as a template. The HBV RT shows sequence similarity with HIV-1 RT, and several inhibitors of HIV-1 replication (e.g. lamivudine, adefovir or tenofovir) have been approved from treating HBV infections (Table 20.1). Unfortunately, there is no available crystal structure for the HBV RT. The existence of this gap of knowledge can be attributed to major difficulties in obtaining a catalytically active polymerase.

RNA polymerases play a key role in the replication of important pathogenic RNA viruses such as HCV, poliovirus, rhinovirus, influenza virus and others. However, drugs targeting viral RNA polymerases have not received approval. At present, HCV RNA polymerase inhibitors are probably the ones in a more advanced stage of development [15, 16]. The HCV replicase (NS5B, an RNA-dependent RNA polymerase) is a 65-kDa protein that contains a C-terminal membrane insertion sequence that traverses the phospholipid bilayer as a transmembrane segment. NS5B inhibitors can be classified into nucleoside and nonnucleoside analogues. Examples of the first group are valopicitabine, 2'–C-methyladenosine, 4'–azidocytidine and mericitabine. The nucleotide prodrug β-D-2'–deoxy-2'-α-fluoro-2'–β-C-methyluridine (PSI-7977) [17] is currently in phase III clinical trials and showed promising results when combined with pegylated interferon and ribavirin. Nonnucleoside inhibitors of NS5B are tegobuvir, filibuvir and pyranoindoles such as HCV-371 and HCV-570 [18].

The non-structural 5A (NS5A) protein of HCV has been identified as the target of daclatasvir (BMS-790052), a thiazolidinone-containing small molecule that was shown to inhibit HCV replication in a cell-based replicon screen [19]. The inhibitor showed picomolar potency in preclinical assays. The enzymatic function of NS5A is not known, although it interacts with NS5B and modulates the host cell interferon response. Resistance to daclatasvir is associated with mutations in the N-terminal region of NS5A [20]. Interestingly, the cyclic endecapeptide alisporivir, currently in Phase III clinical trials, is a cyclophilin A-binding drug that selects for mutations in NS5A, although in a different domain [21].

**Drugs Interacting with Other Targets**

Gene expression is another possible target for antiviral intervention. A phosphorothioate derivative of the oligonucleotide 5'–GCG TTT GCT CTT CTT GCT GCG–3' (known as fomivirsen) has been used as a repressor of the synthesis of early genes in HCMV infections. It binds to the complementary sequence in the viral mRNA, blocking its translation. In retroviruses, integration of the double-stranded proviral DNA (the product of reverse transcription) into the host cell DNA is essential for expression of viral mRNA. Retroviral mRNA is generated by transcription of the integrated provirus by cellular RNA polymerases.
Raltegravir and elvitegravir are inhibitors of HIV-1 integrase [22, 23], an enzyme endowed with 3′ endonucleolytic and strand transfer activities. It is composed of three structural domains: an N-terminal domain of unknown function, the central catalytic domain, and a C-terminal domain with DNA binding activity. Approved drugs bind to the central domain, close to the catalytic residues Asp64, Asp116 and Glu152, and act by blocking the strand transfer activity of the integrase. Crystal structures of catalytic domains have been used in the design of integrase inhibitors, but the full integrase (having all three domains) has become available only very recently [24].

Viral proteins are frequently synthesized as precursor polypeptides that are cleaved by viral proteases. Sometimes, this cleavage occurs before the assembly of the viral capsid, which is formed by the association of mature proteins into capsomers. For example, in poliovirus, rhinovirus and other picornaviruses, 60 protomers made of processed capsid proteins VP0, VP1 and VP3 assemble to form the icosahedral particle. In HCV, its serine protease (designated as NS3) interacts with an NS4A peptidic co-factor that modulates its proteolytic activity. Promising NS3/4A inhibitors were developed after rational structure-based design using a hexapeptide (Asp-Asp-Ile-Val-Pro-Cys) derived from the natural NS5A/5B cleavage site as a lead compound. After systematic replacement of single amino acid residues, important substituents in the protease binding pocket were identified and docked in the crystal structure of the unliganded NS3 protease. Further optimization led to the identification of ciluprevir (BILN-2061), one of the first promising drug candidates (Fig. 20.4). The recently licensed inhibitors boceprevir [25] and telaprevir [26] were developed by using similar approaches.

20.3.2 Inhibitors of Viral Assembly

Despite considerable efforts in recent years, drugs targeting the assembly of viral capsids (see Chaps. 10 and 11) have not yet gained approval for clinical use. At preclinical stages of development, there are interesting examples of drugs inhibiting the assembly of capsids of HBV (e.g. heteroaryldihydropyrimidines) [27, 28] and HIV-1 (CAI, CAP-1, bevirimat, etc.) [29, 30] (see Sects. 20.6.2 and 20.6.3).

