HIV-1 INTEGRASE
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HIV-1 INTEGRASE

Mechanism and Inhibitor Design

Edited by

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Tremendous progress has been achieved since HIV-1 integrase (IN) was first recognized as an important antiretroviral drug target. Starting with our understanding of IN catalysis as essential for the viral life cycle to the interactions the viral enzyme makes with cellular proteins has greatly expanded the number of targets to develop specific IN inhibitors for clinical use. The FDA approval of raltegravir, the first therapeutic IN inhibitor, and the clinical success of elvitegravir have validated IN as an attractive chemotherapeutic target to develop safe and efficacious drugs for the treatment of HIV/AIDS.

This is the first and most comprehensive book dedicated entirely on IN function and inhibitor design. Starting with the initial discovery and isolation of IN as a 32 kD viral protein on a coomassie gel by Grandgenett in 1978, to the first marketed drug in late 2007, the book covers all the major highlights of IN research during the past 30 years. The book is divided into two major areas. The first twelve chapters deal with the basic biology and structural understanding of the enzyme to appreciate the viral mechanism of integration, which was instrumental in developing proper assays for drug screening. The second section, covering Chapters 13–30, details the various technologies used to discover IN inhibitors and discusses all the major chemical classes of inhibitors identified from 2002–2010.

Contributing authors are internationally recognized leaders and pioneers who are intimately involved in retroviral biology and drug discovery and have significantly contributed to our current understanding of IN mechanism, function, and inhibition. In Chapter 1, De Clercq summarizes the life cycle of HIV and discusses other viral targets extensively exploited for the design of antiretroviral drugs. In Chapter 2, Grandgenett delivers a historic perspective of how IN was purified in 1978. Craigie presents the mechanism and function of IN in Chapter 3. Chapters 4 and 10, contributed by the Wlodawer and Cherepanov laboratories, are dedicated to structural aspects of IN. Ciuffi and Bushman discuss retroviral integration site selection in Chapter 5 and Engelman provides a perspective on the outcomes of IN mutagenesis on the HIV-1 life cycle in Chapter 6. The interactions IN makes with DNA and reverse transcriptase are summarized by the Pommier and Chow laboratories, in Chapters 7 and 8, respectively. Debyser and colleagues discuss cellular cofactors for integration in Chapter 9, and Skalka and colleagues deliver a comprehensive and timely overview of the host factors that affect stability and silencing of provirus in Chapter 11. In Chapter 12, Debyser and colleagues thoroughly review the various assays used to test the activity of potential IN inhibitors. Chapter 13 provides a historical perspective of inhibitor design detailing the major discoveries from 2002–2010. Chapter 14 summarizes Merck’s efforts that led to the discovery and approval of raltegravir, while Chapter 15 discusses the design and discovery of elvitegravir by JT Inc, an inhibitor currently undergoing Phase III clinical trials. The Gilead team provides a comprehensive overview of conformationally constrained tricyclic inhibitors in Chapter 16. Models of the slow-onset kinetics of IN inhibitors are discussed by the GlaxoSmithKline group in Chapter 17. The Pfizer team summarizes the azaindole hydroxamic acids in Chapter 18. A simple and accurate assay for predicting protein binding of IN inhibitors is presented by the BMS group in Chapter 19. Chapter 20 deals with the role of metal cofactors in inhibitor design, whereas chapter 21 covers a comprehensive list of natural product IN inhibitors. Mouscadet and colleagues discuss styrylquinoline IN inhibitors in Chapter 22, whereas the chicoric acids and other chemical derivatives are discussed by Crosby and Robinson in
Chapter 23. Chapters 24 and 25 deal with peptide-based and nucleotide-based inhibitors, respectively. Computational methods and affinity-labeled technologies used to design IN inhibitors are discussed in Chapters 26–28. The design of allosteric IN inhibitors, an emerging area of research for the next decade of studies, is covered in Chapter 29. The book is concluded by an in-depth discussion of viral resistance to IN inhibitors in Chapter 30.

