Integrase strand transfer inhibitors as potential anti-HIV agent

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Abstract

Rigorous research over the years has produced a number of potential drugs that target various stages of the human immunodeficiency virus type I (HIV-I) life cycle. Despite remarkable success of anti HIV drug discovery, resistance and cross resistance posses major hurdles and needs attention. The last two decades of research to target virally encoded enzyme integrase, has yielded only one FDA approved drug raltegravir. This slow drug development for integrase may attribute due to its poor 3D structural and catalytic information. This review focus on the perspectives of integrase strand transfer inhibition related to structural and medicinal chemistry approaches as anti HIV target.

Keywords: AIDS, HIV, Raltegravir, Elvitegravir, HIV integrase, Integrase Strand Transfer Inhibitor.

Introduction

Over the past 30 years, the AIDS epidemic has spurred research efforts into successful discovery of a number of effective and clinically efficacious therapeutic agents (Mehellou and De Clercq, 2010). The common antiretroviral drugs target viral entry (co-receptor antagonists and fusion inhibitors), reverse transcription (RT; nucleoside and non-nucleoside inhibitors of the viral reverse transcriptase), integration (IN: integrase inhibitors) and viral maturation (PR: protease inhibitors). The highly active antiretroviral therapy (HAART) is currently in use as standard therapeutic approach, which include either one or two nucleoside RT inhibitors, one non-nucleoside RT inhibitor and/or one protease inhibitor. The approval of newer drug raltegravir as integrase strand transfer inhibitor (INSTI) significantly add the potency of HAART regimens by targeting three viral enzymes: RT, PR and IN (Marchand et al., 2009). The emergence of viral drug resistance posses major threat and open an avenue to discover newer therapeutic approach (Jiang et al., 2011) including new drug candidate (Tang et al., 2011) with novel mechanism (Al-Mawsawi and Neamati, 2011a).

The recent drug raltegravir, developed by Merck and the clinical molecule elvitegravir (phase III), reveals success in the area of viral integrase as target (Al-Mawsawi and Neamati, 2011a). Integrase (IN) is 32-kDa 288-amino acid protein released from the polyprotein by the HIV protease during maturation. The IN enzyme consists mainly three structural domains: amino-terminal domain (NTD), the catalytic core domain (CCD) and the carboxy-terminal domain (CTD) (Chiu and Davies, 2004). The second generation potential molecule S/GSK1349572 (3) (Min et al., 2010) and MK-2048 (4) is distinct from the first-generation (1 and 2) integrase strand transfer inhibitors (INSTIs) and possess a higher genetic barrier to the development of resistance (Serrao et al., 2009; Hadi et al., 2010; Kobayashi et al., 2011). However the never ending problem related to toxicity and antiviral drug resistance (Goethals et al., 2010; Metifiot et al., 2010) enforces to arm HIV research for the search of new candidate with novel mode of action. This opens an avenue towards the detail understanding for structural and medicinal chemistry aspects of INSTIs.

Clinically Significant INSTIs

Raltegravir (1) is the first and still the only FDA approved INSTI. It is a drug with high potency and good pharmacokinetics (Pace and Rowley, 2008). Elvitegravir (2) is the next in clinical development. Elvitegravir like raltegravir, is a potent antiviral (Al-Mawsawi et al., 2008; Iwamoto et al., 2008; Pace and Rowley, 2008) but exhibits a potential higher toxicity in non-infected cells (Sato et al., 2006). The limitation associated with elvitegravir is that unlike raltegravir, it is metabolized by cytochrome P450 (CYP3A4/5), and secondarily by glucuronidation (UGT1A1/3) to produce

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metabolites that are less potent than the parent drug (Pace and Rowley 2008). The clinical status of potential integrase inhibitor has been summarized in table 1.

![Table 1: Status of important clinical molecules as integrase strand transfer inhibitors (Kobayashi, Yoshinaga et. al., 2011).](image)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>IC_{50} values(nM)</th>
<th>Limitation</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raltegravir (1)</td>
<td>3.3</td>
<td>Resistance developed</td>
<td>FDA Approved (2007)</td>
</tr>
<tr>
<td>Elvitegravir (2)</td>
<td>6</td>
<td>High toxicity in non-infected cells</td>
<td>Phase III</td>
</tr>
<tr>
<td>S/GSK1349572 (3)</td>
<td>2.7</td>
<td>Significant toxicity not observed</td>
<td>Phase IIb</td>
</tr>
<tr>
<td>MK-2048 (4)</td>
<td>7</td>
<td>Significant toxicity not observed</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

Fig. 1: The chemical structure of potential INSTIs used in this work. (Serrao, Odde et al., 2009) The metal chelating heteroatoms are colored red and the halogenated benzene groups are green. These advanced HIV integrase inhibitors raltegravir (MK-0518), elvitegravir (GS-9137), GSK572, and MK2048 illustrating coplanar two-metal chelation motifs in red.

