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1. Introduction

Since acquired immunodeficiency syndrome (AIDS) was recognized 27 years ago, 25 million people have died of human immunodeficiency virus (HIV)-related causes. On a global scale, although the HIV epidemic has stabilized since 2000, unacceptably high levels of new HIV infection and AIDS death still occur each year. In 2007, there were an estimated 33 million (30-36 million) people living with HIV, and 2 million (1.8-2.3 million) people died due to AIDS, compared with an estimated 1.7 million (1.5-2.3 million) in 2001. There are two main types of HIV: type 1 (HIV-1) and type 2 (HIV-2) (Buonaguro et al., 2007). HIV-1 is the most prevalent in the worldwide pandemic. HIV-2 is present mainly in West Africa, where it was discovered in 1986, and infects about one million people worldwide. HIV-2 is slowly but continuously spreading throughout Europe, Asia and the Americas, and has reached a significant prevalence in countries such as Portugal and India. After more than 20 years of research, HIV remains a difficult target for a vaccine; thus the treatment of AIDS continues to focus on the search for chemical anti-HIV agents.

A working knowledge of the HIV replication cycle is essential for understanding the mechanism of action of antiviral drugs. The HIV is an enveloped virus that contains two copies of viral genomic RNA in its core. In addition to the copies of RNA, the viral core also contains the enzymes required for HIV replication. The first step in the HIV replication cycle is the interaction between the envelope proteins of the virus (gp120) and specific host-cell surface receptors (e.g. the T-cell receptor CD4 on the cellular membrane) of the host cell. In the second step, the virus binds to the chemokine coreceptors CXC-chemokine receptor 4 (CXCR4) and CC-chemokine receptor 5 (CCR5). This induces a conformational change in gp120 that opens up a high affinity binding site located within the third variable loop (V3) and surrounding surfaces for the chemokine coreceptors CXCR4 and CCR5. This gives rise to further conformational rearrangements of gp120 that expose the transmembrane glycoprotein gp41, and the heptad repeat (HR) regions of the three subunits of gp41, HR1 and HR2, fold into a six-helical bundle. This ultimately results in the “fusion” of the viral envelope and the cytoplasmic membrane. Fusion creates a pore through which the viral capsid enters the cells.

HIV encodes three enzymes required for replication: HIV-1 reverse transcriptase (RT), HIV-integrase (IN) and HIV-protease (PT). Following entry into the cell, the viral RT enzyme catalyzes the conversion of viral RNA into DNA. This viral DNA enters the nucleus and
becomes inserted into the chromosomal DNA of the host cell (integration). This process is facilitated by the viral enzyme IN. Expression of the viral genes leads to production of precursor viral proteins. These proteins and viral RNA are assembled at the cell surface into new viral particles and leave the host cell by a process called budding. During the budding process, they acquire the outer layer and envelope. At this stage, the PT enzyme cleaves the precursor viral proteins into their mature products. If this final phase of the replication cycle does not take place, the released viral particles are non-infectious and not competent to initiate the replication cycle in other susceptible cells.

Once HIV has entered the cell, it must disarm and hijack the intracellular machinery for its own benefit. Normal cell functionality of viral hosts is altered by invading virus proteins to the benefit of the virus. Viral proteins are known to compete with the host proteins, thus disrupting the normal host protein-protein interaction network. HIV-1 encodes the regulatory proteins, Tat and Rev, and four accessory proteins: viral infectivity factor (Vif), viral protein R (Vpr), viral protein U (Vpu) and negative factor (Nef) (Romani & Engelbrecht, 2009, Romani et al., 2010). The regulatory proteins are essential for virus replication by controlling HIV gene expression in host cells. In contrast, accessory proteins are often dispensable for virus replication in vitro. The Vif directly binds to and inactivates cellular deoxycytidine deaminase APOBEC3G, a natural antiviral factor that promotes G- to A-hypermutation of viral DNA during reverse transcription. The Vpu has been shown to down-regulate the CD4 receptor, and is also required for effective release of newly formed viral particles.

