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Mini-review

Recent status of HIV-1 gene expression inhibitors

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Human immunodeficiency virus type 1 (HIV-1) gene expression and transcription is a crucial step in the viral replication cycle, which is considered to be a potential target for inhibition of HIV-1. Among the factors involved in this step, the cellular protein nuclear factor (NF)-κB is the most powerful inducer of HIV-1 gene expression. On the other hand, the viral protein Tat plays a central role in sustaining a high level of HIV-1 replication. Several compounds have been reported to selectively inhibit the functions of Tat and NF-κB. Tat inhibitors target either the Tat/TAR RNA interaction or the Tat cofactor cyclin-dependent kinase 9/cyclin T1. Antioxidants, protein kinase C inhibitors, and IκB kinase inhibitors are known to suppress the activation of NF-κB. Although some of the compounds inhibit HIV-1 replication in cell cultures at low concentrations, they also have considerable toxicity to the host cells. Considering the increase of treatment failure cases in highly active antiretroviral therapy due to the emergence of multidrug resistance, HIV-1 gene expression inhibitors should be extensively studied as alternative approach to effective anti-HIV-1 chemotherapy.

Keywords: HIV-1; Chemotherapy; Transcription; Tat; NF-κB

Contents

1. Introduction

The establishment of highly active antiretroviral therapy (HAART) with reverse transcriptase and protease inhibitors has dramatically altered the prognosis of human immunodeficiency virus type 1 (HIV-1) infection (Yeni et al., 2002; Struble et al., 2005). In fact, more than 20 drugs, including the viral entry inhibitor enfuvirtide, are available in clinic for the treatment of HIV-1 infection. Since reverse transcriptase and protease inhibitors target viral-specific enzymes, emergence of drug-resistant viruses caused by amino acids mutations of the

Abbreviations: HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; TAR, transactivation response; P-TEFb, positive transcription elongation factor b; CycT1, cyclin T1; CDK9, cyclin-dependent kinase 9; CTD, carboxy-terminal domain; PBMC, peripheral blood mononuclear cell; NF-κB, nuclear factor κB; TNF, tumor necrosis factor; IL, interleukin; LTR, long terminal repeat; PKC, protein kinase C; NADA, *N*-arachidonoyldopamine; MAP, mitogen-activated protein

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enzymes often results in treatment failure with existing antiretrovirals (Hirsch et al., 2003). Furthermore, there are few treatment options in case of the failure because of cross-resistance to the same class of compounds (Johnson et al., 2005). Therefore, tremendous efforts have been made to identify and develop novel antiretroviral agents with different targets for inhibition of HIV-1 replication.

In the HIV-1 replication cycle, transcription from the integrated proviral DNA is considered to be a crucial step for viral replication, unless the DNA remains latent. Gene expression from proviral DNA is regulated by two viral proteins, Tat and Rev, as well as several known and unknown host-cellular factors (Karn, 1999; Baba, 2004). Therefore, it appears that molecules associated with the transcription process are potential targets for inhibition of HIV-1 replication. I have previously reviewed the molecular mechanisms of HIV-1 gene expression, viral and cellular factors involved in this process, and potential inhibitors (Baba, 2004). In this paper, I will provide updated information of HIV-1 gene expression inhibitors.

2. Tat inhibitors

The viral protein Tat stimulates transcriptional elongation through its interaction with the transactivation response (TAR) RNA structure (Fig. 1). The TAR RNA is a hairpin loop structure in the proximal portion of the nascent viral RNA transcript. Tat also interacts with cellular cofactors. One of the cofactors is positive transcription elongation factor b (P-TEFb), a complex composed of cyclin T1 (CycT1) and cyclin-dependent kinase 9 (CDK9) (Peng et al., 1998; Wei et al., 1998; Price, 2000). CDK9 hyperphosphorylates the carboxy-terminal domain (CTD) of RNA polymerase II and induces efficient promoter clearance and transcriptional elongation. Furthermore, CycT1 remodels the structure of Tat to enhance its affinity for the TAR RNA, and the TAR RNA further enhances the interaction between Tat and CycT1 (Zhang et al., 2000).

