# Novel Targets and Advancements in Drug Discovery: The Case of HIV-AIDS

18

Nihar Ranjan, Umesh Kumar, and Sunil K. Deshmukh

**Abstract**

Since the very first report of acquired immune deficiency syndrome (AIDS) in the early 1980s in the United States, a number of advancements have taken place both in the structural and functional aspects of the human immunodeficiency virus (HIV) life cycle as well as anti-HIV drug design. While new drugs have come to the market and combination therapies have increased life expectancy, resistance and viral mutations have mandated introduction of new drugs in the market. Apart from two main classes of HIV inhibitors (reverse transcriptase and protease), new inhibitors targeting fusion and integration processes have provided additional sites for therapy development. More recently inhibitors of maturation and capsid assembly as well as viral replication have been studied to provide novel anti-HIV drugs. In this chapter, we briefly discuss the HIV life cycle and describe a few of the recent endeavors made to develop new anti-HIV agents. For brevity, we provide a limited number of examples of discoveries made in the main target sites of current HIV drug design.

**Keywords**

Novel targets • HIV • Reverse transcriptase • Antiviral drug

N. Ranjan • U. Kumar • S.K. Deshmukh (✉)
TERI-Deakin Nanobiotechnology Center, The Energy and Resources Institute, TERI Gram, Gwal Pahari, Gurugram, Haryana, India, 122003
e-mail: nihar.ranjan@teri.res.in; sunil.deshmukh@teri.res.in


S. Abdulhameed et al. (eds.), *Bio-resources and Bioprocess in Biotechnology*,
DOI 10.1007/978-981-10-4284-3_18

## 18.1 Introduction

### 18.1.1 Current State of HIV-AIDS

Despite the decline in the number of new HIV-infected population globally in recent years, HIV-AIDS remains one of the leading causes of disease-related death. According to the latest UNAIDS data (UNAIDS 2016), 36.7 million people are currently living with HIV-AIDS worldwide. In 2015, 1.1 million people died from AIDS related illness and 2.1 million new HIV infections have been reported. Several awareness efforts and regulatory practices have led to many positive outcomes that augur well for significant control over HIV spread in the coming decade. However, success of all concerted AIDS control efforts is centrally linked to therapeutic control of the HIV virus using multifaceted drug combinations. These drug combinations have consequentially helped to increase the life span of HIV-infected population, arresting the viral proliferation for several years and curbing the viral growth to nondetectable levels. Introduction of new and more potent drugs by the Food and Drug Administration (FDA) in recent years that bind to new HIV targets has greatly boosted the available therapeutic arsenal, albeit these inclusions have been simultaneously countered with viral mutations leading to ineffectiveness of few clinically used drugs. Therefore, drug design efforts that target new sites to stop viral function and growth are needed to augment currently used multidrug combination therapy.

### 18.1.2 Target Identification and Drug Discovery Process

Drug discovery is an arduous process that takes several years for a new drug to come to the market (Fig. 18.1). There are more than a thousand (1222) new chemical entities (NCEs) that have been approved by the FDA for use as pharmaceutical therapies since 1950. Among them, only 8% NCEs developed at the benches are eventually approved and enter the market after surviving a process of drug development that takes an average of 13.5 years (Paul et al. 2010).

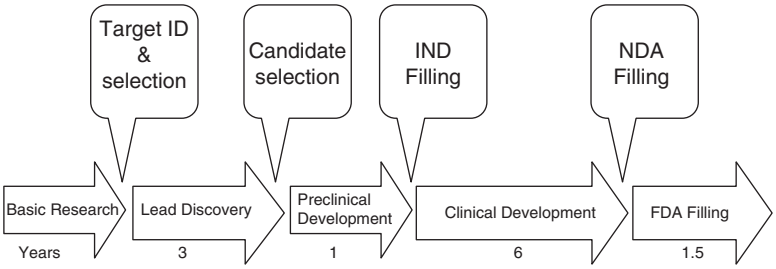


**Fig. 18.1** Drug discovery process from target identification and validation through to filing of a compound and the approximate timescale for these processes. *FDA* Food and Drug Administration, *IND* investigational new drug, *NDA* new drug application (~~Reproduced from Hughes et al. 2010~~)

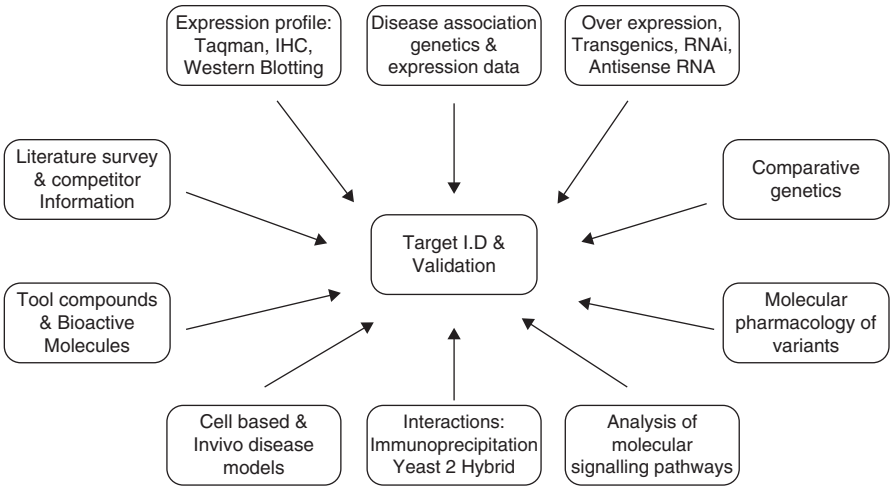


**Fig. 18.2** Multifunctional process of target identification and validation. IHC: immunohistochemistry (~~Reproduced from Hughes et al. 2010~~)

Despite the advancement in the techniques involved in genetics, proteomics, and high-throughput screening, there hasn't been any increase in the rate of NCEs gaining marketing approval. Therefore, there are increasing concerns about the viability of the current model of drug development (Munos 2009). The pharmaceutical industry is looking for new biological targets and innovative ways of generating NCEs and novel pharmacotherapy. One of the key steps in developing a new drug is target identification and its validation (Fig. 18.2). Target is a broad term for a range of biological entities which includes proteins, genes, and RNA. A good target should be efficacious, safe, meet clinical and commercial needs, and, above all, be 'druggable'. A 'druggable' target is something that is accessible to the potential drug molecule, which can be a small molecule or larger biomolecule, and upon binding elicit a measurable biological response both in vitro and in vivo. It is now well established that certain target classes are more suitable for small molecule drug discovery like G-protein-coupled receptors (GPCRs), whereas antibodies, which are good at blocking protein/protein interactions, are good for large molecule drug discovery (Hughes et al. 2010). Identification approaches also include examining mRNA/protein levels to determine whether they are upregulated or down-regulated with disease exacerbation or progression.