Anti-HIV drugs are classified into different groups according to their activity on the replicative cycle of HIV. These are virus-cell adsorption, virus-cell fusion, uncoating, reverse transcription, integration, DNA replication, transcription, translation, budding (assembly/release) and maturation. There are currently 25 compounds approved for the treatment of HIV, and most of these are nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs) (Warnke & Barreto, 2007, Zhan et al., 2009) (Table 1). Highly active antiretroviral therapy (HAART), which combines several such drugs (typically three or four), has dramatically improved patients’ lives. The therapeutic effects are limited, however, by adverse effects and toxicities caused by long-term use and the emergence of drug resistance.

<table>
<thead>
<tr>
<th>GENERIC NAME</th>
<th>ADVERSE REACTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Entry inhibitors</strong></td>
<td>Upper respiratory tract infection, cough, pyrexia</td>
</tr>
<tr>
<td>Maraviroc (UK-427)</td>
<td></td>
</tr>
<tr>
<td><strong>Fusion inhibitors</strong></td>
<td>Pruritus, pain, discomfort</td>
</tr>
<tr>
<td>Enfuvirtide (T20)</td>
<td></td>
</tr>
<tr>
<td><strong>Reverse transcriptase inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Nucleoside inhibitors</strong></td>
<td>Diarrhea, nausea, headache</td>
</tr>
<tr>
<td>Abacavir (ABC)</td>
<td>Rash, abdominal pain, peripheral neuropathy</td>
</tr>
<tr>
<td>Didanosine (ddl)</td>
<td>Hyperpigmentation of skin, rash, diarrhea</td>
</tr>
<tr>
<td>Emtricitabine (FTC)</td>
<td>Rash, nausea, lipoatrophy</td>
</tr>
<tr>
<td>Stavudine (d4T)</td>
<td>Decrease in appetite, headache, fatigue</td>
</tr>
<tr>
<td>Lamivudine (3TC)</td>
<td>Diarrhea, nausea osteopenia</td>
</tr>
<tr>
<td>Tenofovir (DF)</td>
<td>Hepatic steatosis, peripheral neuropathy</td>
</tr>
<tr>
<td>Zalcitabine (ddC)</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Approved antiretroviral drugs for the treatment of HIV infection

<table>
<thead>
<tr>
<th>GENERIC NAME</th>
<th>ADVERSE REACTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine (AZT)</td>
<td>Headache, anorexia, leukopenia</td>
</tr>
<tr>
<td>Non-nucleoside inhibitors</td>
<td></td>
</tr>
<tr>
<td>Delavirdine (DLV)</td>
<td>Rash</td>
</tr>
<tr>
<td>Efavirenz (EFV)</td>
<td>Dizziness, hallucinations, insomnia</td>
</tr>
<tr>
<td>Etravirine (THC125)</td>
<td>Rash</td>
</tr>
<tr>
<td>Nevirapine (NVP)</td>
<td>Rash, hepatotoxicity</td>
</tr>
<tr>
<td>Integrase inhibitors</td>
<td></td>
</tr>
<tr>
<td>Raltegravir (MK-0518)</td>
<td>Diarrhea, injection-site reactions, headache</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td></td>
</tr>
<tr>
<td>Amprenavir (AMP)</td>
<td>Stomach upset, diarrhea, nausea</td>
</tr>
<tr>
<td>Atazanavir (ATZ)</td>
<td>Rash, elevated bilirubin, depression</td>
</tr>
<tr>
<td>Darunavir (TMC-114)</td>
<td>Rash, hypertriglyceridemia, diarrhea</td>
</tr>
<tr>
<td>Fosamprenavir (GW-433908)</td>
<td>Stomach upset, diarrhea, nausea</td>
</tr>
<tr>
<td>Indinavir (IDV)</td>
<td>Kidney stones, vomiting, headache</td>
</tr>
<tr>
<td>Lopinavir (ABT-378)</td>
<td>Diarrhea, headache, fatigue</td>
</tr>
<tr>
<td>Nelfinavir (NFV)</td>
<td>Diarrhea, nausea, rash</td>
</tr>
<tr>
<td>Ritonavir (RTV)</td>
<td>Stomach upset, vomiting, taste disturbance</td>
</tr>
<tr>
<td>Saquinavir (SQV)</td>
<td>Stomach upset, headache, abdominal pain</td>
</tr>
<tr>
<td>Tripanavir (TPV)</td>
<td>Hypercholesterolemia, diarrhea, nausea</td>
</tr>
</tbody>
</table>