**Diketo Acid Derivatives as potential INSTIs**

Diketoacids (DKA) were the first selective INSTIs reported (Figure 2). Historically, the two first reported molecules are 5CITEP (5) from Shinogi and Co. Ltd and the L-731,988 (6) from Merck and Co. (Hazuda et al., 2000; Grobler et al., 2002; Pais et al., 2002). The DKA derivatives selectively inhibit strand transfer (ST) at nanomolar IC_{50} and inhibit integration without interfering with viral DNA synthesis (Marchand et al., 2003).

This inhibition is mediated through metal chelation (Marchand et al., 2003). L-870,810 (7) is a naphthydrine carboxamide derivative derived from the DKA family (Hazuda et al., 2004). L-870,810 is a potent ST inhibitor with improved bioavailability compare to previously reported DKA (Hazuda et al., 2004). L-870,810 inhibits the integrase strand transfer (INST) activity in vitro with an IC_{50} value of 8 nM. During clinical trial it was terminated in Phase II due to toxicity found in dogs (Semenova et al., 2006). The L-731,988 is inhibits the IN ST activity in vitro with an IC_{50} value of 50 nM (Hazuda, Felock et al., 2000). The IC_{50} value of GS-9160 (8) is found to be 28 nM in vitro (Jones et al., 2009).
The first reported natural product INSTIs were Caffeic acid phenyl ethyl ester 11 (CAPE, Fig. 3) obtained from bee hives. They act selectively over 3’P with IC_{50} values of 5 µM (Fesen et al., 1993; Fesen et al., 1994). L-chicoric acid 12 (L-CA, Fig. 3) is a related bidendate cathechol with micromolar concentrations through a noncompetitive reversible mechanism both in vitro and ex vivo (Fesen et al., 1994). These two molecules have served as parent structures for the identification of new molecules with improved properties. The Caffeic acid derivative 13 (Fig. 3) is active at micro molar concentrations (Reinke et al., 2002). The Chichoric acid derivative 14 (Fig. 3) represents a potent inhibitor of IN with an IC_{50} values of 3.8 µM (Lee et al., 2003) while the Caffeoylamino acid derivative 15 (Fig. 3) has IC_{50} of 12 µM. A second set of compounds have been also designed based on the L-CA and dicaffeoyl derivatives (Lee, Yoon et al., 2003; Sechi et al., 2004). These molecules contain a dicaffeoyl group connected by a furan ring linker containing hydrogen bond accepting or donating groups and exhibit IC_{50} of 0.5-12 µM.

Fig. 2: The chemical structures of potential Diketo Acid Derivatives.

L-Chichoric and Caffeic Acid Derivatives

Fig. 3: The chemical structures of potential L-Chichoric and Caffeic Acid Derivatives
These groups of compounds are potent INSTIs but do not display selectivity for 3’P or ST (Zhao et. al., 2008) and inhibition is observed both with magnesium and manganese as cofactors. Rather it was found that the dihydroisoindoline derivative is somewhat more selective for ST. To improve potency and reduce toxicity the aromatic ring was halogenated and the 3-chloro-4-floro-derivative (16) was the most potent inhibitor with an IC$_{50}$ for ST of 0.016 µM (Zhao et. al., 2009).

The dihydroxynapthyl derivative 17 (Fig. 4) with catechol-DKA hybrid is active at low micro molar concentrations with two-three fold decrease in selectivity for ST compared to DKA (Maurin et. al., 2006). Dinitrorosamic acid 18 (Fig. 4) is very potent with a high selectivity for ST (Mekouar et. al., 1998). The IC$_{50}$ for 3’P and ST are 52 µM and 70 nM, respectively. The coumarin caffeoyl derivative 19 (Fig. 4) exhibits potent inhibitory activity at micro molar concentration in vitro by shifting its oligomerization state (Maroun et. al., 2006). Another catechol derivative dihydroxyindole dimeric with piperazine 20 (Fig. 4) has shown IC$_{50}$ value at 7 µM (Al-Mawsawi et. al., 2006).