20.3.3 Budding Inhibitors

At present, antiviral treatment and prophylaxis of human influenza infections, including aggressive strains such as avian H5N1, are based on the use of oseltamivir and zanamivir that inhibit the release of infectious virus. The influenza virus membrane contains a glycoprotein (hemagglutinin) that recognizes sialic acid in cell-surface glycoproteins and glycolipids [31]. Sialic acid is the receptor for virus entry. However, newly formed virions may remain attached to the cell membrane
because of the interaction between hemagglutinin and sialic acid. The viral neuraminidase (also known as sialidase) facilitates the release of virions from infected cells by cleaving sialic acid residues. Oseltamivir and zanamivir inhibit this hydrolytic activity.

### 20.3.4 Maturation Inhibitors

Maturation is a relatively common process in animal and human viruses (see Chap. 13). In contrast to HCV and other virus families, specific cleavage of the polypeptides encoding the mature viral proteins occurs only on assembled immature particles. HIV-1 protease inhibitors block the proteolytic processing of precursor polypeptides Gag and Gag-Pol. Cleavage of Gag and Gag-Pol leads to the formation of structural proteins (*e.g.* MA, CA and NC), p6 and the viral enzymes protease, RT and integrase (Fig. 20.5). This process occurs extracellularly and is required for the formation of an infectious virion. The HIV-1 protease is a homodimeric enzyme composed of subunits of 99 amino acids with a symmetric substrate binding pocket. Inhibitors were initially designed as peptide derivatives mimicking the ‘transition-state’ and contained a non-hydrolysable bond at the position where the cleavage was expected to occur. All of the approved HIV protease inhibitors, except tipranavir, can be considered as peptidomimetics [2].
**Fig. 20.5** Protein arrangement in Gag and Gag-Pol precursor polyproteins, HIV-1 protease structure and model substrate. Cartoon representation of the protease bound to darunavir (PDB entry 1T3R). Inhibitor C, O and N atoms are displayed as green, red and purple spheres, respectively. The minimal peptide substrate SQNY*PIV (asterisk indicates cleavage site) is shown below the protease structure.
20.3.5 Drugs Targeting Viral Entry

Drugs acting at this step include compounds that block cell attachment, receptor recognition or the mechanisms leading to the translocation of the viral capsid to the host cell cytoplasm (see Chaps. 15 and 16). Two anti-HIV drugs belonging to this category have been approved: maraviroc and enfuvirtide. The first interferes with coreceptor recognition, while the second blocks the fusion of the viral envelope with the cell membrane.

Receptor and Coreceptor Recognition in HIV-1 Infection

HIV-1 enters target cells through a multi-step process that requires non-specific interactions with surface heparan sulfates, followed by binding of the viral envelope glycoprotein gp120 to its primary cellular receptor CD4. Attachment inhibitors in HIV-1 include dextran sulphate and other polyanions, as well as proteins binding glycosidic residues (e.g. mannos-specific lectins or cyanovirin N).

CD4 binds into a carbohydrate-free depression in gp120 that is poorly accessible to immunoglobulins. However, crystallographic studies carried out with complexes of gp120, CD4 and Fab fragments of neutralizing antibodies have shown that Phe43 of CD4 and residues 365–371 and 425–430 of gp120 make the largest contributions to interatomic contacts [32]. Phe43 is located on the CDR2-like loop of CD4 and binds within a hydrophobic pocket of gp120, termed the “Phe43 cavity”, while Arg59 (in CD4) is located on a neighboring β-strand and forms an ion pair with the gp120 residue Asp368. The disruption of this interaction by small molecule binding has been validated as a promising approach to prevent HIV-1 entry. Inhibitors containing a tetramethylpiperidine ring such as NBD-556 displayed remarkable antiviral activity and altered the gp120 conformation in a similar way as observed upon CD4 binding [33, 34].

CCR5 and CXCR4 are the necessary coreceptors for cellular entry of HIV-1. These molecules are members of the seven-transmembrane G protein-coupled receptor superfamily, and can be blocked by natural ligands such as MIP-1α, MIP-1β and RANTES for CCR5 and SDF-1 for CXCR4. RANTES derivatives and SDF-1α have been investigated as potential inhibitors of viral entry. However, smaller CCR5 antagonists such as TAK-652 and TAK-779, aplaviroc, vicriviroc and maraviroc were further studied as potential anti-HIV agents. So far, maraviroc remains as the only clinically approved coreceptor antagonist. However, resistance to this drug appears mostly as a result of HIV-1 using CXCR4 as an alternate coreceptor for entry [3].