The multiple steps of the HIV replication cycle present novel therapeutic targets other than the viral enzyme RT and PT for drug development. Continued efforts have been made to discover new inhibitors that target not only RT and PT but also other viral targets, achievements that have been reviewed comprehensively in the literature. Several recent novel target inhibitors were discovered using virus-based screening approaches (Table 2). Alternatively, PIs, the next generation of NNRTIs, CCR5 antagonist and IN inhibitors were identified by structure-based drug design, receptor pharmacology and biochemical screening approaches (Westby et al., 2005, Menéndez-Arias & Tözser, 2008, Greene et al., 2008, Liang, 2008, Pang et al., 2009, Marchand et al., 2009, Tan et al., 2010). Historical precedent therefore suggests that diverse screening strategies should be employed for the discovery of new HIV-1 agents. In this review we present a brief overview of various HIV-1 screening strategies and highlight novel approaches and/or significant advances in HIV-1 screening technology.

2. HIV-1 Entry

As mentioned above, HIV cellular entry is a multistep process that requires the interaction of a viral envelope glycoprotein (gp120) and a host receptor (CD4), followed by binding to a coreceptor (CCR5 and CXCR4). The proteins involved in the entry process have become attractive targets for drug design, and HIV-1 replication screens have successfully identified compounds with antiviral activity that act at each of these three steps of HIV entry (Grande et al., 2008, Wang & Duan, 2009).

The chemokine receptors CCR5 and CXCR4, membrane proteins belonging to the G-protein coupled receptor super-family, have been identified as essential coreceptors for HIV entry into the cells, and molecules that inhibit HIV entry by targeting CCR5 and CXCR4 have been
### Attachment inhibitors
- CD4 binding peptides
- Aminoglycoside-arginine conjugates
- Poly-arginine aminoglycoside conjugates
- Cyclotriazadisulfonamide
- Sulfated polysaccharides

### CCR5 antagonists
- 3-(4-benzylpiperidin-1-yl)-N-phenylpropylamine derivatives
- 1-(3,3-diphenylpropyl)-piperidyl amides
- Ureas
- 1,3,4-trisubstituted pyrroloidines
- 1-amino-2-phenyl-4-(piperidin-1-yl) butane analogs
- Prostatin (12-deoxyphorbol ester)

### CXCR4 inhibitors
- Prostatin (12-deoxyphorbol ester)
- Bicyclams
- Non-cyclam polynitrogenated compounds
- Cyclic penta- and tetrapeptides
- Diketopiperazine mimetics
- Tetrahydroquinolines
- Thiazolylisothiourea derivatives
- Benzodiazepines
- Alkylamine analogs
- Non-peptide derivatives

### Fusion inhibitors
- Fusion inhibitors peptides
- Pyrrole derivatives

### Reverse transcriptase inhibitors
- Nevirapine analogs
- Efavirenz analogs
- Tetrahydroimidazo-[4,5,1-jk][1,4]-benzodiazepinone derivatives
- Tetrazole thioacetililide derivatives
- Calanolide A

### Integrase inhibitors
- β-diketo acids
- GS-9137
- Chalcones

### Protease inhibitors
- Cyclic urea derivatives
- Peptidomimetic protease inhibitors

| Table 2. Selected novel target inhibitors with potential application for the treatment of HIV infection in rapid development as antiviral agents. Additionally, the envelope glycoprotein gp120 exists in its native form as a homopolymeric trimer, held on the outer surface of the virion by non-covalent interactions with a fusion glycoprotein gp41 trimer. The crystal structure of gp120 core bound to CD4 reveals specific targets for developing anti-HIV drugs. |  
High-throughput screening technologies designed to identify compounds that inhibit binding of natural ligands to their cognate G-protein-coupled receptor have been used successfully by the pharmaceutical industry for many years. The disadvantage of this approach is the dependence upon a radiolabeled ligand, which involved a high cost and arouses significant environmental concern when screening large chemical libraries. It is therefore unlikely that radiolabeled ligand binding assays will be widely used in the future. More recently, assays have been developed which identify compounds that inhibit receptor function rather than ligand binding (and thus avoid the need for radiolabeled chemokines).