This book aims to serve as a reference textbook for scientists who face challenging issues in drug design, and for researchers who are interested in antiviral drug discovery in particular. Fundamental progress in state-of-the-art antiviral drug design relies heavily on scientific involvement within multiple disciplines, including computational biology, synthetic chemistry, pharmacology, and virology. However, most scientists are not familiar with aspects of antiviral drug design outside their particular field. This book bridges the knowledge gap that exists in the currently available published reviews on drug design dealing with antiviral therapies. By compiling the latest information, this book will be a very useful reference to any scientists interested in drug design, and it is also a relevant guide for other professions intimately involved in the successful commercial development of antiviral drugs, including governmental, regulatory, and intellectual property agencies. Although there have been numerous publications covering different aspects of IN function and inhibition, there has been no existing resource providing the “big picture,” and therefore a major need was present to bring into focus a coherent and a comprehensive compilation of all the efforts. This book accomplishes that objective.

By bringing together and presenting all the major multi-disciplinary scientific advances that collectively makeup IN mechanism and inhibitor design, it is hoped that potential new investigators will be inspired to perform the much needed additional research for this field to continue to grow and build on what has already been accomplished. At the time of this writing, additional IN inhibitors are already undergoing advanced clinical trials. It is expected that such inhibitors will be safer than previously approved drugs for HIV/AIDS. These great achievements serve as an indication of all the hard work already realized, and the determination of many scientists in the future will likely bring more great advances for IN targeted HIV/AIDS therapeutics.

Finally, I would like to offer my sincere gratitude to all the eminent scientists who dedicated their valuable time to prepare a book of this depth. Without their outstanding contribution and dedication, such a collection would not have been possible. There are other prominent scientists that have significantly contributed to this field, and despite not authoring a dedicated chapter, their discoveries are nonetheless reflected in this book. Last but not least, I am indebted to my mentors, previous and current graduate students, postdoctoral fellows, visiting scientists, and collaborators, who have all provided significant support for writing this book.

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1

HIV LIFE CYCLE: TARGETS FOR ANTI-HIV AGENTS

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1.1 Introduction: Overview of HIV Life Cycle
1.2 Virus Adsorption: Interaction of HIV with CD4 Receptor
1.3 Prelude to Fusion: Interaction of HIV with CXCR4 or CCR5 Coreceptor
1.4 Virus–Cell Fusion: Six-Helical Bundle Formation and Insertion of Fusion Peptide into Cell Membrane
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1.1 INTRODUCTION: OVERVIEW OF HIV LIFE CYCLE

The life cycle of human immunodeficiency virus (HIV) encompasses several crucial steps which can be considered as targets for chemotherapeutic intervention (Fig. 1.1). The life cycle starts with adsorption of virions (virus particles) to the host cell, where the viral envelope glycoprotein gp120 interacts, first, nonspecifically, with heparan sulfate; second, specifically, with the CD4 (cluster of differentiation 4) receptor; and, third, specifically, with the coreceptor CXCR4 or CCR5. Like other enveloped viruses, HIV enters the cell by fusion between the viral envelope and the cellular plasma membrane: for HIV; this process is mediated by the viral glycoprotein gp41 and allows the penetration of the nucleocapsid into the cell. After the viral RNA (ribonucleic acid) genome has been freed from its capsid through an ill-characterized process termed decapsidation (or uncoating), the viral (+)RNA is converted into proviral double-stranded (ds, ±)DNA (deoxyribonucleic acid) through the virion-associated reverse transcriptase. This progenomic viral DNA is then integrated into the host cell genome through HIV integrase (like reverse transcriptase, a virion-associated enzyme). Being an integral part of the host genome the proviral DNA is subject to the normal transcription and translation machinery of the cell for expression of its genes, although for HIV this is additionally regulated by specifically induced viral regulatory proteins such as Tat (transcription trans activator) and Rev (regulator of expression of viral proteins). Viral RNA and proteins will then assemble at the cell membrane to produce progeny virus particles that are released through the process termed budding. Following translation of the viral precursor proteins pp55 gag and pp160 gag–pol, the viral protease (which is autocatalytically cleaved) will cleave these precursor proteins into the structural (gag) proteins [MA (matrix antigen) p17, CA (capsid antigen) p24, NC (nucleocapsid) p7] and functional (pol) proteins [PR (protease) p11/p11, RT (reverse transcriptase) p66/p51, IN (integrase) p32 tetramer]. This proteolytic processing is inherent to the production of infectious progeny virions, and, while coinciding with the assembly of the new virus particles, it may continue after the budding has taken place.