Since Tat is crucial for viral replication, Tat has been considered to be one of the best targets for inhibition of HIV-1 transcription. In fact, several attempts, including a gene therapy approach, have been made to block the Tat/TAR RNA interaction. The peptidomimetic compound of the Tat basic domain ALX40-4C (O'Brien et al., 1996), the hybrid peptoid/peptide oligomer CGP64222 (Hamy et al., 1997), and amino-glycoside–arginine conjugates (Litovchick et al., 2000) were previously reported to block the Tat/TAR interaction. However, their anti-HIV-1 activities were found to be primarily attributed to the inhibition of viral entry via the chemokine receptor CXCR4 (Doranz et al., 1997; Daelemans et al., 2000; Cabrera et al., 2000).

Recently, approximately 39,000 small molecules were screened for their binding to the TAR RNA. After optimization of chemical structures, a set of molecules were identified as effective inhibitors of HIV-1 replication by disrupting the Tat/TAR interactions (Hwang et al., 2003). The representative compound TR87 achieved sustained suppression of HIV-1 replication in cell cultures over 24 days at a concentration of 5 μM. The fluoroquinoline derivative K-37 is a potent and selective inhibitor of HIV-1 replication in both acutely and chronically

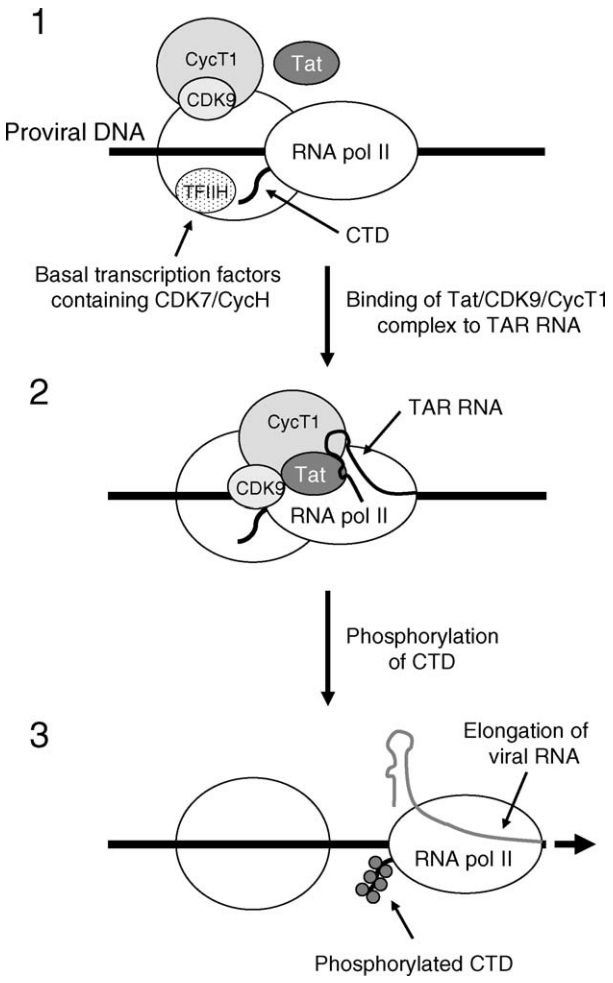

Fig. 1. Mechanism of HIV-1 transcriptional elongation by Tat. (1) HIV-1 mRNA synthesis from proviral DNA is initiated by basal transcription factors containing CDK7/CycH (TFIIH). (2) After synthesis of the TAR RNA, HIV-1 Tat interacts with CDK9/CycT1 by the transactivation domain, and this interaction highly enhances the affinity and specificity of the binding of Tat to the bulge region of the TAR RNA. (3) Consequently, the CTD of RNA polymerase II can be hyperphosphorylated, leading to efficient elongation of viral mRNA.

infected cells at nanomolar concentrations (Baba et al., 1998). K-37 could inhibit Tat-dependent transactivation and was not an inhibitor of CDK9 (Okamoto et al., 2000). Although its target molecule remains to be elucidated, the aminoquinolone WM5, which is structurally related to K-37, proved to interact with the bulge region of the TAR (Parolin et al., 2003; Richter et al., 2004).