Once a target has been identified, drug discovery in recent decades has relied on extensive screening of chemical libraries (Table 18.1) to detect compounds with activity against the target. Commercial libraries including either combinatorial or natural products can now exceed one million different compounds. Furthermore, it has been estimated that there might be as many as $10^{40}$–$10^{100}$ possible small compounds that are potential drugs (Macarron 2006). Until now, major enzymes that have been targeted for drug discoveries included kinases, proteases, phosphatases, oxidoreductases, and transferases, whereas cellular targets include GPCRs, nuclear hormone receptors, and some ion channels. Further, it is interesting to note that

**Table 18.1** Screening strategies for drug discovery (~~Reproduced from Hughes et al. 2010~~)

| Screening strategies | Description | Remarks |
|---|---|---|
| High throughput | Large numbers of samples or compounds can be analyzed in a single assay. Generally designed to run in plates of 384 wells and above | Large compound collections are often run by big pharmaceutical companies but small compound banks can also be run in either pharmaceutical companies or academia, which can help reduce costs. Companies are also now trying to provide coverage across a wide chemical space using computer-assisted analysis to reduce the numbers of compounds screened. |
| Focused screening | Compounds previously identified as hitting specific classes of targets (e.g. kinases) and compounds with similar structures | Provide a cheaper avenue to find a hit molecule but completely novel structures may not be discovered, and there may be difficulties in obtaining a patent position in a well-covered IP area |
| Fragment screening | Soak small compounds into crystals to obtain compounds with low millimolar activity which can then be used as building blocks for larger molecules | Can join selected fragments together to fit into the chemical space to increase potency. Requires a crystal structure to be available |
| Structure-aided drug design | Use of crystal structures to help design molecules | Often used as an adjunct to other screening strategies within big pharma companies. Here a compound is docked into the crystal structure and this is used to help predict where modifications could be added to provide increased potency or selectivity |
| Virtual screening | Docking models: interrogation of a virtual compound library with the X-ray structure of the protein or, if have a known ligand, as a base to develop further compounds | Can provide the starting structures for a focused screen without the need to use expensive large library screens. Can also be used to look for novel patent space around existing compound structures |
| Physiological screening | A tissue-based approach for determination of the effects of a drug at the tissue rather than the cellular or subcellular level, for example, muscle contractility | Bespoke screens of lower throughput. Aim to more closely mimic the complexity of tissue rather than just looking at single readouts, which helps to screen smaller number of compounds to give a more disease-relevant readout |
| NMR screening | Screen small compounds (fragments) by soaking into protein targets of known crystal or NMR structure to look for hits with low mM activity which can then be used as building blocks for larger molecules | NMR is used as a structure-determining tool |
| Integrated screen | Combined phenotypic screening of a directed small molecule library with competitive activity-based protein profiling to map and functionally characterize the targets of screening hits | Accelerate the identification and pharmacologic validation of new therapeutic targets |

there are 20,000 genes and around 100,000 proteins in humans; among these, only 324 targets resulted in approved drugs (Mayr and Bojanic 2009). Of these, only 266 are human genome-derived proteins, the rest are microbial targets. Despite the potential profits and the extraordinary capacity of drug discovery technology, there is a scarcity of new drugs in the development pipeline, particularly for those medications that are likely to be highly profitable because they are used long term and by a large proportion of the population. Validation techniques range from in vitro tools through the use of whole animal models, to modulation of desired target in patients. While each approach is valid in its own right, confidence in the observed outcome is significantly increased by a multivalidation approach (Fig. 18.2).

Development of antiviral drugs is a very lengthy process as it involves many stages such as target identification and screening (Table 18.1), lead generation and optimization, preclinical and clinical studies, and final registration of the drug (Fig. 18.1). Increasing knowledge about viruses, mechanism of their infections, and the rapid evolvement of novel antiviral strategies and techniques will speed up the development of novel antiviral drugs. Here we emphasize one such advancement toward the development of HIV-AIDS therapeutics.

## 18.2 HIV Life Cycle in a Nutshell

### 18.2.1 Functions and Targets

In the last three decades, a number of efforts have led to better understanding of the virus life cycle and design of therapeutics for its control. The primary targets of the HIV virus are $CD^+$ T cells, monocytes, macrophages, and dendritic cells (Ramana et al. 2014). The decrease in the number of $CD^+$ T cells leads to failure of the immune response system, which eventually leads to fatality. From the point of entry into the human T cells, a number of processes are required for virus maturation. Each of the important stages of the viral maturation serves as a target for potential development of AIDS therapeutics. At present, there are about 30 FDA-approved (Table 18.2, Fig. 18.3) drugs of five different classes that are part of highly active antiretroviral therapy (HAART) (Zhan et al. 2016).

After the HIV infection, which is contracted via three major routes – sexual transmission, blood transfusion, and by passage from mother to child – seven key steps determine the life cycle of the virus, which are as follows: (1) binding, (2) fusion, (3) reverse transcription, (4) integration, (5) replication, (6) assembly, and (7) budding.

The HIV virus begins its life cycle by binding to CD4 receptor cells of the host. This binding is initiated by glycoprotein gp120 on the outer envelope of HIV and is promoted by chemokine receptors CCR5/CXCR4. The binding of gp120 to CD4 receptor leads to conformational changes in gp120, which promotes gp41-mediated fusion of the virus particle to the host cell (O'Hara and Olson 2002). The fusion is followed by release of HIV capsid which contains HIV RNA and three key enzymes, reverse transcriptase, integrase, and protease, that are essential to virus life and its proliferation. At this stage, within CD4 membrane, HIV capsid releases HIV RNA, which undergoes reverse transcription to make HIV DNA. The newly formed HIV

**Table 18.2** Chronological discovery of anti-HIV drugs by class[a]

| Class | Generic name | Role/function | Side effects | FDA approval date |
|---|---|---|---|---|
| Entry inhibitor | Maraviroc | Allosteric modulator of CCR5 | Liver problems, skin reactions | August 6, 2007 |
| Fusion inhibitor | Enfuvirtide | gp41binding | Insomnia, depression, anorexia | March 13, 2003 |
| NRTI[b] | Zidovudine | NTP[c] mimic | Anemia, myopathy, neutropenia | March 19, 1987 |
| NRTI[b] | Didanosine | NTP[c] mimic | Diarrhea, nausea, vomiting, abdominal pain | October 9, 1991 |
| NRTI[b] | Stavudine | NTP[c] mimic | Headache, dizziness, abnormal thinking | June 24, 1994 |
| NRTI[b] | Lamivudine | NTP[c] mimic | Headache, nausea, fatigue | November 17, 1995 |
| NRTI[b] | Abacavir | NTP[c] mimic | Nausea, headache, fatigue, vomiting | December 17, 1998 |
| NRTI[b] | Tenofovirdisoproxilfumerate | NTP[c] mimic | Diarrhea, nausea, vomiting, | October 26, 2001 |
| NRTI[b] | Emtricitabine | NTP mimic | Diarrhea, nausea, | July 2, 2003 |
| NNRTI[d] | Nevirapine | Binding to RT and blocking DNA- and RNA-dependent polymerase activity | Moderate rash | June 21, 1996 |
| NNRTI[d] | Delavirdine | Blocking RT catalytic site | Rash, fatigue, nausea | April 4, 1997 |
| NNRTI[d] | Efavirenz | Inhibiting viral DNA polymerase activity | Insomnia, confusion, memory loss | September 17, 1998 |
| NNRTI[d] | Etravirine | Binding to RT and blocking DNA and RNA dependent polymerase activity | Skin rash and allergic reactions | January 18, 2008 |
| NNRTI[c] | Rilpivirine | Binding to RT and blocking DNA and RNA dependent polymerase activity | Depression, headache, insomnia | May 20, 2011 |
| Integrase inhibitor | Dolutegravir | Integrase-DNA complex binding | Insomnia and headache | August 13, 2003 |
| Integrase inhibitor | Raltegravir | Integrase-DNA binding inhibition | Headache. Stomach pain, | October 12, 2007 |
| Integrase inhibitor | Elvitegravir | Integrase-DNA binding inhibition | Diarrhea | September 24, 2014 |
| Protease inhibitors | Saquinavir | Enzyme active site binding | Diarrhea, nausea | December 6, 1995 |

| | | | | |
|---|---|---|---|---|
| Protease inhibitors | Ritonavir | Enzyme active site binding | Diarrhea, nausea, vomiting | March 1, 1996 |
| Protease inhibitors | Indinavir | Enzyme active site binding | Diarrhea, nausea, vomiting | March 13, 1996 |
| Protease inhibitors | Nelfinavir | Enzyme binding | Flatulence, diarrhea, rash | March 14, 1997 |
| Protease inhibitors | Atazanavir | Enzyme binding | Headache, nausea, rash, vomiting | June 20, 2003 |
| Protease inhibitors | Fosamprenavir | Enzyme active site binding | Diarrhea, dizziness | October 20, 2003 |
| Protease inhibitors | Tipranavir | Enzyme active site binding | Intracranial hemorrhage, hyperglycemia | June 22, 2005 |
| Protease inhibitors | Darunavir | Enzyme active site binding | Diarrhea, headache, abdominal pain | June 23, 2006 |
| Pharmacokinetic enhancers | Cobicistat | Inhibit functions of liver enzymes | Nausea, diarrhea, fatigue | September 24, 2014 |