HIV is an enveloped virus, and its envelope proteins complex (Env) controls the key process of viral entry. Env is a complex composed of a transmembrane gp41 subunit and a noncovalently-associated surface gp120 subunit. Infection is initiated by the binding of the virion gp120 Env protein to the CD4 molecule present on some T-cells, macrophages and microglial cells. The interaction induces a conformational change that promotes secondary gp120 binding to the coreceptor CCR5 and CXCR4. Both coreceptors are members of the chemokine receptor family, but CCR5 is the coreceptor for HIV-1 strains that infect macrophages (M-tropic or R5 strains), while CXCR4 is the coreceptor for HIV-1 strains that infect T-cells (T-tropic or X4 strains). Ochsnerbauer-Jambor et al. (2006) introduced a T-cell based receptor reporter cell line (JLTRG-R5) that expresses both HIV-1 coreceptors, CXCR4 and CCR5, and offers the convenience of using enhanced green fluorescent protein (EGFP) as a direct and quantitative marker. Unlike previous EGFP-based reporter cell lines, JLTRG-R5 cells have an unusually high dynamic signal range, sufficient for plate reader detection using a 384-well format. Because EGFP can be directly and continuously quantified in cell culture, the reporter cell line requires no manipulation during assay preparation or analysis. These characteristics make the system extremely flexible, rapid and inexpensive. Due to its intrinsic flexibility, the JLTRG-R5 cell-based reporter system provides a powerful tool which will considerably facilitate future screening for HIV inhibitors.

Immortalized cell lines, transfected with the HIV-1 Env gene, express gp120/gp41 on their surface and can fuse to cells co-expressing CD4 and either CCR5 and CXCR4. Screens based on this approach have been described by a number of laboratories. A cell-based enzyme-linked immunosorbent assay (ELISA) was developed using and anti-CXCR4 monoclonal antibody, 12G5, and cells expressing CD4 and CXCR5, the U373-MAGI-CXCR4 (CEM) cell line (Zhao et al., 2003). The assay was sensitive to the well-characterized CXCR4 antagonists, T22, T14012 (a downsized analog of T22) and AMD3100, which effectively inhibited 12G5 binding to CXCR4-expressing cells whereas HIV-1 entry inhibitors targeting CD4 and gp41 in addition to HIV-1 RT and PT inhibitors, did not block the binding of 12G5 to U373-MAGI-CXCR4 (CEM) cells. This suggests that the cell-based ELISA is specific, sensitive, convenient, rapid and economical.

More recently, two new T-cell-based reporter cell lines were established to measure HIV-1 infectivity (Chilba-Mizutani et al., 2007). One cell naturally expresses CD4 and CXCR4, making it susceptible to X4-tropic viruses, and the other cell line, in which a CCR5 expression vector was introduced, is susceptible to both X4- and R5-tropic viruses. Reporter cells were constructed by transfecting the human T-cell line HPB-Ma, which demonstrated high susceptibility to HIV-1, with genomes expressing two different luciferase reporters: HIV-1 long terminal repeat (LTR)-driven firefly luciferase and cytomegalovirus promoter-driven renilla luciferase. The cell lines were also beneficial for screening new antiretroviral agents, as false inhibition caused by the cytotoxicity of test compounds was easily detected by monitoring renilla luciferase activity.
3. HIV-1 enzyme targets

HIV-1 encodes three enzymes required for replication: HIV-1 RT, HIV-1 IN and HIV-1 PT. A number of assays have been developed for screening test compounds against these well-known targets for drug discovery. Utilization in screening campaigns of RT or PT enzymes that contain drug-resistant mutations is a common strategy for identifying next-generation HIV-1 inhibitors against these targets.

HIV-1 RT is a multifunctional enzyme involved in several essential activities for viral replication (Sarafianos et al., 2009, Herschhorn & Hiz, 2010). These activities include DNA- and RNA-dependent DNA polymerase, ribonuclease H (RNase H), strand transfer and strand displacement activities. RT has been the main target of current antiviral therapies against AIDS. NRTIs have been widely used in HAART, combined with PIs and/or NNRTIs. The high error rates characteristic of HIV-1 RT, however, are a presumed source of the viral hypermutability that contributes mainly to the emergence of resistant variants, although the significant toxicity associated with current anti-HIV drugs also results in treatment failure. These factors in combination drive pharmacologists to develop more potent and less toxic RT inhibitors against the native and drug-resistant variants, which will most certainly remain critical components of future drug regimens.