As mentioned above, hyperphosphorylation of the CTD of RNA polymerase II by the Tat cofactor CDK9/CycT1 is necessary for transcriptional elongation. Thus, CDK9/CycT1 inhibitors are also assumed to suppress Tat functions. Although several compounds, such as 5,6-dichloro-1-β-D-robofuranosylbenzimidazole (DRB) and its derivatives, were found to be active against this process, they were not selective enough (Mancebo et al., 1997; Nekhai et al., 1997). Flavopiridol (Fig. 2) is a CDK inhibitor and has been in clinical trials as an anticancer agent because of its antiproliferative properties (Zhai et al., 2002). The compound inhibited HIV-1 replication

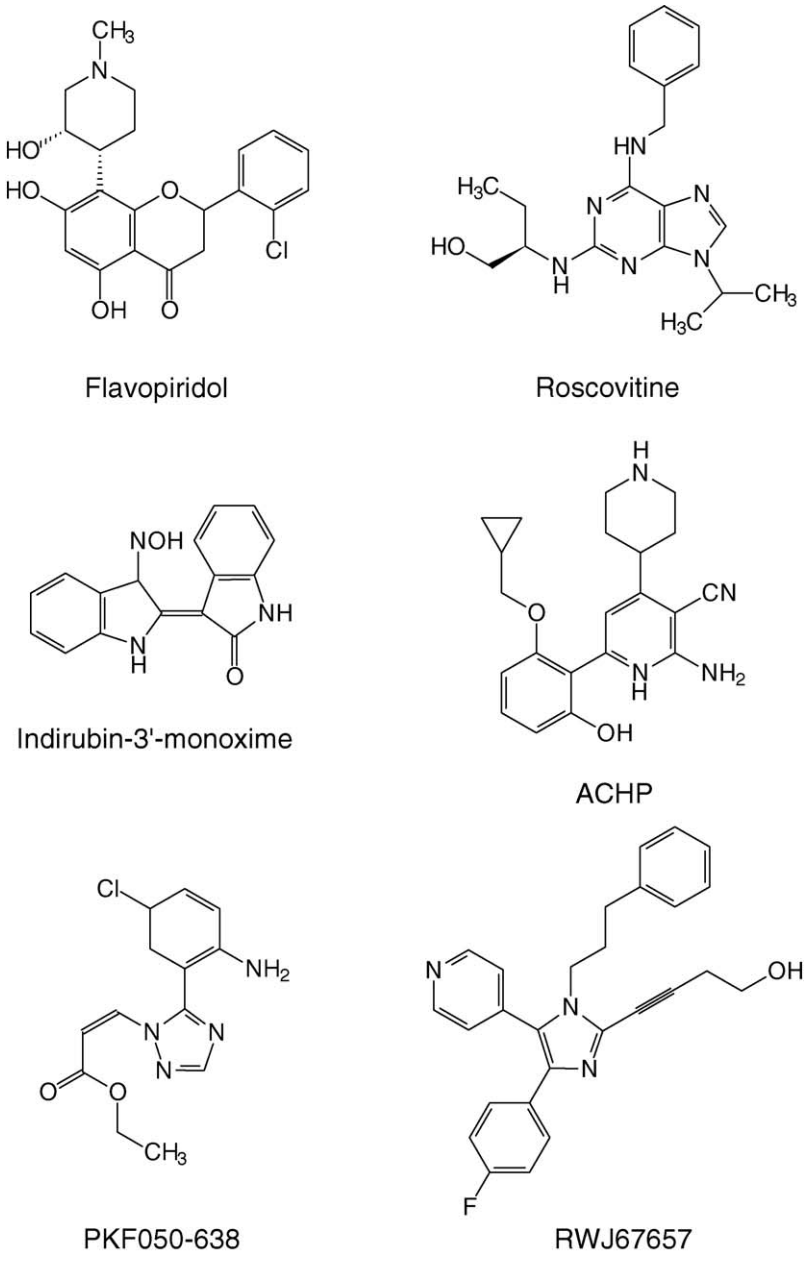

Fig. 2. Chemical structures of HIV-1 transcription inhibitors recently identified.

in cell cultures at a concentration less than 10 nM. However, according to the results in phase II clinical trials against cancers, development of this compound as an anti-HIV-1 agent does not seem to be feasible because of its side effects (Stadler et al., 2000; Schwartz et al., 2001). Roscovitine (Fig. 2), another CDK inhibitor, almost completely blocked Tat-induced HIV-1 gene expression in latently infected cells at a concentration of 10 μM (Wang et al., 2001). A recent study demonstrated that it effectively inhibited wild-type and resistant HIV-1 mutants in T cell lines, monocytes, and peripheral blood mononuclear cells (PBMCs) at low concentrations and sensitized these cells to apoptosis resulting in a dramatic drop in viral titers (Agbottah et al., 2005). R-Roscovitine (CYC202) is currently undergoing phase II clinical trials in non-small-cell lung cancer and breast tumor. More recently, the antileukemic agent indirubin-3′-monoxime (Fig. 2) was reported to be a potent and selective inhibitor of CDK9 (Heredia et al., 2005). This compound inhibited HIV-1 replication in PBMCs and macrophages at a concentration of 0.5–1 μM and was effective against wild-type and drug-resistant mutants of HIV-1.