[a]Partly adapted from aidsinfo.nih.gov factsheet (Retrieved May 30, 2016)
[b]Nucleotide reverse transcriptase (RT) inhibitors
[c]Nucleotide triphosphate
[d]Nonnucleotide reverse transcriptase (RT) inhibitors

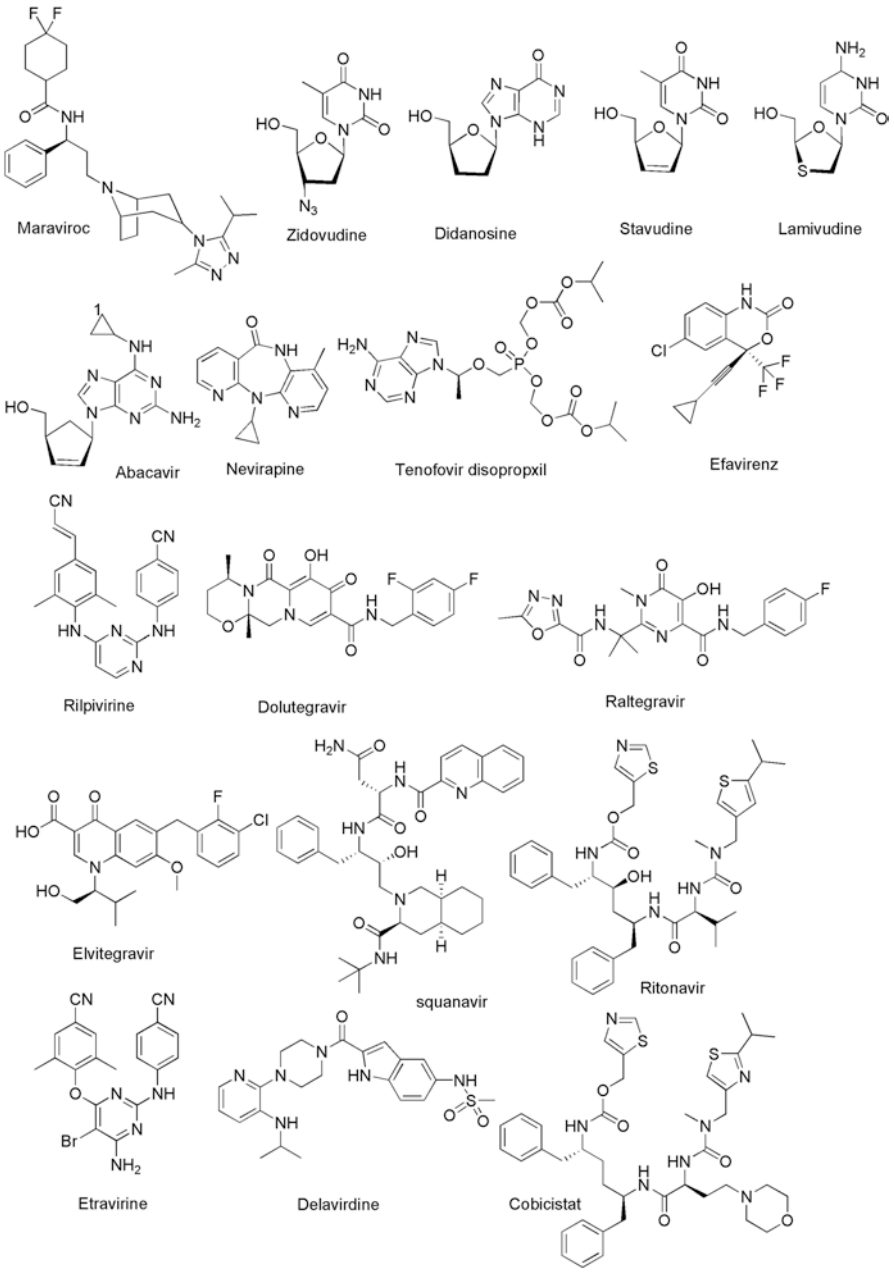


**Fig. 18.3** Chemical structures of FDA-approved HIV drugs

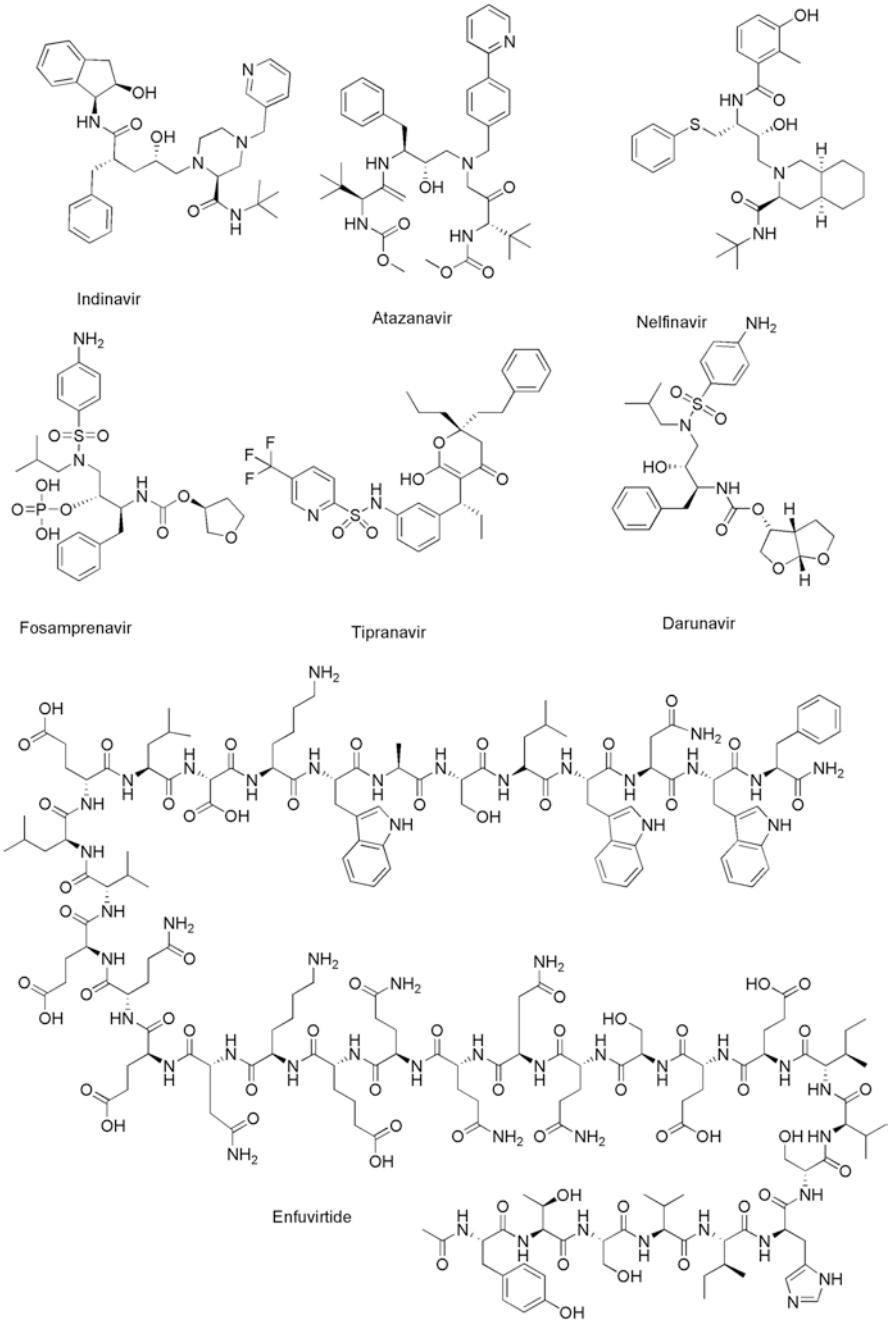


**Fig. 18.3** (continued)

DNA then passes through the CD4 nuclear membrane where it undergoes integration with the host cell DNA facilitated by the enzyme integrase. Using this machinery, the proviral DNA hides itself within the host cell DNA and leads to the higher-latency period of the virus. Within the host nucleus, the proviral DNA undergoes routine steps of transcription and translation to make new HIV viral proteins. The final stage of the virus life cycle is assembly and maturation of the new HIV RNA where the RNA and proteins exit out of the host cell membrane during which they remain noninfectious. The long chains of polypeptides then get broken down into smaller fragments of proteins by proteases and finally get processed as infectious viral particles at the maturation stage.

All seven important steps of the HIV virus life cycle have been envisaged as potential targets for developing therapy. A number of advances toward inhibition of these processes have led to the development of HAART and other agents of chemotherapy which have curtailed virus proliferation and enhanced life expectancy. Here we list therapeutic advances made toward HIV therapy and highlight some of the current drug discovery approaches. Traditionally, discovery approaches have screened a large number of drug-like molecules, often using high-throughput assays to identify lead compounds and subsequently perform structure-based modifications to enhance their efficacy. But several new screening approaches have identified novel structural motifs for AIDS therapy. For example, fragment-based screening has led to the identification of molecules containing indole core as protease inhibitors. This work led to subsequent discovery of brominated benzoic and naphthoic acid derivatives as new protease inhibitors. These discoveries demonstrate that fragment-based design of new molecules can provide complementary approaches to established drug discovery screening essays. A number of other approaches such as privileged fragment-based reconstruction approach, dynamic ligation scattering, rapid diversity-oriented and in situ screening, and hierarchical virtual screening are in practice to supplement traditional methods of drug discovery (Ghosh et al. 2016).

## 18.3 Advances in the HIV Drug Development

Despite the progress in antiretroviral therapy, the significantly high mutation rate of the HIV virus has mandated newer alternatives to currently used drugs not just to have a different line of therapeutics but also to discover more efficacious drugs. Here we outline recent advances made toward inhibition of seven key steps required for virus life cycle.

### 18.3.1 Entry Inhibitors

The enveloped HIV virus undergoes cell surface fusion with the CD4 cells as the first step of its entry to the host cell. The virion is surrounded by a cell-surface glycoprotein gp120 and a transmembrane glycoprotein gp41, both of which can act as