1.2 VIRUS ADSORPTION: INTERACTION OF HIV WITH CD4 RECEPTOR

Before HIV finds its specific receptor CD4, it first interacts nonspecifically with heparan sulfate, which is widely
expressed on animal cells and involved in virus–cell binding of a broad array of enveloped viruses, including herpes simplex virus and Dengue virus. Numerous polyanionic substances (i.e., polysulfates, polysulfonates, polycarboxylates, polynucleotides, poloxomelalates, and negatively charged albumins) have been shown to inhibit HIV replication by preventing virus adsorption to the surface of the host cells, but none of these substances have yet found their way to a clinical application, whether systemic or topical.

The specific interaction of HIV with CD4, a marker for HIV-sensitive host cells, is considered a much more specific target for potential therapeutic intervention. CD4 is an integral membrane glycoprotein, belonging to the immunoglobulin gene superfamily, that is expressed mainly on the surface of T lymphocytes and cells of the macrophage/monocyte lineage. It consists of an extracellular region of 370-amino-acid residues organized in four domains (D1–D4), a hydrophobic membrane-spanning region of 25 amino acids, and a highly charged cytoplasmic tail of 38 amino acids. The D1 loop has been identified as the primary binding site for the HIV envelope protein gp120. Of particular importance for the binding of gp120 are the positively charged amino acid residues at positions 46 and 59 surrounding the phenylalanine residue at position 43 (denoted as the Phe 43 cavity) (Fig. 1.2).

Various analogues of cyclotriazadisulfonamide (CADA, or 9-benzyl-3-methylene-1,5-di-p-toluenesulfonyl-1,5,9-triazacyclododecane) (Fig. 1.3) have been found to specifically downmodulate the expression of CD4 without altering the expression of any other cell receptor examined, including HIV coreceptors, and a close correlation was found between the anti-HIV potency of the CADA analogues and their ability to downmodulate the CD4 receptor. The potential of CADA and its analogues in the treatment of HIV infections and, possibly, other diseases (whether infectious or immunological) that are mediated by CD4 needs to be further explored.

1.3 PRELUDE TO FUSION: INTERACTION OF HIV WITH CXCR4 OR CCR5 CORECEPTOR

Following its interaction with the CD4 receptor, the viral envelope gp120 must interact with its coreceptor, CXCR4 (C–X–C chemokine receptor 4) for T-tropic or X4 HIV strains or CCR5 (C–C chemokine receptor 5) for M-tropic or R5 HIV strains. CXCR4 and CCR5 normally act as the receptors for the C–X–C chemokine SDF1 (stromal cell-derived factor 1) and the C–C chemokines RANTES (regulated upon activation, normal T cell expressed and secreted) and MIP-1 (macrophage inflammatory protein 1), respectively. The coincidental use of CXCR4 and CCR5 by HIV as coreceptors to enter cells has prompted the search for CXCR4 and CCR5 antagonists, which, through blockade of...
the corresponding coreceptor, might be able to block HIV entry into the cells (Fig. 1.4).