The most advanced CXCR4 antagonist, AMD3100 (Mozobil) has not been further studied as a therapeutic agent against HIV-1 [18]. However, in combination with granulocyte colony stimulating factor (and under the name of plerixafor), it has been approved for the mobilization of hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin’s lymphoma and multiple myeloma.
Fusion Inhibitors: Targeting the Formation of Coiled-Coil Structures

Viral recognition by cellular receptors triggers a mechanism that leads to the fusion of the viral envelope and the target cell membrane [35] (see Chap. 16). Crystallographic studies of pre- and post-fusion conformations of the influenza virus hemagglutinin showed that the formation of a six-helix bundle played a major role in the fusogenic events. Drugs targeting the helix bundle have been developed to block entry of HIV-1 and respiratory syncytial virus (RSV).

In the case of HIV-1, enfuvirtide is a 36-amino acid peptide that derives from residues 127–162 of the transmembrane protein gp41. This glycoprotein has a trimeric structure, and each monomer contains a transmembrane region of 21 residues, located between an N-terminal ectodomain (175 residues) and a long cytoplasmic domain of 150 amino acids. The sequence of enfuvirtide overlaps with the heptad repeat two (HR2) region (residues 117–154) that interacts with the HR1 region (residues 29–82) of gp41. Enfuvirtide binds HR1 and prevents the formation of the trimeric coiled-coil structure required for membrane fusion. Despite showing efficacy in vivo, the clinical use of the drug has been limited by its reduced bioavailability and its large production costs. Novel anti-HIV drugs based on similar principles include tifuvirtide, sifuvirtide and T-2635. These peptides mimic in part the HR2 structure and exert their effects through a mechanism of action similar to that shown by enfuvirtide [35].

In the case of the human RSV, an orally active candidate (BMS-433771) was proposed to bind in a hydrophobic cavity within the trimeric N-terminal heptad repeat (equivalent to HR1), which is the region that associates with a C-terminal HR2 to form a six-helical coiled-coil bundle during the fusion process. X-ray crystallography of another drug candidate (TMC353121) bound to the human RSV fusion protein (Protein Data Bank (PDB) entry 3KPE) showed that this drug, rather than preventing formation of the coiled-coil, produces a local disturbance of the six-helix bundle by stabilizing the HR1–HR2 interactions in an alternate conformation of the six-helix bundle [36].

20.3.6 Uncoating Inhibitors

Amantadine and rimantadine were used more than 20 years ago to prevent influenza A virus infections. However, they never gained wide acceptance and today virtually all strains are resistant to these agents. These compounds block the release of the viral capsid to the cytoplasm of the infected cell, because they interact with M2, an integral protein located in the viral envelope. It consists of four identical units, each one having three distinct domains: (i) an ectodomain made up of 24 residues exposed to the outside environment, (ii) a transmembrane region (mostly hydrophobic) composed of 22 amino acids, and (iii) 52 amino acids at the C-terminal region exposed to the intraviral milieu. M2 is activated by low pH and functions as an ion channel that facilitates entry of protons into the viral particle. Amantadine and rimantadine bind to the transmembrane region (Fig. 20.6), sterically blocking the channel and inhibiting the release of the viral capsid to the host cell cytoplasm.
20.4 Strategies in the Development of Antiviral Drugs: From Random Screening to Structure-Based Design

Most of the antiviral drugs clinically used today and mentioned in previous sections of this chapter were discovered serendipitously or as a result of screening campaigns that made use of large libraries of synthetic compounds or natural products. In the traditional drug discovery process, it is estimated that screening of around 80,000 molecules can render one hit that needs to be further optimized to a lead compound by improving its activity and reducing its toxicity before entering clinical trials. Usually, these huge efforts are needed because of the lack of structures for ligands and protein targets. In this scenario, pharmaceutical companies invested in the development of high-throughput screening (HTS) assays and in the generation of large chemical libraries obtained by combinatorial chemistry. Nevertheless, this process is expensive, time-consuming and requires further efforts in target validation.