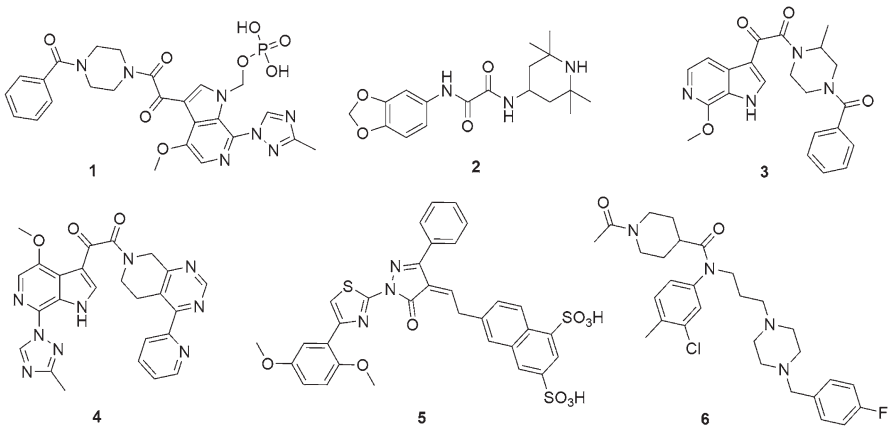


**Fig. 18.4** Chemical structures of a few recent small molecule inhibitors of HIV entry

targets of viral entry inhibition and are collectively known as entry inhibitors. The attachment of virus is believed to be facilitated by electrostatic interactions (nonspecific) between the positively charged domains of gp120 and negatively charged domains of proteoglycans of the host cell surface. However, certain specific interactions such as those between gp120 and integrin α4β7 are also known to mediate this process. Once attachment to the CD4 receptor is achieved, gp120 undergoes conformational changes. At this stage, chemokine receptors CCR5/CXCR4 help in gp120 binding, which is followed by further conformational changes in gp120.These events lead to conformational changes in the transmembrane glycoprotein gp41, which anchors fusion of viral and host cell membrane. Therefore, all these three stages serve as targets of therapeutic intervention at the entry stage.

Some of the earliest small molecule gp120-CD4 interaction inhibitors included BMS 378806 and BMS 448043, which were soon replaced by BMS 626529 and its prodrug BMS 663068. A line of small molecule inhibitors targeting coreceptor CCR5 were developed such as Aplaviroc, Vicriviroc, Cenicriviroc and Maraviroc. Of these, Maraviroc was granted FDA approval in 2007. Another set of small molecules aimed at blocking gp41-mediated membrane fusion were developed, of which Enfuvirtide (initially known as T-20, a 36-amino acid synthetic peptide based upon the heptad repeat sequence 2 of gp41) was granted FDA approval in 2003, and it remains the only FDA-approved fusion inhibitor to date. Several new molecules (Fig. 18.4), some of which are in advanced phases of clinical trials, have since been discovered and tested for their efficacy as entry inhibitors. These include Fostemsavir (Fig. 18.4(1)); NBD-556-based minimally toxic CD4 mimics (Fig. 18.4(2)) (Mizuguchi et al. 2016); nonnatural amino acids, pyrroloaryls, and pyrrolohetroaryls (Fig. 18.4(3)) (Patel and Park 2015); substituted tetrahydroisoquinolines (Fig. 18.4(4)) (Swidorski et al. 2016); small molecule sulfotyrosine mimics (Fig. 18.4(5)) (Dogo-Isonagie et al. 2016); and 1, 4 disubstituted piperazine derivatives (Fig. 18.4(6)) (Dong et al. 2012).

### 18.3.2 Reverse Transcriptase Inhibitors

Once the virus enters the cytoplasm of the host cell, the reverse transcriptase (RT) of the virus is activated. In addition to other assisting cellular factors, RT performs reverse transcription reactions using DNA polymerase and RNase H to make new copies of double-stranded DNA. The reverse transcription process begins with the binding of the tRNA primer to the 5′ end of viral RNA known as the primer binding site. The initiated minus-strand DNA synthesis is transferred to the 3′ end of either of the two copies of viral RNA, which is released from the capsid in the host cell cytoplasm. The minus-strand DNA synthesis is continued along with RNase H degradation. A polypurine tract in the RNA resists RNA degradation and ultimately serves as the template for plus-strand DNA synthesis. The plus-strand DNA synthesis also generates 18 nucleotides for the tRNA primer.