The best characterized of the CXCR4 antagonists is AMD3100 (previously called JM3100)\(^{10}\) (Fig. 1.5). It was originally discovered as an anti-HIV agent with strong inhibitory effect on the replication of X4 HIV strains\(^{11}\) and was later found to inhibit X4 HIV replication by a selective antagonization of CXCR4\(^{12}\) then found to specifically mobilize hematopoietic stem CD34\(^+\) cells from the bone marrow into the bloodstream (by interruption of the interaction of CXCR4 with its normal ligand SDF1, which is responsible for the “homing” of the stem cells in the bone marrow).

The most advanced among the CCR5 antagonists is maraviroc\(^{13}\) (Fig. 1.5), which has been approved for clinical use in the treatment of HIV infection. Limitation in the clinical use of maraviroc is that it is only effective against CCR5-using R5 HIV-1 strains. From dual (CCR5 and CXCR4)-tropic or mixed HIV populations that use both CCR5 and CXCR4 (which are common among highly treatment experienced patients), maraviroc may select for the outgrowth of pure CXCR4-tropic X4 strains.\(^{14}\) In addition, R5 HIV-1 strains may develop resistance to maraviroc while still utilizing the inhibitor-bound receptor for entry.\(^{15}\) Obviously, to cope with dual-tropic or mixed X4/R5 HIV-1 populations, a combination of CXCR4 inhibitors with CCR5 inhibitors will be ultimately needed.

### 1.4 VIRUS–CELL FUSION: SIX-HEXALIC BUNDLE FORMATION AND INSERTION OF FUSION PEPTIDE INTO CELL MEMBRANE

While the viral glycoprotein gp120 is responsible for virus interaction with the CD4 receptor and CCR5 or CXCR4 coreceptor, the viral gp41 glycoprotein (which has remained noncovalently attached to gp120 after their precursor glycoprotein gp160 has been cleaved by cellular proteases to yield the gp120/gp41 heterodimer) is responsible for the fusion of the viral envelope with the cell membrane. The gp41 glycoprotein contains four major functional domains, starting from the N-terminus toward the C-terminus: (i) the fusion peptide, (ii) heptad repeat 1 (HR1), (iii) heptad repeat 2 (HR2), and (iv) the transmembrane domain that anchors gp41 into the viral lipid bilayer. When the N-terminal fusion peptide of gp41 is inserted into the host
cell membrane, the three HR2 domains of the gp41 trimer loop back in a triple hairpin and “zip” themselves into three highly conserved hydrophobic grooves on the outer face of the HR1 trimeric bundle. This conformational change results in the formation of a six-helical bundle which pulls the outer membranes of both virus and cell into close physical proximity, thus enabling the two membranes to fuse\textsuperscript{16–18} (Fig. 1.6).

Enfuvirtide (originally designated DP-178, pentafuside, or T-20) (Fig. 1.7) is homologous to part of the HR2 region, and, while T-20 will itself engage in a coiled-coil interaction with HR1, it prevents the six-helical bundle formation required to initiate the fusion process. Enfuvirtide (Fuzeon\textsuperscript{®}), the only HIV drug injected subcutaneously, is generally used in combination with HAART (highly active antiretroviral therapy) regimens. Even if added onto highly active four-drug regimens, enfuvirtide has still proved able to afford only an incremental benefit.\textsuperscript{19} However, enfuvirtide has a low genetic barrier to resistance with mutations primarily arising in the HR1 domain. This low genetic barrier to resistance\textsuperscript{20} underscores the importance of combining enfuvirtide with other anti-HIV agents.

**Figure 1.4** Coreceptor (CXCR4 or CCR5) antagonists. During the viral adsorption process, (a) the viral envelope glycoprotein gp120 interacts with the CD4 receptor at the cell membrane; (b) subsequently, gp120 interacts with the coreceptor CXCR4 for T-tropic (X4) HIV strains or CCR5 for M-tropic (R5) HIV strains, whereupon (c) the viral glycoprotein gp41 anchors into the cell membrane. CXCR4 and CCR5 antagonists bind to CXCR4 or CCR5 and thus block their interaction with HIV gp120. (Data from De Clercq.\textsuperscript{19})