Fig 4: The chemical structures of potential Cathechol Derivatives

Discussion

Molecular Mechanism of Integrase (IN) in Viral Replication

HIV-1 integration encompasses a series of molecular events. The viral core is released into the cytoplasm of host cell after the binding of HIV-1 viral particle to CD-4 and CCR5 co-receptor (Delelis et. al., 2008). Now within 1–2 h the viral RNA is processed by HIV-1 reverse transcriptase and leads to the production of proviral cDNA. In the first step of integration process (Delelis et. al., 2008) (Fig. 5), two nucleotides are removed from 3’ end of the proviral DNA by SN$_2$ mechanism through 3’ processing (3’P). In this step water molecule serves as a nucleophile for the cleaving of the terminal dinucleotide GT. This process generates a preintegration complex (PIC) (Chiu and Davies, 2004; Pommier et. al., 2005; Jaskolski et. al., 2009). In the second step the PIC migrates into host nucleus where the 3’P processed end of proviral DNA are inserted into the target host DNA through ST. This process consists of ligation of the two 3’P hydroxyl ends into the host DNA with a five base pair stagger across the major groove. The final processes of integration is the repair of junctions between the viral and host DNA, probably by the cellular proteins known as lens epithelium derived growth factor (LEDG) leading to integrated HIV-1 provirus. The provirus is silent until triggering of DNA transcription, which is then followed by viral RNA translation, maturation, packaging leading to new viral particles.
Integrate Structure

HIV-1 IN (Fig. 6) is a 32 kDa enzyme of 288 amino acid residues that comprises three structural domains, amino terminal domain (NTD), the catalytic core domain (CCD) and the carboxy terminal domain (CTD) (Jaskolski et al., 2009). The NTD consists of amino acids 1-49 and a zinc binding motif to involve in the oligomerization of IN. The CCD contains amino acids 50-212 and harbors the catalytic DDE motif (D<sub>64</sub>D<sub>116</sub>E<sub>152</sub>) (Chiu and Davies, 2004). This triad (DDE) coordinates with two metal co-factors for DNA binding. The CTD contains amino acids 213-288 and carries a SH3 (SRC – homology -3-domain) domain implicated in DNA binding (Delelis et al., 2008).

Structural perspective of IN for the development of anti-HIV agent

Among the several HIV-integrase models published, the crystal structure of prototype foamy virus (PFV) IN complexed with INSTIs (Hare et al., 2010a; Hare et al., 2010b) reveals significant information. However, it further requires translation of structural information into an original HIV-I integrase in place of PFV IN. Therefore, we intended to develop a valid 3D catalytic model of HIV-I integrase based on crystal structure of PFV IN. The developed model with raltegravir has been shown in Fig. 7 to demonstrate the recognition phenomenon between raltegravir and the active site of HIV integrase. The overall 3D catalytic integrase model (Fig. 7), demonstrate the highly conserved regions surrounding the DDE motif near to two metal ion interacts with INSTIs using di-keto pharmacophore (Fig. 1: shown in red color). Further the presence of DNA cofactors along with two metal-ion, virtually mimics the presence of viral DNA (vDNA) in the active site.

Fig. 5: The different reactions during integration;
Step 1: IN catalyzes the cleavage of dinucleotide GT at both 3’ extremities of the viral DNA (red) by 3’P leading to PIC,
Step 2 After nuclear import of PIC, the strand transfer (ST) reaction leads to the integration of the viral DNA with cellular DNA (black).

Fig. 6: Schematic diagram of the HIV-1 integrase protein highlighting NTD (red), CTD (yellow) and CCD (green) domain.

Fig. 7: The binding mode of raltegravir in the active site of HIV-1 integrase showing two metal ion associations with diketo pharmacophore.

From the integration mechanism, it seems 3’P step and strand transfer steps can be potential targets for HIV integrase inhibition. But, as the 3’P step is kinetically favored due to involvement of only viral in-house complex it is obviously difficult to bring about any inhibition at this step (Fig. 5, PIC). Thus the strand transfer (Fig. 5) is considered as rational target, which requires the involvement of vDNA and host DNA (hDNA) along with metal cofactors. The observed mechanism of metal-chelating functions associated with vDNA/hDNA has been exploited significantly for the rational design of INSTI and the successful inhibitors belongs to this class (INSTIs) (Prada and Markowitz, 2010; Kobayashi, Yoshinaga et al., 2011; Lenz and Rockstroh, 2011). The discussed 3D-strucutural perspectives (Fig. 7) along with ligand based pharmacophoric discussion (Fig. 1) opens an avenue for future anti-viral drug development.
Conclusions

The progresses of IN inhibitors search are in part due to our progress in the knowledge on integrase and development of more robust and effective therapies. Molecules that inhibit IN on the micromolar range are numerous but there is not a great chemical diversity for the much closed group of molecules active at nanomolar level. Therefore new original structures are warranted to continue to feed the pipeline of drug candidates. The successful development of integrase inhibitors would target an additional viral component in addition to the actually available antiretroviral combination therapy and will provide a new treatment option in the field of AIDS therapy.

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