Inhibiting HIV RT activity has been one of the most sought after targets in HIV therapy. Of currently approved FDA drugs for HIV therapy, nearly half of them belong to the class of RT inhibitors. In fact, the very first anti-HIV drug, azidothymidine (AZT), belonged to the class of RT inhibitors. The RT inhibitors have been classified into two main classes (Table 18.2): nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs act as mimics of natural substrates of DNA synthesis. The sugar moiety of the nucleoside analogs lacks the 3′-OH functionality which is catalytically essential. Once these nucleoside mimics are incorporated inside the cells, they are converted as their triphosphate analogs by cellular enzymes (kinases) to be active. The triphosphate analogs thus produced are used by RT for making DNA, which, in turn, acts as chain terminators blocking the DNA synthesis. On the other hand, NNRTIs act by a non-competing mechanism where they induce allosteric conformational changes to the catalytic activity of the enzyme.

The success of AZT led to research focus on the development of NRTIs, which resulted in a few more FDA drugs such as Stavudine and Lamivudine by the mid-1990s which acted by mimicking natural nucleoside substrate thymidine triphosphate. But soon viral resistance necessitated alternatives, which resulted in the development of NNRTIs with Nevirapine being the first FDA-approved drug in this class. Several new molecules have been reported with excellent activity and promise (Fig. 18.5). From a large sample size (over 20,000 compounds) and using high-throughput assay, novel phenylaminopyridine derivatives (Fig. 18.5(7)) were identified as NNRTIs with low nanomolar inhibitory concentrations against RT activity (Kim et al. 2012). Using a molecular hybridization strategy, 6-substituted diarylpyridine derivatives (Fig. 18.5(8)) have been designed which show more potent activities than Nevirapine (Yang et al. 2016). RT-related RNase H activity has also been targeted recently with reasonable cell-based antiviral activity using hydroxypyridonecarboxylic acid derivatives (Fig. 18.5(9)) (Kankanala et al. 2016). Compounds containing the Imidazole-amide biaryl scaffold (Fig. 18.5(10)) have also shown good overall RT activity, which promises a new class of alternative compounds (Chong et al. 2012). Molecular simulation-based design of pyrimidine sulfonylacetanalides (Fig. 18.5(11)) has shown very good activity against clinically relevant

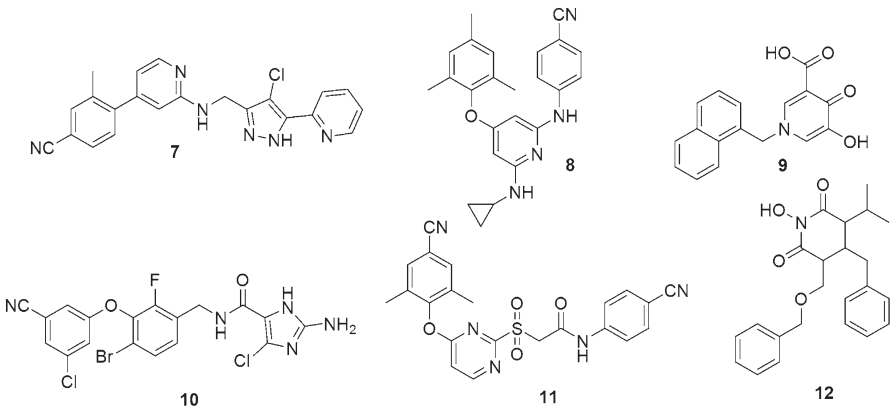


**Fig. 18.5** Examples of some reverse transcriptase inhibitors

single- and double-mutant strains of HIV-1 (Wan et al. 2015). A new molecular scaffold containing N-hydroxyimide moiety (Fig. 18.5(12)) has been reported to have dual activity against both RT and integrase. Development of these dual binders as drug candidates could help in reducing drug dosage and the number of components in HAART therapy (Tang et al. 2011).

### 18.3.3 Integrase Inhibitors

Following reverse transcription, the viral DNA is transported into the nucleus where it undergoes integration with the host DNA using the enzyme integrase. The viral DNA undergoes highly specific 3′-processing (also known as dinucleotide processing) by the integrase enzyme, which removes two 3′-end nucleotides from both strands of the DNA. The 3′-OH on the viral DNA then attacks the phosphodiester bonds of the host DNA. The site of attack is separated by five nucleotides in the case of HIV. The resulting intermediate integration is repaired by cellular enzymes which conclude the integration process. Both the enzyme and the conserved DNA sequence that acts as primer for DNA synthesis of the provirus have been envisaged as potential targets for inhibiting integrase activity. Hydroxyisoquinolinediones (Fig. 18.6(12)) have recently been reported to have nanomolar affinity for integrase with good antiviral activity (Billamboz et al. 2016). A relatively weaker but new class of small molecule inhibitor of integrase has been reported recently. Based on molecular-docking simulations, naphthalene derivatives (Fig. 18.6(14)) have been designed (Gu et al. 2014). The potential of flavanoids (Fig. 18.6(15)) to act as metal chelates has been used to develop integrase activity. Furthermore, 8-hydroxyquinoline tetracyclic lactams (Fig. 18.6(16)) have also been identified as potent integrase strand transfer inhibitors (Velthuisen et al. 2016). Derivatives of 3-hydroxypyrimidine 2,4 diones (Fig. 18.6(17)) have recently been discovered to possess dual binding abilities where they have shown extremely potent activity against integrase and RT-associated

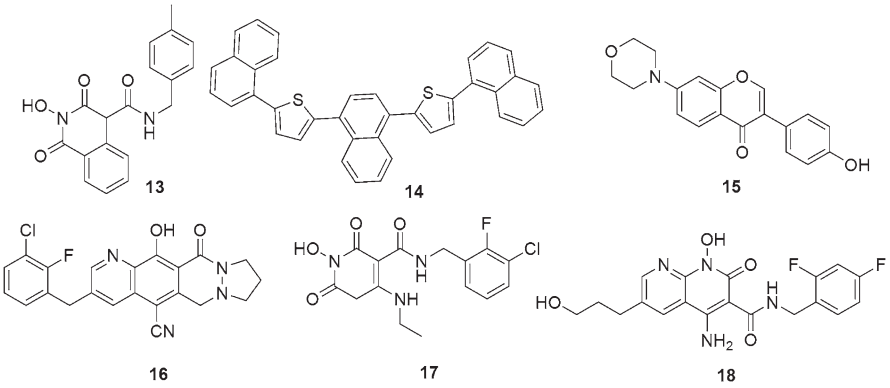


**Fig. 18.6** Chemical structures of some recently discovered integrase inhibitors

RNase H (Wu et al. 2016). New agents based on naphthyridine moiety (Fig. 18.6(18)) have shown interstrand inhibitory effects with susceptibility to mutant integrases (Zhao et al. 2016).

## 18.4 Inhibitors of Tat-TAR Binding

The HIV life cycle is heavily reliant on the transcriptional and translational machineries of the host cell. The successful and fast transcription of the proviral DNA requires interaction of viral protein *tat* and the transactivating region (TAR) RNA with the human positive transcription elongation factor through a ternary complex formation. Prior to the ternary complex formation, the viral protein *tat* interacts with the TAR RNA (from the newly formed viral mRNA transcripts) in a highly specific manner with high affinity. TAR is a 59-nucleotide RNA structure that contains loops and bulges, a characteristic typical of the RNA structures. The three-nucleotide bulge (U23, C24, and U25) is crucial for *tat* binding where the arginine 52 residue of the *tat* protein binds. Therefore, inhibiting *tat*-TAR interaction has been a long sought after target as HIV provirus replication inhibitor despite the fact that none of the FDA-approved drugs belong to this class.

