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MINI-REVIEW

HIV-1 reverse transcriptase inhibitors

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Abstract Reverse transcriptase (RT) is one of the three enzymes encoded by the human immunodeficiency virus type 1 (HIV-1), the etiological agent of AIDS. Together with protease inhibitors, drugs inhibiting the RNA- and DNA-dependant DNA polymerase activity of RT are the major components of highly active antiretroviral therapy (HAART), which has dramatically reduced mortality and morbidity of people living with HIV-1/AIDS in developed countries. In this study, we focus on RT inhibitors approved by the US Food and Drugs Administration (FDA) or in phases II and III clinical trials. RT inhibitors belong to two main classes acting by distinct mechanisms. Nucleoside RT inhibitors (NRTIs) lack a 3′ hydroxyl group on their ribose or ribose mimic moiety and thus act as chain terminators. Non-NRTIs bind into a hydrophobic pocket close to the polymerase active site and inhibit the chemical step of the polymerization reaction. For each class of inhibitors, we review the mechanism of action, the resistance mechanisms selected by the virus, and the side effects of the drugs. We also discuss the main perspectives for the development of new RT inhibitors.

Introduction

Human immunodeficiency virus type 1 (HIV-1), the etiological agent of AIDS, has been identified in the beginning of the 1980s (Barre-Sinoussi et al. 1983; Gallo et al. 1983), and nowadays, more than 40 million people are infected by this retrovirus, which targets the immune system's CD4+ cells for replication. There is currently no cure for AIDS. However, a combination of drugs can be used to control viral replication. Highly active antiretroviral therapy (HAART, protocols that associate at least three drugs) has been prescribed for more than a decade and has dramatically reduced mortality rates (Schneider et al. 2005).

Twenty-three drugs targeting several stages of viral replication have been approved by the US Food and Drug Administration (FDA) for treating AIDS. Eleven drugs are protease inhibitors that affect maturation of HIV particles by inhibiting processing of Gag and Gag–Pol precursors. A fusion inhibitor prevents viral particles from fusing with the cellular membrane. The 11 other drugs target viral reverse transcriptase (RT).

RT is a key enzyme in the retroviral life cycle that catalyzes conversion of the single-stranded genomic RNA into double-stranded DNA with duplicated long terminal repeats, which is integrated into cellular DNA by the viral integrase. The mature p66/p51 heterodomeric HIV-1 RT is generated by the viral protease from a p66/p66 homodimer by cleavage of the C-terminal RNase H domain during maturation of the viral particle. The polymerase and RNase H catalytic sites are located on p66, while p51 plays a structural role (Kohlstaedt et al. 1992).

This review focuses on RT inhibitors that have been approved by FDA as well as those in advanced clinical trials (phase II or III; http://aidsinfo.nih.gov/ and http://www.thebody.com/). Noticeably, all those inhibitors target the polymerase activity of RT. RNase H inhibitors are only in preclinical development. Inhibitors in advanced clinical trials and approved drugs inhibiting the RT polymerase activity comprise two classes of compounds: Nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs), which act by totally different mechanisms.

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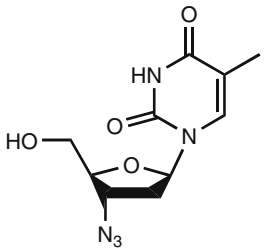
Nucleoside and nucleotide reverse transcriptase inhibitors

FDA-approved nucleoside reverse transcriptase inhibitors

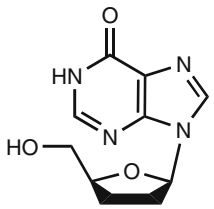
Nucleoside or nucleotide RT inhibitors are integral components of almost all anti-HIV treatments. Although they are administrated under different forms, these two classes of compounds act via the same active species: a nucleoside 5′-triphosphate. Therefore, in this review, both nucleoside and nucleotide RT inhibitors will be referred to as NRTIs.

The first anti-HIV drug approved by FDA was zidovudine (AZT), a modified thymidine bearing an azide group instead of the hydroxyl group at the 3′ position of the deoxyribose (Fig. 1). It was originally developed in 1964 as a possible cancer treatment but was found to be ineffective against tumor cells. However, collaboration between the National Cancer Institute and the Burroughs Wellcome Company led to the discovery of AZT's ability to suppress HIV replication (Mitsuya et al. 1985) and paved the way for clinical trials of AZT.