Although currently marketed agents inhibit the DNA polymerase activity of HIV-1 RT, inhibition of any of the step in the reverse transcription process would result in inhibition of viral replication. Therefore various assays suitable for testing compounds in a high-throughput screening format have been described for measuring the DNA polymerase, RNase H and DNA strand transfer activities of HIV-1 RT.

Examples of isotopic assays for measuring DNA polymerase activity include “microarray compound screening technology”, and “scintillation proximity assay technology” (Xuei et al., 2003). Inhibition reverse transcription by targeting the RNase H activity of HIV-1 RT is another approach of interest, since mutations in the NNRTI allosteric domain or the RT active site are not expected to affect inhibitors that bind to the RNase H domain. Although RNase H-mediated cleavage of hybrid RNA/DNA duplex occurs either concurrently with DNA polymerization or independently, most RNase H assays target the latter. Parniak et al. (2003) described a homogeneous “fluorescence resonance energy transfer” (FRET) assay for measuring RNase H activity. The duplex substrate contains a fluorescein label on the 3'-end of the RNA, which is quenched by a Dabcyl label on the 5'-end of the DNA strand. When the substrate is cleaved by RNase H, the interaction between the fluorescein and Dabcyl is removed, resulting in an increase in the fluorescence signal.

A fluorescence polarization (FP) microplate assay for screening compounds against the RNase H activity of HIV-1 RT has also been developed (Nakayama et al., 2006). This homogeneous assay uses a hybrid 18-mer DNA/RNA duplex substrate composed of an RNA oligonucleotide labelled with 6-carboxytetramethyl rhodamine at the 3'-end, that is annealed to a complementary unlabeled DNA strand substrate cleavage by RNase H to produce small RNA fragments (1-4 mer), resulting in a significant change in the measured FP value. More recently, a 6-phenylpyrrolocytidine (PhpC)-based assay has been incorporated into high-throughput microplate assay format, and may form the basis for a new screen for inhibitors of HIV-1 RNase H (Wahba et al., 2010). The PhpC-containing RNA formed native-like duplex structures with complementary DNA or RNA. The PhpC-modification was found to act as a sensitive reporter group, and was non-disruptive to structure and the enzymatic activity of RNase H. A RNA/DNA hybrid possessing a single PhpC insert was an excellent substrate for
HIV-1 RT RNase, and rapidly reported cleavage of the RNA strand with a 14-fold increase in fluorescence intensity. The PhpC-based assay for RNase H was superior to the traditional molecular beacon approach in terms of responsiveness, speed and ease.

HIV-IN represents a potential target for the development of new anti-HIV chemotherapeutic agents. This viral enzyme is required for the integration of viral DNA into the host DNA, which catalyzes two reactions known as processing and strand transfer. The viral DNA is first cleaved by HIV IN at a CA dinucleotide at the 3’-end to leave the two-nucleotide overhanging. This step is known as processing. Then, the protein-DNA complex is transported into the nucleus. The host DNA is cleaved to leave a 5’ overhang of five bases, and the 3’-ends of the viral DNA are covalently linked to the 5’-end of the host DNA. Finally, the 5-bases gap between the 5’-end of the viral DNA and the 3’-end of the host DNA is filled in by host cell enzymes. Since IN-negative mutants of HIV do not produce infectious virus particles, and no cellular homologue of HIV IN has been described, IN is considered to be an attractive target. However, in contrast to RT and PT, not a single IN inhibitor has yet entered the anti-HIV drug market. However, using in vitro assay systems and the recombinant HIV-1 IN, a variety of HIV IN inhibitors have been identified.

Most currently used assays for HIV-1 IN target the strand transfer process and follow a similar premise. HIV IN is combined with donor dsDNA, which has been immobilized onto a solid support, to form an enzyme/DNA complex. The reaction is then initiated by the addition of target dsDNA labelled in some manner, and after an incubation period, the ligated product is quantified. John et al. (2005) reported a highly efficient and sensitive high-throughput screen, HIV IN Target SRI Assay for HIV-1 IN activity, using 5’ biotin-labelled DNA (5’ BIO donor) and 3’ digoxygenin-labelled DNA (3’ DIG target). Following 3’ processing of the 5’ BIO donor, strand transfer proceeds with integration of the 5’ BIO donor into the 3’ DIG target. The assay was used to screen drugs in a high-throughput format, and the assay was also adapted to study mechanistic questions regarding the integration process. For example, using variations of the assay format, it showed a high preference of the E strand of the LTR viral DNA as a target strand compared with its complementary A strand. Wang et al. (2005) described two homogeneous time-resolved FRET-based assays for the measurement of HIV-1 IN 3’-processing and strand transfer activities. These assays have also proven their utility for the identification of mechanistically interesting and biologically active inhibitors of HIV-1 IN that hold potential for further development into potential antiviral drugs.