A number of these efforts have used TAR RNA binding by small molecules as a direct/allosteric competitor of *tat* binding. Based on the observations that arginine and arginine derivatives induced conformational changes in the TAR, ethidium-arginine conjugates were synthesized which showed micromolar anti-HIV activities (Peytou et al. 1999). Structure-guided peptidomimetic design led to β-hairpin inhibitors of *tat*-TAR interaction with nanomolar affinities (Athanassiou et al. 2004, 2007). Aminoglycosides, which are known to bind to various RNA structures including bulges, were investigated to assess their TAR RNA binding. Ribonuclease protection experiments showed that the binding site of neomycin (Fig. 18.7(19)), an aminoglycoside, on TAR was immediately below the three-nucleotide bulge UCU (Wang et al. 1998). Similarly, a DNA minor groove binder Hoechst 33258 (Fig. 18.7(20)) was

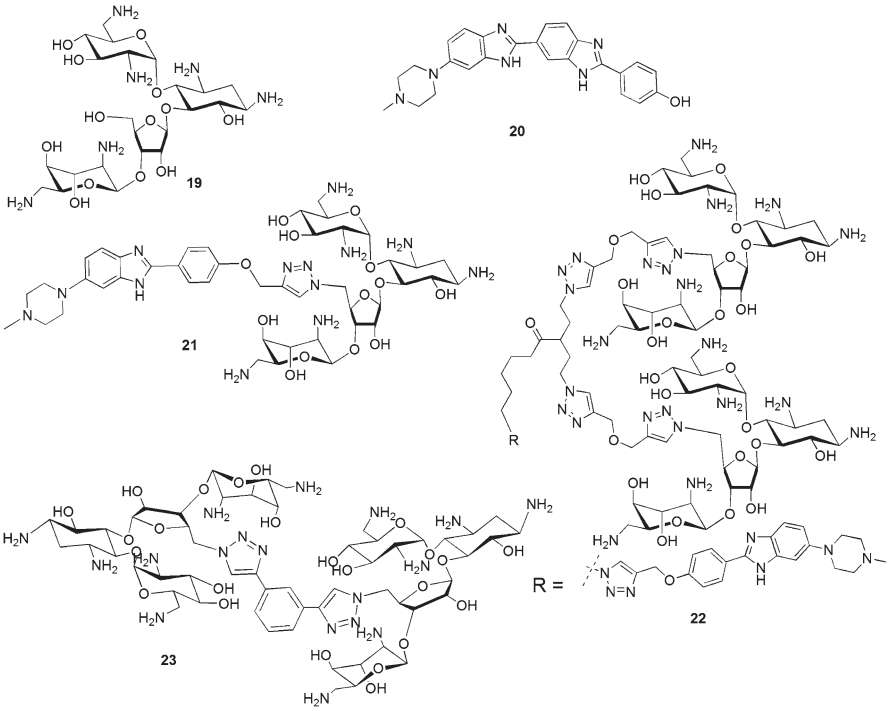


**Fig. 18.7** Chemical structures of neomycin conjugates, the new inhibitors of *tat*-TAR interaction

found to bind TAR at unique GC-rich site GCUCU, unlike its natural preference for AT-rich sites in B-DNA duplexes (Dassonneville et al. 1997). Recently, conjugation approaches have been undertaken to enhance the affinity of TAR RNA binding drugs. Conjugation of neomycin to a Hoechst 33258-derived benzimidazole (Fig. 18.7(21)) led to a nearly five-fold increase in the neomycin affinity toward TAR RNA in a competitive binding assay with the *tat* protein (Ranjan et al. 2013). Further enhancement toward TAR RNA binding was achieved by synthesis of a multivalent, in principle, triple-recognition agent (Fig. 18.7(22)) that showed nanomolar affinity (Kumar et al. 2016). Triazole-linked dimeric neomycin conjugates (Fig. 18.7(23)) have also shown remarkable binding toward TAR RNA with affinity constants $K_a \sim 10^7$–$10^8\ M^{-1}$ and inhibit the release of RT at low concentrations (Kumar et al. 2012). Clearly, these promising results require further follow-up in terms of toxicity studies for them to be developed as specific *tat*-TAR interaction inhibitors.

### 18.4.1 Protease Inhibitors

HIV protease is one of the most important enzymes for the HIV life cycle and is responsible for production of all necessary viral proteins. Subsequent to HIV integrase function, the proviral DNA undergoes transcription and translation to give long chains

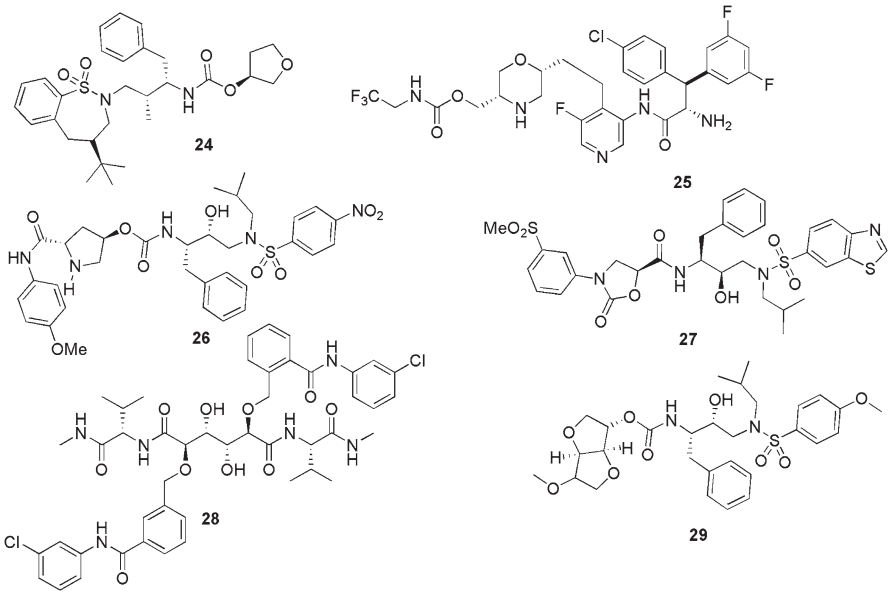


**Fig. 18.8** Chemical structures of recently developed protease inhibitors

of proteins that contain Gag and Gag-Pol polyproteins. Protease cleaves the long polypeptides at nine processing sites to give mature active proteins eventually leading to final digested products as new protease, RT (p51), RNAse H (p15), and integrase. Not surprisingly, protease has been a central target of antiretroviral therapy.

The majority of FDA-approved anti-HIV drugs belong to the class of protease inhibitors and have been an essential part of combination therapies. Despite the success of this class of retrovirus inhibitors, a number of efforts have been going on toward combating resistance issues and increasing their efficacy both in monotherapy and combination therapies. These have included (Fig. 18.8) cyclic sulfonamide-based inhibitors (Fig. 18.8(24)) with picomolar affinities (Ganguly et al. 2014) that resulted from structural study-based design. Recently, a series of morpholine-based aspartate-binding group compounds were developed which identified MK-8718 (Fig. 18.8(25)) as an orally bioavailable agent with a good overall effect including a favorable pharmacokinetic profile (Bungard et al. 2016). A series of new HIV protease inhibitors with hydroxyethylamine core (Fig. 18.8(26)), which resemble the FDA-approved drug Darunavir, have been reported to have potent activity similar to Darunavir (Gao et al. 2011). Similar compounds, based on Darunavir, containing phenyloxazolidinone (Fig. 18.8(27)) showed picomolar binding affinities and low nanomolar antiviral activity against patient-derived HIV-1 virus (Ali et al. 2010). Tertiary alcohol containing transition state mimics bearing P2 and P1 substituents have shown excellent inhibitor properties (Ohrngren et al. 2011). A new class of C2 symmetric protease inhibitors (Fig. 18.8(28)) has been reported with low nanomolar

affinities (Wannberg et al. 2006). Novel isosorbide-derived P2 ligands (Fig. 18.8(29)) sharing the Darunavir cores have also shown picomolar affinities, thus opening several avenues to take drug-resistance challenges (Qiu et al. 2014).