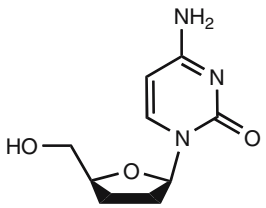
There are now eight modified nucleosides used against HIV (Fig. 1), and several others are in development. Although these drugs have different structures, they all lack the 3′ hydroxyl group of the deoxyribose and act as chain terminators. NRTIs compete with natural nucleoside triphosphates for binding at the nucleotide (N) site of the RT active site, whereas the 3′ hydroxyl group of the primer is bound at the priming (P) site. Once bound in the N site, NRTIs can be incorporated into the growing primer chain. Translocation of the primer/template complex relative to the active site then places the NRTI at the 3′ end of the primer



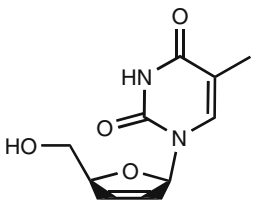
AZT-Zidovudine-Azidothymidine
Retrovir® GlaxoSmithKline-1987



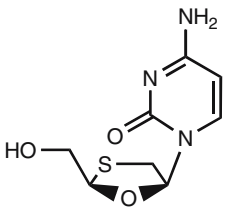
ddI-Didanosine-Dideoxyinosine
Videx® Bristol Myers-Squibb-1991



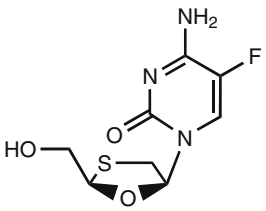
ddC-Zalcitabine-Dideoxycytidine
Hivid® Hoffman-LaRoche-1992



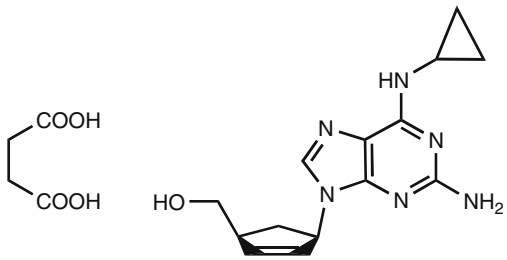
d4T-Stavudine
Zerit® Bristol Myers-Squibb-1994



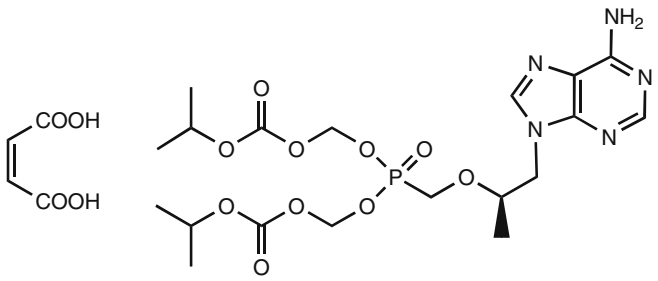
3TC-Lamivudine-
Epivir® GlaxoSmithKline-1995



FTC-Emtricitabine
Emtriva® Gilead Sciences-2003



ABC-Abacavir
Ziagen® GlaxoSmithKline-1998



PMPA-Tenofovir
Viread® Gilead-2001

Fig. 1 FDA-approved NRTIs for the treatment of HIV

into the P site, allowing binding of the next incoming nucleotide in the N site. However, the absence of an hydroxyl group at the 3′-end of the primer prevents the nucleophilic attack of the 5′ phosphate group of the incoming nucleotide, and DNA synthesis is blocked. The incorporation rates and the affinities of the most efficient NRTIs are similar to those of natural nucleotides (Feng et al. 1999; Reardon 1992; Rigourd et al. 2000). However, the intracellular concentrations of NRTI-triphosphates are very low compared to natural deoxyribonucleotide triphosphate (dNTP) concentrations (Becher et al. 2002a, b; Font et al. 1999). NRTIs are efficient inhibitors of reverse transcription despite the low intracellular concentration of their active form because incorporation of a single chain terminator during polymerization of the ~20,000 nucleotides of the double-stranded provirus is in principle sufficient to block the process.

To be incorporated into the nascent DNA chain, NRTIs must conserve their base-pairing properties with the template strand and avoid steric clashes with the RT active site. Therefore, most NRTIs have no or minimal modification of the base moiety (Fig. 1). AZT, 2′-3′-dideoxycytidine (ddC), d4T, 3TC and tenofovir carry unmodified bases. Emtricitabine (FTC) is a thymidine analog to which a fluorine atom was added to stabilize the nucleoside. Abacavir is a guanosine analog with a cyclopropyl amine modification on position 6. However, the active form of this compound is the guanine analog (Ray et al. 2002), obtained by action of the adenosine monophosphate deaminase (AMPD). Compared to the guanosine analog, this prodrug displays increased solubility in water. Hypoxanthine, the base moiety of didanosine (ddI), is a modified adenine, and the active form of ddI is actually the adenosine analog (Johnson and Fridland 1989). Under the stomach's acidic conditions, ddA and ddI are cleaved into 2′,3′-dideoxyribose and the free base. Adenine, the free base of ddA, is subsequently metabolized to 2,8-dihydroxyadenine, which is insoluble and can cause renal failure. By contrast, hypoxanthine is metabolized into uric acid, a physiological waste product.