**Figure 1.5** Structures of AMD3100 (Mozobil) and UK-427857 (Maraviroc).
1.5 FROM VIRAL RNA TO PROVIRAL DNA: REVERSE TRANSCRIPTION

To convert the genomic viral RNA to pregenomic proviral DNA, three successive enzymatic reactions, all ensured by the p66 subunit of the p66/p51 RT (reverse transcriptase) heterodimer, are required: (i) transcription of the (+)RNA strand to a (−)DNA strain, which, being complementary to the (+)RNA, remains hybridized with its template; (ii) degradation of the (+)RNA strand of the (−)DNA−(+)(+)(+)RNA hybrid by the p15 [RNaseH (H for hybrid)] subdomain of p66; and (iii) formation of the (+)DNA strand from the

\[ \text{YTSLIHSLIEESQNEKNEQELLEDKWASLWNWF} \]

Figure 1.7  Structure of enfuvirtide (DP-178, pentafuside, T20).
DNA template, thus producing a (±)DNA duplex. Both functions (i) and (ii) are catalyzed by reverse transcriptase, the catalytic site being located in the palm domain (Fig. 1.8), which contains a substrate [deoxyribonucleotide triphosphate (dNTP)] binding site (indicated by the dot in Fig. 1.8) and an allosteric site (indicated by the asterisk in Fig. 1.8) at about 15 (1.5 nm) distance from the catalytic site. Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs, NtRTIs) are targeted to the catalytic (dNTP binding) site, whereas the nonnucleoside reverse transcriptase inhibitors (NNRTIs) are targeted to the allosteric (NNRTI binding) site.

To interact with their target sites, the NRTIs [i.e., zidovudine (Retrovir®), didanosine (Videx®), zalcitabine (Hivid®), stavudine (Zerit®), lamivudine (Epivir®), abacavir (Ziagen®), and emtricitabine (Emtriva®)] and NtRTIs [i.e., tenofovir (marketed as tenofovir disoproxil fumarate, Viread®)] must first be phosphorylated to their triphosphate derivatives (NRTI triphosphates) or diphosphate derivatives (NtRTI diphosphates), which can be readily accomplished by two or three consecutive intracellular phosphorylations. NNRTIs [i.e., nevirapine (Viramune®), delavirdine (Recriptor®), efavirenz (Sustiva®, Stocrin®)] do not need any intracellular metabolism and are able to interact as such with their target site. RT has proven to be the favored target enzyme for drug development against HIV infection (AIDS). In addition to those that have been mentioned (Fig. 1.9) (and which have all been approved for the treatment of AIDS), several other NRTIs (i.e., apicritabine, amoxovir, Racivir®, Reverset®) and NNRTIs (i.e., etravirine, rilpivirine, dapivirine) are in clinical development. These and yet other NRTIs, NtRTIs, or NNRTIs may join the anti-HIV drug armamentarium in the (near) future.22,23

1.6 INTEGRATION OF PROVIRAL DNA INTO HOST CELL GENOME BY HIV INTEGRASE

Approximately 40–100 integrase molecules are packaged within each HIV particle.24 The primary role of integrase is to catalyze the insertion of the proviral DNA into the genome of infected cells. Integration is required for viral replication because efficient transcription of the viral genome and production of viral proteins require that the proviral DNA is fully integrated into the cellular genome. Following reverse transcription, the proviral DNA is primed for integration by integrase-mediated 3' processing, which corresponds to an endonucleolytic cleavage of the 3' ends of the proviral DNA, thereby generating CA-3'-hydroxyl ends (Fig. 1.10). Following 3' processing, integrase remains bound to the proviral DNA as a multimeric complex that bridges both ends of the viral DNA within intracellular particles termed PICs (preintegration complexes). PICs are able to cross the nuclear membrane. Once in the nucleus, the integrase catalyzes the insertion of the proviral DNA into the host chromosome by a strand transfer reaction, consisting of the ligation of the viral 3'-OH DNA ends (generated by 3' processing) to the 5'-phosphates of host chromosomal DNA. Completion of integration can only take place after trimming of the last two nucleotides at the proviral DNA 5' ends and extension (gap filling from the 3'-OH ends of the genomic DNA).24