### 18.4.2 Other New Targets

In addition to the above discussed inhibitors, a few other processes are being targeted to develop a new class of inhibitors. Prime of them are maturation and capsid assembly inhibitors. Maturation inhibitors, albeit inhibiting the functions of protease, are different from protease inhibitors in that they do not directly bind to the protease enzyme to block its catalytic activity. Rather they bind to a segment of Gag polyprotein disrupting the protease-mediated conversion of p25Gag (CA/SP1) to mature p24 protein (Liu et al. 2016). Proteolytic cleavage of Gag polyprotein releases HIV-1 capsid protein, which reassembles into a cone-shaped structure containing viral RNA and other necessary proteins (e.g. RT, integrase, protease) (Tremblay et al. 2012). Proper assembly of capsid is mandatory for the infectivity of the new virus. Therefore, capsid assembly inhibitors are also being explored as a new type of anti-HIV drug. The interaction of *rev* response element (RRE) with the *rev* protein is essential for viral replication (Luedtke and Tor 2003). A line of *rev*-RRE interaction inhibitors have been designed and are being explored as an alternative drug target. Immunotherapy and gene therapy-based efforts have also been tried for HIV treatment.

## 18.5 Future Perspectives

Development of combination therapies such as the HIV-AIDS-related HAART have helped us explore new cumulative and complementary approaches to a desired action. Advancements in the computational methods have significantly curtailed lengthy screening efforts for the lead identification and have provided alternatives for structural understanding of a drug-receptor binding. Despite this progress, the growing emergence of drug resistance and fast viral mutation has always reminded us to be vigilant to meet the challenges of the future. While lead optimization has been relatively faster in recent years, the long path of clinical trials has always been a limiting factor. A drug making it to the market has to go through all stringent checks for its adverse actions, which requires a longer time frame for data compilation and the willingness of several patient groups for a new drug trial. Clearly these issues are big roadblocks in a fast drug development and continue to be a problem whose efficient solutions are not in place yet.

However, advancements in structural biology, most notably by the development of high-resolution crystallographic and Nuclear Magnetic Resonance (NMR) techniques, have helped us understand several biologically relevant targets with fairly decent details of their active site. Understanding of the active site and chemically important functional groups in the vicinity of the active binding site has greatly aided in structure-guided drug-design efforts. Moreover, the biochemical functional

mapping has allowed us to think of novel targets at fusion, maturation, and capsid assembly stage. These new targets need to be followed with dedicated discovery programs to bring new drugs to the market.

## References


Ali A, Reddy GSK, Nalam MNL, Anjum SG, Cao H, Schiffer CA, Rana TM (2010) Structure-based design, synthesis, and structure activity relationship studies of HIV-1 protease inhibitors incorporating phenyloxazolidinones. J Med Chem 53(21):7699–7708

Athanassiou Z, Dias RLA, Moehle K, Dobson N, Varani G, Robinson JA (2004) Structural mimicry of retroviral tat proteins by constrained beta-hairpin peptidomimetics: ligands with high affinity and selectivity for viral TAR RNA regulatory elements. J Am Chem Soc 126(22):6906–6913

Athanassiou Z, Patora K, Dias RLA, Moehle K, Robinson JA, Varani G (2007) Structure-guided peptidomimetic design leads to nanomolar beta-hairpin inhibitors of the Tat-TAR interaction of bovine immunodeficiency virus. Biochemistry 46(3):741–751

Billamboz M, Suchaud V, Bailly F, Lion C, Andréola M, Christ F, Debyser Z, Cotelle P (2016) 2-hydroxyisoquinoline-1,3(2H,4H)-diones (HIDs) as human immunodeficiency virus type 1 integrase inhibitors: influence of the alkylcarboxamide substitution of position 4. Eur J Med Chem 117:256–268

Bungard CJ, Williams PD, Ballard JE, Bennett DJ, Beaulieu C, Bahnck-Teets C, Carroll SS, Chang RK, Dubost DC, Fay JF, Diamond TL, Greshock TJ, Hao L, Holloway MK, Felock PJ, Gesell JJ, Su HP, Manikowski JJ, McKay DJ, Miller M, Min X, Molinaro C, Moradei OM, Nantermet PG, Nadeau C, Sanchez RI, Satyanarayana T, Shipe WD, Singh SK, Truong VL, Vijayasaradhi S, Wiscount CM, Vacca JP, Crane SN, McCauley JA (2016) Discovery of MK-8718, an HIV protease inhibitor containing a novel morpholine aspartate binding group. ACS Med Chem Lett 7(7):702–707

Chong P, Sebahar P, Youngman M, Garrido D, Zhang H, Stewart EL, Nolte RT, Wang L, Ferris RG, Edelstein M, Weaver K, Mathis A, Peat A (2012) Rational design of potent non-nucleoside inhibitors of HIV-1 reverse transcriptase. J Med Chem 55(23):10601–10609

Dassonneville L, Hamy F, Colson P, Houssier C, Bailly C (1997) Binding of hoechst 33258 to the TAR RNA of HIV-1. Recognition of a pyrimidine bulge-dependent structure. Nucleic Acids Res 25(22):4487–4492

Dogo-Isonagie C, Lee S, Lohith K, Liu H, Mandadapu S. R, Lusvarghi S, O'Connor RD, Bewley CA (2016) Design and synthesis of small molecule-sulfotyrosine mimetics that inhibit HIV-1 entry. Bioorg Med Chem 24(8): 1718–1728.

Dong M, Lu L, Li H, Wang X, Lu H, Jiang S, Dai QY (2012) Design, synthesis, and biological activity of novel 1,4-disubstituted piperidine/piperazine derivatives as CCR5 antagonist-based HIV-1 entry inhibitors. Bioorg Med Chem Lett 22(9):3284–3286

Ganguly AK, Alluri SS, Wang C, Antropow A, White A, Caroccia D, Biswas D, Kang E, Zhang LK, Carroll SS, Burlein C, Fay J, Orth P, Strickland C (2014) Structural optimization of cyclic sulfonamide based novel HIV-1 protease inhibitors to picomolar affinities guided by X-ray crystallographic analysis. Tetrahedron 70(18):2894–2904

Gao B, Zhang C, Yin Y, Tang L, Liu Z (2011) Design and synthesis of potent HIV-1 protease inhibitors incorporating hydroxyprolinamides as novel P2 ligands. Bioorg Med Chem Lett 21(12):3730–3733

Ghosh AK, Osswald HL, Prato G (2016) Recent progress in the development of HIV-1 protease inhibitors for the treatment of HIV/AIDS. J Med Chem 59(11):5172–5208

Gu W, Ip DT, Liu S, Chan JH, Wang Y, Zhang X, Zheng YT, Wan DC (2014) 1,4-bis(5-(naphthalen-1-yl) thiophen-2-yl)naphthalene, a small molecule, functions as a novel anti-HIV-1 inhibitor targeting the interaction between integrase and cellular lens epithelium-derived growth factor. Chem Biol Interact 213:21–27

Hughes JP, Rees S, Kalindjian SB, Philpott KL (2010) Principles of early drug discovery. Br J Pharmacol 162(6):1239–1249

Kankanala J, Kirby KA, Liu F, Miller L, Nagy E, Wilson DJ, Parniak MA, Sarafianos SG, Wang Z (2016) Design, synthesis, and biological evaluations of hydroxypyridonecarboxylic acids as inhibitors of HIV reverse transcriptase associated RNase H. J Med Chem 59(10):5051–5062