Random approaches including HTS and combinatorial chemistry are used in the absence of structural information about ligands or target proteins or macromolecular complexes. Over the last two decades we have been able to witness many profound changes in the drug discovery paradigm due to major advances in such diverse areas as HTS methods, genome sequence projects, macromolecular structure determination, quality and scope of publicly accessible databases, computer performance and new algorithms for ‘in silico’ approaches [37] (Fig. 20.7). Among the latter, structure-based and ligand-based virtual screening (SBVS and LBVS, respectively) stand out as powerful tools that are being increasingly used in both
industry and academia [38]. These techniques rely on the three-dimensional coordinates of either the macromolecular target (SBVS) or one or more known ligands (LBVS) to search in chemical or fragment libraries for putative hits that can then be tested experimentally for confirmation of affinity/activity and then eventually transformed into lead compounds. Due to the huge amount of ligands that can be found in currently available databases (e.g. 13 million in ZINC [39]), it is customary to employ a series of computational cost-effective filters to narrow down the number of molecules that will be subjected to virtual screening. Lipinski’s rule of five\(^1\) [40] and/or other physico-chemical-based principles can be used for filtering, as well as pharmacophoric hypotheses. One goal of LBVS is to find similarly shaped molecules that belong to novel chemotypes (“scaffold hopping”) [41].

### 20.4.1 Structure-Based Drug Design

Structure-based drug design relies on knowledge of the three-dimensional structure of the biological target gained by means of techniques such as X-ray

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\(^1\) Lipinski’s rule states that, in general, an orally active drug should meet at least three of the following criteria: (i) Not more than five hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms), (ii) not more than ten hydrogen bond acceptors (nitrogen or oxygen atoms), (iii) a molecular mass \(<500\) daltons, and (iv) an octanol-water partition coefficient \(\log P\) not greater than five.
crystallography (Chap. 4), nuclear magnetic resonance (NMR) spectroscopy (Chap. 5) and electron microscopy (Chap. 3). A number of computational methods have been developed to identify putative ligands that can fit into an active site or functional epitopes in the target protein or molecular complex. Success in molecular docking, which may be defined as an optimization problem that describes the “best-fit” orientation of a ligand within a binding site [42], very often depends on the quality of the three-dimensional target protein structure. Structures determined by X-ray crystallography at atomic or near-atomic resolution are the most adequate for docking. More specifically, successful identification of lead compounds usually requires a crystallographic resolution below 2.5 Å and an R factor well below 25 % (see Chap. 4).

20.4.2 Ligand Docking and Virtual Screening

Docking methods are calibrated and tested for their ability to predict, at the top of the list of possible solutions, the native conformation of a ligand bound to a protein as found in a representative series of crystallographic complexes. To assess the performance of a docking program in SBVS its ability to discriminate true binders from a pool of fake ligands (“decoys”) is also examined. The goodness of fit between the ligand and the receptor is evaluated by means of a scoring function composed of different terms that attempt to account for the forces driving the binding event. Although the underlying physical laws describing the association process are well understood, accuracy and computational resources (mainly execution time) evolve in opposite directions, and fine tuning the appropriate balance between them is not an easy task. Therefore, accuracy is normally sacrificed for speed, especially in SBVS where very often too simplistic scoring functions are employed which plainly cannot capture the true free energy of binding that is measured experimentally [43]. Ligand conformers can be generated within the binding site itself “on the fly” for each target, or created beforehand as a collection of all major theoretically possible conformers following some pre-defined rules. The former method is more computer-intensive but, as an advantage, it can generate strained conformations that adapt better to each specific binding site environment. The latter method allows the stored coordinates to be reused again as many times as needed in different SBVS campaigns but the drawback is that a relevant conformation that deviates slightly from a canonical one can be missed.

Fragments [molecular weight ≤ 250 Da, log P ≤ 2.5, (where P refers to the octanol-water partition coefficient) and less than 6 rotatable bonds] are usually endowed with reduced affinities compared to conventional ligands but are better suited for chemical modifications aimed at producing novel drug candidates [44]. They can sample chemical space more effectively than do regular ligands. It is generally accepted that fragment docking outperforms traditional small-molecule docking in terms of hit rates even though erroneous binding modes can be obtained for them because their volumes are much smaller than those of typical binding site
Docked fragments can then evolve virtually (by adding different chemical decorations), be linked (to join two or more fragments that occupy different regions of the binding site), self-assemble (through direct bond formation between different reacting fragments), and/or be optimized to better fulfill drug-like properties. Since the binding affinity of compounds has been shown to grow approximately linearly with their molecular weight, the affinity of fragments and standard drugs are usually compared in terms of a metric called ligand efficiency index, which is defined as the binding free energy per heavy atom. Given that affinity/activity information related to ligand-target complexes is currently stored in several databases (e.g. BindingDB, ChEMBL, and PDBBind, among others), ligand efficiency indices have been proposed as useful variables to chart the chemico-biological space and visualize it in an atlas-like type of representation [45].