Among the furthest advanced integrase inhibitors in clinical development are MK-0518 (raltegravir) and GS-9137 (elvitegravir) (Fig. 1.11). Raltegravir has been approved by the U.S. Food and Drug Administration (FDA) in 2007 and elvitegravir’s approval is pending. Both compounds inhibit the strand transfer reaction in the integration process (Fig. 1.10) and were validated as genuine HIV
integrase inhibitors in cell culture assays. Clinical studies have indicated that both raltegravir and elvitegravir upon 10-day monotherapy can achieve 2 log₁₀ reductions in viral load. When added onto an optimized background regimen, raltegravir, at all three doses tested (200, 400, or 600 mg orally twice daily), offered better viral suppression than placebo over a 24-week treatment period. Clearly, the integrase inhibitors (INIs) may be welcomed [following the NtRTIs, NNRTIs, Protease inhibitors (PIs), and virus entry inhibitors] as the next new class of anti-HIV drugs. As for all other classes of HIV inhibitors, INIs should be used in combination drug regimens and carefully monitored for the emergence of drug-resistant virus strains.

1.7 HIV TRANSCRIPTION AND ITS REGULATION (ACTIVATION)

The regulation of transcription of HIV is an extremely complex process requiring the cooperative action of both viral and cellular components. In latently infected resting CD⁴⁺ T cells, HIV transcription seems to be repressed by deacetylation events mediated by histone deacetylases (HDACs). Upon reactivation of HIV from latency, HDACs are displaced in response to the recruitment of histone acetyltransferases (HATs) by nuclear factor κB (NF-κB) or the viral transcriptional activator Tat, and this results in multiple acetylation events. Following chromatin
remodeling of the viral promoter region, transcription is initiated. The complex of Tat with p-TEFb then binds to the TAR (Tat response) element, thereby positioning CDK9 to phosphorylate the cellular RNA polymerase and thus ensuring transcription elongation. Other phosphorylation and acetylation events accompany and may at least partially account for the (activation of the) HIV transcription process (Fig. 1.12).

Numerous inhibitors of the HIV transcription process have been described. They may be targeted at the stages of NF-κB activation (e.g., α-tocopherol, coumarins, acridone derivatives, iron chelators), NF-κB binding (pyridine N-oxide derivatives), the NF-κB signaling pathway (cephalanthine, carboxyamidotriazole), p-TEFb (flavopiridol, roscovitine), p300/CBP (curcumin), or, most interestingly, the Tat–TAR interaction (CGP64222, Tat peptide mimetics, quinolone derivatives, arginine–aminoglycoside conjugates RNA aptamers, and TAR RNA decoys). Although these HIV transcription inhibitors may be expected to prevent HIV gene expression in both acute and chronic, as well as

Figure 1.10 The two integrase catalytic reactions (3′ processing and strand transfer). Att = viral DNA recombination sites (a). The 3′ processing takes place in the cytoplasm following reverse transcription. The 3′ processing generates reactive 3′-hydroxyls at both ends of the viral DNA [red circles (b); other 3′-hydroxyl ends and 5′-phosphate ends are shown as red and green dots, respectively]. Integrase multimers (not shown) remain bound to the ends of the viral DNA as the preintegration complexes (PICs) translocate to the nucleus. The second reaction [(c) to (d)] catalyzed by integrase is strand transfer (3′-end joining), which inserts both viral DNA ends into a host–cell chromosome (acceptor DNA in blue). Strand transfer is coordinated in such a way that each of the two 3′-hydroxyl viral DNA ends (red circles) attacks a DNA phosphodiester bond on each strand of the host DNA acceptor with a 5-bp stagger across the DNA major groove (d). Strand transfer leaves a five-base, single-stranded gap at each junction between the integrated viral DNA and the host acceptor DNA, and a two-base flap at the 5′ ends of the viral DNA [(d) and (e)]. Gap filling and release of the unpaired 5′ ends of the viral DNA [arrows in (e)] are carried out in coordination with cellular repair enzymes. (Data from Pommier et al.24) (See insert for color representation of this figure.)
latent, infected cells, none of these therapeutic options has been pursued clinically.