Kim J, Lee D, Park C, So W, Jo M, Ok T, Kwon J, Kong S, Jo S, Kim Y, Choi J, Kim HC, Ko Y, Choi I, Park Y, Yoon J, Ju MK, Kim J, Han SJ, Kim TH, Cechetto J, Nam J, Sommer P, Liuzzi M, Lee J, No Z (2012) Discovery of phenylaminopyridine derivatives as novel HIV-1 non-nucleoside reverse transcriptase inhibitors. ACS Med Chem Lett 3(8):678–682

Kumar S, Kellish P, Robinson WE, Wang D, Appella DH, Arya DP (2012) Click dimers to target HIV TAR RNA conformation. Biochemistry 51:2331–2347

Kumar S, Ranjan N, Kellish P, Gong C, Watkins D, Arya DP (2016) Multivalency in the recognition and antagonism of a HIV TAR RNA-TAT assembly using an aminoglycoside benzimidazole scaffold. Org Biomol Chem 14(6):2052–2056

Liu Z, Swidorski JJ, Nowicka-Sans B, Terry B, Protack T, Lin Z, Samanta H, Zhang S, Li Z, Parker DD, Rahematpura S, Jenkins S, Beno BR, Krystal M, Meanwell NA, Dicker IB, Regueiro-Ren A (2016) C-3 benzoic acid derivatives of C-3 deoxybetulinic acid and deoxybetulin as HIV-1 maturation inhibitors. Bioorg Med Chem 24(8):1757–1770

Luedtke NW, Tor Y (2003) Fluorescence-based methods for evaluating the RNA affinity and specificity of HIV-1 rev? RRE inhibitors. Biopolymers 70(1):103–119

Macarron R (2006) Critical review of the role of HTS in drug discovery. Drug Discov Today 11(7):277–279

Mayr LM, Bojanic D (2009) Novel trends in high-throughput screening. Curr Opin Pharmacol; Anti-infect/New Technol 9(5):580–588

Mizuguchi T, Harada S, Miura T, Ohashi N, Narumi T, Mori H, Irahara Y, Yamada Y, Nomura W, Matsushita S, Yoshimura K, Tamamura H (2016) A minimally cytotoxic CD4 mimic as an HIV entry inhibitor. Bioorg Med Chem Lett 26(2):397–400

Munos B (2009) Lessons from 60 years of pharmaceutical innovation. Nat Rev Drug Discov 8(12):959–968

O'Hara BM, Olson WC (2002) HIV entry inhibitors in clinical development. Curr Opin Pharmacol 2(5):523–528

Ohrngren P, Wu X, Persson M, Ekegren JK, Wallberg H, Vrang L, Rosenquist A, Samuelsson B, Unge T, Larhed M (2011) HIV-1 protease inhibitors with a tertiary alcohol containing transition-state mimic and various P2 and P1prime or minute substituents. Med Chem Commun 2(8):701–709

Patel RV, Park SW (2015) Pyrroloaryls and pyrroloheteroaryls: inhibitors of the HIV fusion/attachment, reverse transcriptase and integrase. Bioorg Med Chem 23(17):5247–5263

Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, Schacht AL (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov 9(3):203–214

Peytou V, Condom R, Patino N, Guedj R, Aubertin A, Gelus N, Bailly C, Terreux R, Cabrol-Bass D (1999) Synthesis and antiviral activity of ethidium-arginine conjugates directed against the TAR RNA of HIV-1. J Med Chem 42(20):4042–4053

Qiu X, Zhao G, Tang L, Liu Z (2014) Design and synthesis of highly potent HIV-1 protease inhibitors with novel isosorbide-derived P2 ligands. Bioorg Med Chem Lett 24(11):2465–2468

Ramana LN, Anand AR, Sethuraman S, Krishnan UM (2014) Targeting strategies for delivery of anti-HIV drugs. J Control Release 192:271–283

Ranjan N, Kumar S, Watkins D, Wang D, Appella DH, Arya DP (2013) Recognition of HIV-TAR RNA using neomycin–benzimidazole conjugates. Bioorg Med Chem Lett 23(20):5689–5693

Swidorski JJ, Liu Z, Yin Z, Wang T, Carini DJ, Rahematpura S, Zheng M, Johnson K, Zhang S, Lin PF, Parker DD, Li W, Meanwell NA, Hamann LG, Regueiro-Ren A (2016) Inhibitors of HIV-1 attachment: the discovery and structure–activity relationships of tetrahydroisoquinolines as replacements for the piperazine benzamide in the 3-glyoxylyl 6-azaindole pharmacophore. Bioorg Med Chem Lett 26(1):160–167

Tang J, Maddali K, Dreis CD, Sham YY, Vince R, Pommier Y, Wang Z (2011) N-3 hydroxylation of pyrimidine-2,4-diones yields dual inhibitors of HIV reverse transcriptase and integrase. ACS Med Chem Lett 2(1):63–67

Tremblay M, Bonneau P, Bousquet Y, DeRoy P, Duan J, Duplessis M, Gagnon A, Garneau M, Goudreau N, Guse I, Hucke O, Kawai SH, Lemke CT, Mason SW, Simoneau B, Surprenant S, Titolo S, Yoakim C (2012) Inhibition of HIV-1 capsid assembly: optimization of the antiviral potency by site selective modifications at N1, C2 and C16 of a 5-(5-furan-2-yl-pyrazol-1-yl)-1H-benzimidazole scaffold. Bioorg Med Chem Lett 22(24):7512–7517

UNAIDS (2016) Global AIDS update, 2016. Retrieved June 29, 2016, from http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016

Velthuisen EJ, Johns BA, Temelkoff DP, Brown KW, Danehower SC (2016) The design of 8-hydroxyquinoline tetracyclic lactams as HIV-1 integrase strand transfer inhibitors. Eur J Med Chem 117:99–112

Wan Z, Yao J, Mao T, Wang X, Wang H, Chen W, Yin H, Chen FE, De Clercq E, Daelemans D, Pannecouque C (2015) Pyrimidine sulfonylacetanilides with improved potency against key mutant viruses of HIV-1 by specific targeting of a highly conserved residue. Eur J Med Chem 102:215–222

Wang S, Huber PW, Cui M, Czarnik AW, Mei H (1998) Binding of neomycin to the TAR element of HIV-1 RNA induces dissociation of tat protein by an allosteric mechanism. Biochemistry 37(16):5549–5557

Wannberg J, Sabnis YA, Vrang L, Samuelsson B, Karlén A, Hallberg A, Larhed M (2006) A new structural theme in C2-symmetric HIV-1 protease inhibitors: ortho-substituted P1/P1′ side chains. Bioorg Med Chem 14(15):5303–5315

Wu B, Tang J, Wilson DJ, Huber AD, Casey MC, Ji J, Kankanala J, Xie J, Sarafianos SG, Wang Z (2016) 3-hydroxypyrimidine-2,4-dione-5-N-benzylcarboxamides potently inhibit HIV-1 integrase and RNase H. J Med Chem 59(13):6136–6148

Yang J, Chen W, Kang D, Lu X, Li X, Liu Z et al (2016) Design, synthesis and anti-HIV evaluation of novel diarylpyridine derivatives targeting the entrance channel of NNRTI binding pocket. Eur J Med Chem 109:294–304

Zhan P, Pannecouque C, De Clercq E, Liu X (2016) Anti-HIV drug discovery and development: current innovations and future trends. J Med Chem 59(7):2849–2878

Zhao XZ, Smith SJ, Maskell DP, Metifiot M, Pye VE, Fesen K et al (2016) HIV-1 integrase strand transfer inhibitors with reduced susceptibility to drug resistant mutant integrases. ACS Chem Biol 11(4):1074–1081