The binding pockets that are explored in most SBVS campaigns are relatively small cavities that are meant for natural agonists, substrates or cofactors in a limited number of macromolecular targets. These represent what are known as the “orthosteric” binding sites for drugs. However, other drugs (e.g. NNRTIs) exert their action by altering the receptor’s conformation in such a way that it would affect the binding and/or functional properties of agonists or substrates (“allosteric modulators”) whereas yet others are known to modulate or prevent some key protein-protein interactions.

Most protein-protein interfaces have been usually regarded as “undruggable” because conventional analysis reveals them as lacking well-defined binding pockets. However, it has been noted that a large percentage of the free energy of interaction arises from small regions designated as “hot spots”. These facts supported the concept by which protein-protein interaction disruptors do not need
to mimic the entire protein binding surface but rather a smaller subset of key residues in a relatively compact region of one of the binding partners.

Different computational tools have been developed for exploring the properties of protein-protein interfaces and facilitate the identification of starting points for small-molecule design (e.g. PocketQuery [46], http://pocketquery.csb.pitt.edu/). More generally, “druggable” sites can be located using FTMap [47], which is an efficient fast Fourier transform correlation method that samples multiple positions of up to 16 different probes on a three-dimensional grid centered on a protein of interest. A simple energy expression then allows the identification of consensus sites where probes tend to cluster, giving rise to favorable interactions. These sites correlate with locations where high-affinity ligands are found in protein complexes. The method is sensitive to conformational changes in the protein and can be used to map affinity sites prior to docking. The FTMap algorithm is implemented in the public domain Web server http://ftmap.bu.edu/.

### 20.4.3 Structure-Activity Relationships

Finally, visual examination of the crystal structure of a single ligand-receptor complex is usually highly informative and can guide the design of new analogues that will display graded affinities toward the target. However, a quantitative assessment of these variations will generally be difficult because the observed free energy differences result from a subtle interplay of binding forces within the receptor binding site that normally take place in the face of competition with water molecules. An approach that has been shown to be useful in these cases is to model the whole set of complexes and decompose the ligand-receptor interaction energies into a number of van der Waals and electrostatic contributions emanating from individual receptor amino acids, together with additional terms representing the desolvation of both binding partners. The resulting matrix of energy terms can then be projected onto a small number of orthogonal “latent variables” or principal components using partial least squares in a way similar to that used in comparative molecular field analysis (CoMFA). At the end of the procedure, those pair-wise interactions between the ligands and individual protein residues that are predictive of binding affinity or activity are selected and weighted according to their importance in the model. Since its inception, this chemometric method, termed comparative binding energy (COMBINE) analysis, has been successfully applied to a variety of biologically relevant targets including the protease and the RT of HIV-1 [48].

### 20.5 Case Studies in Structure-Based Antiviral Drug Development

During the last three decades we have witnessed an unprecedented effort to discover new antiviral drugs, most of them directed towards HIV, and more recently towards HCV. Many of those studies have benefited from the availability
of high-resolution crystal structures. We have selected a few case studies to illustrate how structure-based design has contributed to antiviral drug discovery. In this section we describe two successful examples that include oseltamivir and zanamivir acting on influenza virus neuraminidase, and mutation-resilient inhibitors of HIV-1 protease (e.g. darunavir). In the next section (20.6) we describe current experimental efforts in structure-based design, applied to inhibitors acting on the assembly or disassembly of viral capsids.

### 20.5.1 Neuraminidase Inhibitors Against the Threat of Influenza Pandemics

Although the crystal structure of influenza virus neuraminidase (also known as sialidase) was determined in 1983 [49], its resolution was relatively low (approx. 3.0 Å) and therefore, of limited value in structure-based drug design. Initial efforts to design neuraminidase inhibitors were based on substrate-like compounds such as N-acetylneuraminic acid (Neu5Ac) derivatives (Fig. 20.9). One of them, the 2-deoxy-α-Neu5Ac demonstrated good activity in mouse model experiments. The improvement in resolution of the neuraminidase X-ray structures obtained in complex with Neu5Ac or with the derivative Neu5Ac2en facilitated the design of more potent inhibitors. These studies revealed that sialic acid adopts a quite deformed conformation when bound to neuraminidase, due to strong ionic interactions of the sialic acid carboxyl moiety with three arginine residues of the enzyme (Arg118, Arg192 and Arg371). Starting with Neu5Ac2en, the substitution of the C-4 hydroxyl group with a guanidinyl functionality rendered the 4-deoxy-4-guanidino-Neu5Ac2en (zanamivir) that showed significantly improved affinity promoted by interactions between the guanidinyl moiety and the neuraminidase residues Glu119 and Glu227 [50]. Zanamivir was found to be highly selective for influenza virus sialidase. It was approved in 1999 as the first neuraminidase-targeting anti-influenza drug (Fig. 20.9) even though it can be administered only as a powder for oral inhalation.