3. Nuclear factor κB (NF-κB) inhibitors

NF-κB is an inducible transcription factor that plays an important role in cellular gene expression associated with immune responses, inflammation, and cell survival (Ghosh et al., 1998; Viatour et al., 2005). Two major signaling pathways, the classical (canonical) and alternative (noncanonical) path-

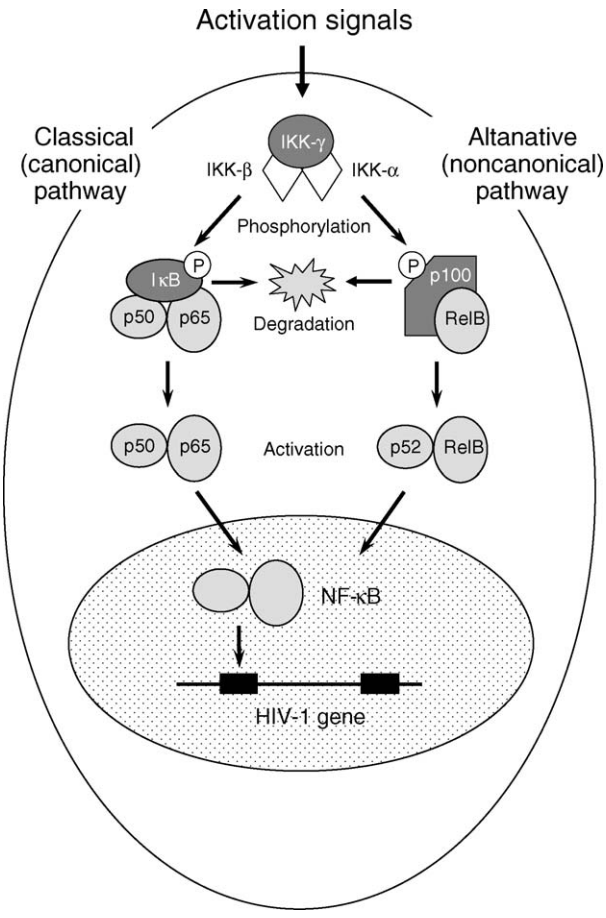

Fig. 3. NF-κB activation pathways. In the classical pathway, NF-κB is a heterodimeric molecule (p50/p65) existing in the cytoplasm as an inactive complex with its inhibitor IκB. Stimulation leads to immediate phosphorylation and subsequent degradation of IκB, resulting in the translocation of NF-κB from the cytoplasm to nucleus. In this pathway, the phosphorylation of IκB is mainly mediated by IκB kinase (IKK)-β. In the alternative pathway, IKK-α phosphorylates and processes p100, generating p52/RelB heterodimers.

ways, leading to NF-κB activation have recently been identified (Fig. 3). In the classical pathway, NF-κB is a heterodimeric molecule (p50/p65) existing in the host cell cytoplasm as an inactive complex with its inhibitor IκB. Stimulation with inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, viral and bacterial antigens, and stress-inducing agents leads to immediate phosphorylation and subsequent degradation of IκB, resulting in the translocation of NF-κB from the cytoplasm to nucleus. The IκB kinase (IKK) complex composed of the catalytic subunits IKK-α and -β and the regulatory subunit IKK-γ is responsible for the phosphorylation of IκB. In this pathway, the phosphorylation of IκB is mainly mediated by IKK-β (Ghosh and Karin, 2002). On the other hand, the alternative pathway is activated by different stimuli, such as lymphotoxin B, B cell activating factor, and CD40 ligand. This pathway involves IKK-α and leads to the phosphorylation and processing of p100, generating p52/RelB heterodimers.