In addition to recombinant enzyme screens, biochemical assays have been developed that measure HIV-1 IN activity in the context of the preintegration complex (PIC), which mediates the integration of the retroviral genome into host cell DNA. The HIV PIC is a large nucleoprotein complex containing the viral CDNA and IN as well as matrix Vpr, RT and a number of host proteins including histones and members of the non-homologous end joining pathway. It is possible that screening for PIC activity, analogous to that in a true infection, may offer an expanded set of targets and yield more biologically relevant compounds. A polymerase chain reaction-based assay for integration has been reported which employs HIV-1 PICs derived from cells infected with single-cycle HIV-1 reporter viruses.

4. HIV-1 protease

In a later stage of the HIV-1 life cycle, HIV PT hydrolyzes precursor polyproteins into functional proteins that are essential for viral assembly and subsequent activity. HIV-1 Gag and Pol polypeptide precursors are cleaved by the viral encoded aspartyl protease to form
the mature structural and enzymatic gene products. During virus assembly, the viral Gag polyprotein must be effectively processed and transported to the cell membrane. Cofactors such as the phospholipid phosphatidylinositol (4,5) biphosphate, the ADP ribosylation factor binding proteins or tumour susceptibility gene 101, are required for the intracellular transport and budding of HIV particles. While these are just a few examples of virus-host cell interactions, each one represents a potential new target under rigorous research with their validation being actively pursued.

The functional structure of HIV-1 PT is a homodimer containing an active site created in the cleft between the monomers as part of a four-stranded \( \beta \) turn. The active site region is capped by two identical \( \beta \)-hairpin loops (the flaps, residues 45-55 in each monomer), which undergo significant conformational changes upon substrate binding. All PIs currently licensed for the treatment of HIV infections mimic the substrate and block the active site. Another strategy is to develop compounds that bind to the subunit interface and thus block dimerization. As a result, drug discovery efforts continue to focus on the identification of new inhibitors against this validated target that are active against HIV-1 variants which are resistant to the currently available HIV-1 PIs. In line with these efforts, the assays described here may be conducted with wild-type proteins or variants that contain mutations conferring resistance to current HIV-1 PIs.

FRET assays are more commonly used for HIV-1 PT. Synthetic peptide substrates typically consist of a cleavage sequence flanked with fluorescent donor and acceptor labels. The fluorescence signal is low in the intact peptide because the donor is quenched by the nearby acceptor. Once the substrate is cleaved by HIV-1 PT, the FRET interaction is removed, and the fluorescence increases. Hamilton et al. (2003) described a biochemical detection method for peptide products of enzymatic reactions, based on the formation of PSD95/Disc-large/ZO-1 (PDZ) domain* peptide ligand complexes. The product sensor involves using masked or cryptic PDZ domain peptide ligands as enzyme substrates. The practical applicability of this PDZ-based detection method is determined by the affinity of the PDZ* peptide ligand interaction, and the efficiency of the enzyme to process the masked peptide ligand. These results showed that the Na\(^+\)/H\(^+\) exchanger regulatory factor, which binds to the consensus sequence Thr/Ser-X-Leu-COOH, can be used to extend the flexibility in the recognition of the carboxy-terminal amino acid of the ligand, and monitor the enzymatic activity of HIV PT.

In addition to enzyme assays, a number of cell-based assays have been reported for HIV-1 PT. A green fluorescent protein (GFP)-PT chimera was developed that can be expressed in mammalian cells, causing minimal toxicity until autocatalytic cleavage occurs (Lindsten et al., 2001). The precursor is activated in vivo by autocatalytic cleavage, resulting in rapid elimination of PT-expressing cells. Treatment with therapeutic doses of HIV-1 PIs results in a dose-dependent accumulation of the fluorescent precursor that can be easily detected and quantified by flow cytometric and fluorimetric assays. More recently, Majerova-Uhlikova et al. (2006) described a new assay that might serve as a non-infectious, rapid, cheap and reliable alternative to the currently used phenotypic assays. These investigations showed that in the GFP-PT reporter, the HIV wild-type PT can be replaced by a drug-resistant HIV PT mutant, yielding a simple and biologically relevant tool for the quantitative analysis of drug-resistant HIV PT mutant susceptibility to HIV PIs.