Oseltamivir was developed as a result of efforts to develop neuraminidase inhibitors based on non-carbohydrate templates [50]. In this context, a cyclohexene ring was considered as a transition-state mimic, and amenable to chemical modifications to optimize its biological activity. X-ray crystallographic studies of sialic acid and related analogues bound to neuraminidase (Fig. 20.9) demonstrated that the C7 hydroxyl group of the glycerol side chain does not interact with amino acid residues in the sialidase active site. Replacement of the glycerol moiety to improve the lipophilicity of those inhibitors was carried out even though the hydroxyl groups at C8 and C9 were involved in interactions with Glu276 and Arg224. A 3-pentyl ether side chain at position C7 gave optimal results and led to the development of oseltamivir. Oseltamivir (originally known as GS 4104) is an ethyl ester prodrug of GS 4071 that can be administered orally as capsules or as a
suspension, and is then converted to the active form in vivo by the action of endogenous esterases. Crystal structures of influenza virus neuraminidase bound to GS 4071 showed a rearrangement of the side-chain of Glu276 that now interacts with Arg228, generating a larger hydrophobic area in this domain. The amino acid substitution H274Y decreases neuraminidase binding affinity for oseltamivir and is the major mutation involved in resistance to this antiviral drug. Crystal structures of oseltamivir-resistant mutant neuraminidases have shown that the presence of Tyr instead of His274 affects the positioning of Glu276 and disrupts the hydrophobic pocket that accommodates the 3-pentyl ether side chain of oseltamivir [35, 51].

20.5.2 Development of Darunavir to Combat Resistance to HIV-1 Protease Inhibitors

The HIV-1 protease is a homodimer that contains two catalytic aspartic acid residues (one in each subunit) in the active site (Fig. 20.5). The Asp25 residues share an acidic proton and interact with a water molecule in the absence of substrate or inhibitor. The hydrolysis mechanism involves activation of a water molecule by these aspartates and nucleophilic attack of the water oxygen to the amide carbonyl
of the bond to be cleaved between P1 and P1'. Breakdown of the resulting tetrahedral intermediate by successive proton transfers leads to the amino and carboxylate products. The scissile bond of the peptide substrate is in close proximity of the active site. Upon binding, flexible flaps (one per monomer) that adopt an open conformation in the apoenzyme fold down over the substrates. The peptide substrates contain at least seven residues extending from P4 to P3', where the cleavage site lies between P1 and P1'. The side chains of the substrate lie in the protease subsites S4 to S3'. Replacement of the hydrolyzable peptide linkage by a non-cleavable bond was a basic principle for the development of peptidomimetic inhibitors that culminated with the approval of saquinavir [52].

Saquinavir and other related protease inhibitors have serious drawbacks that include severe side effects and toxicities, poor bioavailability (high doses required to maintain their therapeutic effect), costly synthesis and the rapid emergence of drug resistance. Extensive analysis of protein-ligand X-ray structures of wild-type and mutant HIV-1 proteases showed that there were minimal alterations in the active site backbone conformation of proteases resistant to inhibitors. Therefore, drugs effective on resistant HIV-1 strains should maintain extensive hydrogen-bonding interactions with the protein backbone of the wild-type enzyme.

The replacement of the asparagine moiety at the P2 position in saquinavir with 3R-tetrahydrofuranyl glycine resulted in a more potent inhibitor (Fig. 20.10). Other studies leading to the development of fosamprenavir/amprenavir showed an improvement in antiviral activity by introducing the 3R-tetrahydrofuranyl urethane moiety onto a hydroxyethylamine sulfonamide isostere. However, crystal structures revealed that 3R-tetrahydrofuranyl urethane-bearing inhibitors had weak hydrogen bonding potential with main chain atoms of residues Asp29 and Asp30. Therefore, a bicyclic tetrahydrofuran (bis-THF) ligand was designed and developed to improve hydrogen bonding with both aspartic acid residues. Inhibitors incorporating a P2 bis-THF and a p-methoxysulfonamide as the P2' ligand (e.g. TMC-126) were remarkably potent in enzyme inhibition assays and displayed strong antiviral activity in cell culture. Darunavir was similar to TMC-126 but contained p-aminosulfonamide instead of p-methoxysulfonamide at the P2' position. The P2'-amine group provided more favorable pharmacokinetic properties compared to the P2'-methoxy group. Darunavir-bound X-ray crystal structures showed that both P2 and P2' moieties are involved in extensive hydrogen bonding with the protein backbone. These tight interactions were consistently observed with mutant proteases and might be responsible for the unusually high resistance profile of darunavir. Detailed reviews describing the design and development of darunavir have been published [53].