1.8 PROTEOLYTIC CLEAVAGE OF PRECURSOR INTO MATURE VIRAL PROTEINS

To be converted to the mature Gag (p17, p24, p7) and Pol (p11/p11, p66/p51, and p32) proteins, the precursor Gag and Gag–Pol proteins have to be cleaved at specific peptide linkages by the HIV protease after this enzyme itself has been cleaved autocatalytically from the Gag–Pol precursor protein. If this proteolytic cleavage is blocked, that is, by PIs, no infectious particles will be produced, and virus spread will be halted. At present, 10 PIs have been approved: saquinavir (Invirase®, Fortovase®), ritonavir (Norvir®), indinavir (Crixivan®), nelfinavir (Viracept®), amprenavir (Agenerase®, Prozei®), lopinavir (combined with ritonavir at 4/1 ratio, Kaletra®), atazanavir (Reyataz®), fosamprenavir (Lexiva®, Telzir®), tipranavir (Aptivus®), and darunavir (Prezista®). The development of an eleventh PI, brecanavir, was recently discontinued because of “formulation” problems.

Except for tipranavir (which is based on the coumarin lactone scaffold), all available protease inhibitors can be considered as peptidomimetic (Fig. 1.13). They are built upon an hydroxyethylene motif, which mimics the peptide linkage. Whereas the peptide linkage can be readily hydrolyzed by the HIV protease, the hydroxyethylene bond cannot. The surrounding parts of the protease inhibitor are very much similar to the amino acid residues around the peptide linkage that is cleaved in the normal substrate, so the HIV protease is “fooled” and, if it were, imprisoned by its “funny” substrate, the protease inhibitor. Recently, it has been demonstrated that some protease inhibitors, that is, darunavir and tipranavir, may also block dimerization at its nascent stage of protease.30

The structures of tipranavir and darunavir, the last PI to be licensed for clinical use, are depicted in Fig. 1.14. Darunavir has, akin to the other PIs, proven efficacious in the treatment of HIV infections when used as an integral part of drug combination therapy.31 As was first demonstrated with lopinavir, and now is customary for all PIs, ritonavir is added onto a therapeutic PI-containing regime just to “boost” its activity. Ritonavir-boosted tipranavir has proved more efficacious than other ritonavir-boosted protease inhibitors following a 24-week treatment period.32,33 In lopinavir-naïve, treatment-experienced patients, darunavir–ritonavir was noninferior to lopinavir–ritonavir treatment from a virological viewpoint and may therefore be considered as a treatment option for this population.34

1.9 VIRAL CAPSID FORMATION: ULTIMATE STEP TO BLOCK VIRUS PRODUCTION?

A very late and perhaps the ultimate step in the HIV life cycle that could serve as target for therapeutic intervention involves the conversion of the capsid precursor p25 (CA-SP1) to mature capsid protein p24 (CA), which depends on CA-SP1 cleavage (Fig. 1.15).35 This CA-SP1 cleavage can be blocked by bevirimat [3-O-(3’3’,3’0-dimethylsuccinyl)betulenic acid (PA-457)] (Fig. 1.16).36 Virions generated in the presence of bevirimat exhibit aberrant capsid morphology and are no longer infectious. Resistance mutations to bevirimat have been localized near the CA-SP1 cleavage site (Fig. 1.15).35 As bevirimat is the first-in-class HIV maturation inhibitor and, therefore, represents a new assault on HIV, in vivo efficacy data are eagerly awaited (http://www.panacos.com/product_2.htm). Bevirimat is well absorbed after oral administration and its half-life is unexpectedly long (60–80 h),37 which may facilitate infrequent, that is, twice-weekly, dosing.