HIV-1 gene expression is initiated and enhanced by the activation and subsequent binding of NF-κB to the enhancer region in the long terminal repeat (LTR) of HIV-1. Several compounds were reported to suppress HIV-1 gene expression and replication through the inhibition of NF-κB activation (Pande and Ramos, 2003; Baba, 2004). These include a variety of antioxidants, such as *N*-acetyl-L-cysteine (Roederer et al., 1990), cepharanthine (Okamoto et al., 1998), and α-tocopherol (Vitamin E) (Israel et al., 1992). Protein kinase C (PKC) inhibitors, such as staurosporine (Laurence et al., 1990) and Gö 6976 (Qatsha et al., 1993), were also shown to suppress HIV-1 replication through the inhibition of NF-κB activation. However, these antioxidants and PKC inhibitors have not been clinically licensed as anti-HIV-1 agents because of their insufficient antiviral activity or potential toxicity in vivo. *N*-arachidonoyldopamine (NADA), an endogenous *N*-acyl dopamine, was recently identified as a new class of brain neurotransmitters. NADA was also found to be a potent inhibitor of receptor-mediated T cell activation. NADA inhibited NF-κB-dependent transcriptional activities without affecting either IκB degradation or DNA binding activity of NF-κB (Sancho et al., 2004). Furthermore, NADA could inhibit the replication of vesicular stomatitis virus-pseudotyped HIV-1 in cell cultures (Sancho et al., 2005). 2-Amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl-nicotinonitrile (ACHP) (Fig. 2) was identified as a potent and selective inhibitor of IKK-β (Murata et al., 2003). The compound was less inhibitory to IKK-α and not inhibitory to IKK-γ. ACHP dramatically inhibited TNF-α-induced HIV-1 gene expression in the latently infected cells OM-10.1 with a 50% effective concentration of 0.56 μM (Victoriano et al., 2006). Although these compounds appear to be more specific to a molecule involved in the NF-κB signaling pathway than "classical" NF-κB inhibitors, further studies are required to elucidate their potential as anti-HIV-1 agents.

4. Other inhibitors

Another important mechanism of HIV-1 gene expression is the regulation of viral mRNA by Rev, which promotes the export of unspliced and partially spliced mRNA (Pollard and Malim, 1998). However, it seems more difficult to find selective inhibitors of Rev than those of Tat. In fact, a few molecules, such as neomycin (Zapp et al., 1993) and leptomycin B (Wolff et al., 1997), were reported to be Rev inhibitors. Neomycin B could block binding of the Rev protein to the Rev response element (RRE). On the other hand, leptomycin B proved to be an inhibitor of nucleo-cytoplasmic translocation of the Rev protein and Rev-dependent mRNA. The compound was, however, highly cytotoxic to the host cells, probably because it targets CRM1 (exportin1), a cellular cofactor required for Rev-mediated export of mRNA from the nucleus (Kudo et al., 1999). PKF050-638 (Fig. 2) is a low molecular weight compound that specifically inhibits Rev functions (Daelemans et al., 2002). Like leptomycin B, PKF050-638 also targets CRM1, yet its cytotoxicity was much lower than that of leptomycin B.

The serine/threonine kinase p38 MAPK, a member of the mitogen-activated protein (MAP) kinase superfamily, is assumed to play an important role in HIV-1 replication. In fact, the p38 MAPK inhibitor RWJ67657 (Fig. 2) was effective in

inhibiting HIV-1 replication in cell cultures (Muthumani et al., 2004). It is likely that RWJ67657 acts as an HIV-1 transcription inhibitor, yet further studies are required to prove this hypothesis. The bis-anthracycline WP631 efficiently inhibited HIV-1 gene expression and viral replication in acutely infected PBMCs (Kutsch et al., 2004). This compound suppressed Tat-induced transactivation but did not directly interfere with the Tat protein or Tat/TAR binding. Thus, it is assumed that WP631 inhibits a cellular transcription factor important for Tat-induced transactivation. Further studies are needed to determine whether this compound can be developed as an anti-HIV-1 agent because of its potential toxicity.

5. Conclusions

Currently, none of the compounds described herein have been approved for clinical use as anti-HIV-1 agents. The use of transcription inhibitors in the treatment of HIV-1 infection would be limited, unless they could have not only potent antiviral activity but also sufficient safety profiles. This class of compounds must be inhibitory to mutant viruses resistant to entry, reverse transcriptase, and protease inhibitors. In addition, HIV-1 transcription inhibitors might allow the interruption of HAART, since they could keep latent HIV-1 silent during the treatment interruption. Considering the rapid increase of treatment failure cases due to the emergence of multidrug resistance, HIV-1 gene expression inhibitors should be extensively studied to overcome such problems in current anti-HIV-1 chemotherapy.

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