### 20.6 Viral Capsids as Targets of Antiviral Intervention

Remarkable examples of structure-based design of preclinical drugs binding to the mature or immature viral capsid or interfering with their assembly or conformational transitions required for genome uncoating have been described for picornaviruses, HBV and, more extensively, for HIV-1.
20.6.1 Picornaviruses

The Picornaviridae family includes enteroviruses and rhinoviruses which are important human pathogens. Picornaviruses are non-enveloped, positive-stranded RNA viruses with an icosahedral capsid. The availability of crystal structures of poliovirus and rhinovirus (see Chaps. 4 and 10) led to the discovery of compounds that could bind to a hydrophobic pocket in the capsid protein VP1. Those molecules, known as WIN compounds (for Winthrop, the firm that developed the drugs), mimic natural factors that bind to the VP1 pocket and stabilize the capsid in a metastable conformation. During the course of a normal infection by poliovirus or rhinovirus, virion binding to the cell receptor allows for the displacement of the pocket-bound natural factors. This event triggers a global rearrangement of the capsid that is required for the release of the viral genome into the host cell cytoplasm [54]. WIN compounds and other related drugs bind to the pocket with
higher affinity than the natural factors and interfere with the receptor-induced conformational change by preventing either receptor binding or the receptor-induced rearrangement that is required for genome uncoating. In a long series of trend-setting studies, structure-function relationship analyses based on the determination of crystal structures of capsid-bound compounds, docking studies, genetic modifications of the virus particles and chemical modifications of the antiviral compounds led to the development of derivatives with improved antiviral activity. These derivatives included pleconaril (WIN-63843) for the treatment of rhinovirus-induced exacerbation of pre-existing asthma or chronic obstructive pulmonary disease [55], BTA-798 for the enterovirus-induced sepsis syndrome [56], and V-073 with potential applications in the treatment of poliovirus infections [57]. Pleconaril binds into the hydrophobic pocket and stabilizes the metastable capsid conformation in a way that results in inhibition of both viral adsorption to cell membranes and RNA uncoating [54]. Natural resistance to this drug is largely confined to VP1 residue 191, which is valine in the vast majority of susceptible viruses and leucine in all resistant serotypes. Full resistance is achieved by an additional Tyr152 to Phe mutation [58].

20.6.2 Hepatitis B Virus

In HBV, the capsid is composed of 120 copies of a dimeric structural protein (known as CA protein) that assemble around an RNA-reverse transcriptase complex that will transcribe the RNA pregenome to the mature DNA genome. Ten years ago the fluorescent probe 5,5'-bis[8-(phenylamino)-1-naphtalenesulfonate] was shown to disturb the geometry of intersubunit contacts and to act both as a noncompetitive inhibitor of capsid assembly in HBV and as an assembly ‘misdirector’ that facilitates the formation of noncapsid polymers [59]. Assembly misdirection has also been shown to occur upon exposure to activator heteroarylhydropyrimidines such as HAP1 [28, 60] and BAY 41–4109 [61].

20.6.3 Human Immunodeficiency Virus

In the case of HIV-1, we have discussed above the role of the viral protease in the release of the capsid protein (CA) from the precursor Gag polyprotein, and how protease inhibitors can prevent the formation of the mature capsid. Another compound that interferes with the maturation process is bevirimat (3-O-(3',3'-dimethylsuccinyl)betulinic acid, also known as PA-457). This compound is a derivative of betulinic acid and interferes with the processing of the Gag polyprotein at the CA-SP1 cleavage site (i.e. between the C-terminal of the capsid protein and a spacer peptide located between CA and the nucleocapsid protein). This inhibitory action is independent of the protease activity. Tomographic reconstructions suggest
that the CA-SP1 of Gag forms a helical bundle that serves to anchor the carboxy-terminal domain (CTD) of CA below the plane of the N-terminal domain (NTD). In the absence of CA-SP1 cleavage, mature cores cannot be formed because the immature particles cannot disassemble (for reviews, see [30, 62]). Virions produced from bevirimat-treated cells display aberrant core morphologies. A similar mechanism of action has been recently described for a pyridine-based compound (PF-46396) whose inhibitory activity results in the accumulation of CA/SP1 (p25) precursor proteins and the blockade of viral core particle maturation [63].