1.10 CONCLUSION: COMBINATION THERAPY

With the advent of the integrase inhibitors (INIs), following the NRTIs, NtRTIs, NNRTIs, PIs, and FIs (and other viral
Figure 1.12 Model for regulation of Tat-mediated transcription. Initially, nonacetylated Tat interacts with P/CAF which was found to be associated with the HIV-1 promoter only in response to Tat. P/CAF acetylates Tat at position Lys28. This subsequently abrogates the interaction between P/CAF and Tat but significantly enhances its interaction with p-TEFb, which is released from its association with MAQ1/HEXIM1 and 7SK snRNA upon different stimuli, including stress, ultraviolet light, DRB, and hypertrophic signals. The complex of Tat with autophosphorylated p-TEFb then binds the upper bulge and loop structures of TAR, thereby positioning CDK9 to phosphorylate also the negative elongation factors NELF and DSIF, which repress transcription by binding to the lower stem in TAR, as well as the CTD of RNAPII on both Ser2 and Ser5. These phosphorylation events result in the release of the transcriptional elongation block. In addition to the productive elongation mediated by the hyperphosphorylated RNAPII, Tat becomes acetylated by p300/CBP on Lys50, resulting in the dissociation of TEFB-Tat from TAR RNA and subsequent binding to the elongating RNAPII. Furthermore the acetylation of Tat on Lys50 serves as a signal to recruit P/CAF generating a p-TEFB-Tat-P/CAF ternary complex associated with RNAPII during transcriptional elongation. (Data from Stevens et al.29)

Figure 1.13 Currently available PIs (protease inhibitors) except for tipranavir act as peptidomimetic inhibitors in that they are built upon an hydroethylene scaffold which mimics the peptide linkage in the normal substrate but cannot be hydrolyzed by the protease.
entry inhibitors), the sixth class of anti-HIV agents has become available for clinical use in the treatment of HIV infections (AIDS). Starting from these different classes of anti-HIV drugs, numerous drug combinations could be conceived, containing multiple (two, three, four, five, or even six) drugs (Fig. 1.17). The increasing availability of new anti-HIV drugs has, on the one hand, increased the number of options, while, on the other hand, made the right choice more difficult.

Over the past decade HAART has gradually evolved from drug regimens with more than 20 pills daily (i.e., stavudine plus lamivudine plus indinavir) in 1996 to 3 pills daily [i.e., zidovudine/lamivudine (Combivir®) twice daily and efavirenz once daily] in 2003 to 2 pills daily [i.e., emtricitabine/tenofovir disoproxil fumarate (Truvada®) and efavirenz] in 2004 and finally to one pill daily in 2006 (Atripla®, containing tenofovir disoproxil fumarate plus emtricitabine and efavirenz).38

The triple-drug combination tenofovir disoproxil fumarate (TDF), emtricitabine ([(-)FTC]), and efavirenz has proved more efficacious (in terms of virological and immunological response) and is less prone to toxic side effects than the other arm of the study (GS934), consisting of combivir (zidovudine/lamivudine) and efavirenz over a period of 48, 96, and recently extended to 144 weeks.23,39,40

The drug combinations which have been most extensively pursued consist of one or two NRTIs (or one NtRTI instead of one of the NRTIs) and one NNRTI (or instead of the NNRTI, one PI boosted with ritonavir). Combinations of NNRTIs with PIs have been rather exceptional. The first study to assess the use of the NNRTI etravirine with the PI darunavir (boosted by ritonavir) in HIV-1-infected subjects with no treatment options showed impressive virological responses (HIV RNA reduction of 2.7 log10 copies per milliliter) over a 24-week treatment period.41 This underscores the high and as yet hardly explored potential of combinations of NNRTIs with PIs in the treatment of HIV infections.
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