5. HIV-1 replication screens

Although biochemical high-throughput screening and structure-based drug design approaches are currently preferred over holistic approaches, HIV-1 replication screens have
historically been used to identify antiviral compounds. HIV-1 replication assays offer the advantage of screening for multiple targets in the context of a natural infection. As the methodology used in the determination of the antiviral activity and the interpretation of the results have been virtually specific to each laboratory and are thus not comparable to one another, simple procedures and guidelines for evaluating antiviral and/or virucidal activities of compounds are needed. Various cell culture-based assays are currently available and can be successfully applied for the antiviral or virucidal determination of substances. Antiviral agents interfere with one or more dynamic processes during virus biosynthesis, making them candidates for clinically useful antiviral drugs; whereas virucidal substances inactivate virus infectivity extracellularly and are therefore better candidates for antiseptics, exhibiting a broad spectrum of germicidal activities. Cost, simplicity, accuracy and reproducibility are the key factors determining the selection of the assay system, but selectivity, specificity and sensitivity also need to be taken into account. The methods commonly used for evaluation of in vitro antiviral activities are based on the different abilities of viruses to replicate in cultured cells. Some viruses can cause cytopathic effect (CPE) or form plaques. Others are capable of producing specialized functions or cell transformation. Virus replications in cell culture may also be monitored by the detection of viral products such as viral DNA, RNA or polypeptides. Thus, the antiviral test selected may be based on inhibition of CPE, reduction or inhibition of plaque formation. Several different HIV-1 replication assays have been described that could be adapted for medium-to-high-throughput screening. Such assays can generally be subdivided into one of three categories: reporter virus assays, reporter cell assays or cell protection assays.

In reporter virus assays, a reporter gene is introduced into the virus genome, usually in place of a viral gene not required for replication, in the target cells of interest. The concept of using HIV-1 reporter viruses to monitor HIV-1 replication was first introduced using a replication competent HIV-1 reporter virus containing the chloramphenicol acetyltransferase gene in place of HIV-1 Nef sequences. Cells are then infected with the recombinant reporter virus and virus replication is quantified by measuring the expression of the virally encoded reporter gene (Adelson et al., 2003; Dey & Berger, 2003).

For reporter cell assays, the target cells of interest are engineered to contain a reporter gene, which is activated upon viral infection. Virus replication is measured by monitoring induction of the reporter gene in the infected target cells. These assays have been used for some time to monitor HIV-1 infection and measure the activity of HIV-1 inhibitors. Kremb et al. (2010) presented a full HIV-replication system for the identification and analysis of HIV inhibitors. This technology is based on adherently growing HIV-susceptible cells, with a stable fluorescent reporter gene activated by HIV Tat and Rev. A fluorescence-based assay was designed to measure HIV infection through two parameters relating to the early and the late phases of HIV replication respectively. These results concluded that this technology is a versatile tool for the discovery and characterization of HIV inhibitors. Reporter cell assays have also been adapted to allow analysis of CCR5 as well as CXCR4 tropic HIV strains (Miyake et al., 2003).

In cell protection assays, CPE resulting from virus replication are measured by determining cell viability using a dye reduction method. These assays represent a more conventional approach to antiviral screening and have been used successfully to execute antiviral screens and identify new HIV-1 inhibitors. Although cell protection assay formats have been available for some time, they continue to be the cornerstone of many HIV-1 drug discovery programs.
6. Acknowledgements

The technical assistance of Ms. Brooke-Turner is gratefully acknowledged.

7. References


The collective efforts of HIV/AIDS research scientists from over 16 countries in the world are included in the book. This 27-chapter Open Access book well covers HIV/AIDS translational researches on pathogenesis, diagnosis, treatment, prevention, and also those beyond conventional fields. These are by no means inclusive, but they do offer a good foundation for the development of clinical patient care. The translational model forms the basis for progressing HIV/AIDS clinical research. When linked to the care of the patients, translational researches should result in a direct benefit for HIV/AIDS patients.

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