Assembly of the HIV-1 capsid depends on CA-CA interactions involving structural domains NTD and CTD (Fig. 20.11). The peptides CAI and CAC1 and derivatives, as well as small molecules such as α-hydroxyglycineamide (a C-amidated tripeptide), benzodiazepine-based compounds and CAP-1 have been identified as inhibitors of HIV-1 capsid assembly [29, 30, 65]. CAP-1 contains a urea-based scaffold and two substituted aromatic groups at the molecule ends, and acts in the late stage of the viral life cycle. When added to preformed HIV-1 particles, the drug has no inhibitory effect on HIV-1 infection. The CAI peptide was identified in a screen using a random peptide library. The CAC1 peptide was rationally designed based on structure and thermodynamic information to include most of the residues critically involved in the CTD-CTD dimerization interface that participates in mature HIV-1 capsid assembly [65]. CAP-1, CAI, CAC1 and their combinations and/or derivatives were able to partially inhibit HIV-1 infectivity in cultured cells.

One of the limitations of CAI and other peptide inhibitors of capsid assembly is their inability to penetrate cells. Chemical modifications consisting of covalent crosslinks between amino acids found in those inhibitors led to the development of conformationally restricted peptides that showed increased affinity for monomeric CTD and improved delivery to target cells. Examples of those “stapled” peptides are NYAD-1 (derivative of CAI) and NYAD-201 [65]. NYAD-1 inhibits the formation of both immature and mature virions. At low micromolar concentrations, this drug interferes with early events in the viral life cycle. Despite their higher solubility and low toxicity, further development of HIV-1 CA-derived natural or stapled peptides is limited by their relatively modest binding affinities [30, 66]. Also, problems related with the general metabolic instability of peptides may need to be addressed by using peptidomimetic compounds.

A high-throughput antiviral screening campaign led to the identification of a cluster of structurally related compounds that inhibited HIV-1 replication at submicromolar concentrations by targeting a post-entry stage prior to reverse transcription. Among them, PF-3450074 (or PF74) triggers premature HIV-1 uncoating in target cells [67]. Interestingly, point mutations in CA that stabilize the HIV-1 core conferred strong resistance without influencing inhibitor binding. Structural studies of the complex of the CA N-terminal domain and PF74 (PDB entry 2XDE) revealed a new binding site, different from those described for CAP-1 (PDB entry 2PXR) or CAI (PDB entry 2BUO) (Fig. 20.11), and several in silico screened inhibitors [68]. PF74 increased the rate of HIV-1 CA multimerization in vitro, while CAI and CAP-1 decreased this rate under the same assay conditions. In a model of an assembled CA hexamer in complex with PF74, an indole group
protruding from the N-terminal domain was found to localize to the interface between capsid monomers suggesting both early and late stage effects on viral propagation. The N-terminal domain of CA is also the target of novel benzodiazepines that prevent virus release or benzimidazoles that inhibit the
formation of the mature capsid [69]. These compounds bind to a different site as compared with PF74 and have no effects at the early steps of viral replication. Benzimidazol inhibitors are expected to interfere with the formation of CA\textsubscript{NTD}/CA\textsubscript{CTD} interfaces in the CA hexamer [69].

20.7 Perspectives and Conclusions

Despite the recent success in developing new antiviral drugs, particularly against HIV and HCV, there are still important challenges ahead. Drug resistance constitutes a frequent obstacle that limits the efficacy of antiviral treatments. As learnt from the HIV experience, monotherapies should be considered suboptimal therapies and combination therapies are expected to prevail in the future treatment of viral infections. Good combination therapies rely on the exploitation of multiple targets and/or mechanisms of action. A better knowledge of the biological processes involved in specific viral infections, including interaction with specific host factors, is required to develop efficient screening methods and to identify viable targets. A wealth of information derived from massive sequencing techniques applied to humans and pathogens will be probably helpful in this process. Drug efficacy, toxicity and cost are also issues that need to be considered in the development of future antiviral drugs.

From a technical point of view, high-throughput X-ray crystallography and structural analysis, computational modeling and better synthetic methods will provide tools to accelerate drug discovery, once the target mechanism has been validated. The rapidly increasing availability of high-resolution structures of viral particles, and substantial advances in our understanding of their morphogenesis and conformational dynamics are opening new avenues for the design of novel antiviral agents. Although the odds of finding effective inhibitors of novel targets have probably increased, the outcome still remains unpredictable.

Although HIV and HCV will probably continue to be major actors in the drug development field, other viral pathogens such as influenza virus and flaviviruses (e.g. dengue virus) will probably gain attention in the coming years due to their general impact on human health.

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Further Reading


A collection of reviews written by Dr. Eric De Clercq and published in Medicinal Research Reviews between 2008 and 2011 provide a nice summary on the design and development of many antiviral drugs, from a historical perspective and providing relevant chemical structures. References for these articles are


Also especially recommended for further reading are references [1, 3, 18, 35] listed above.