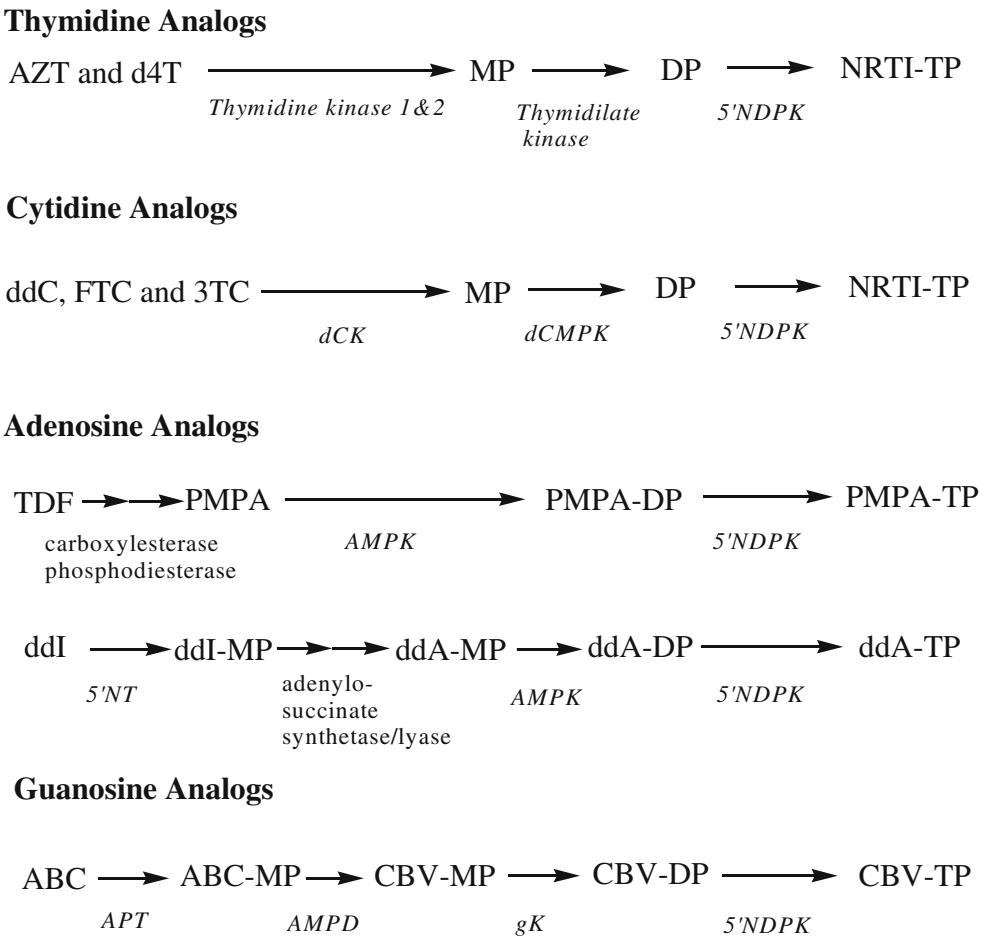
Whereas there are few modifications on the NRTI bases, sugar moieties have more varied and structurally different forms (Fig. 1). AZT is a thymidine analog with an azide group mimicking the 3′ hydroxyl of the ribose. The next generation of approved drugs includes the 2′,3′-dideoxynucleosides ddI and ddC (Dahlberg et al. 1987; Kim et al. 1987). Other NRTIs carry a double bond between positions 2′ and 3′ of the ribose (d4T; Mansuri et al. 1989) or cyclopentene ring (ABC; Daluge et al. 1997). β-L-oxathiolane nucleosides such as 3TC (Soudeyns et al. 1991) and FTC (Schinazi et al. 1992) have also been approved as HIV-1 inhibitors. Surprisingly enough, these nucleosides are active despite their reversed configuration compared to natural nucleosides. The 3′ sulfur present in these molecules reduces their cytotoxicity compared to the 2′-3′-dideoxy compounds (Van Draanen et al. 1994). Tenofovir disoproxil fumarate (TDF; Balzarini et al. 1996), an acyclic nucleoside phosphonate diester, has been approved in 2001. Acyclic analogs have the advantage of being more stable than classical ribose rings. The phosphonate diester is completely hydrolyzed by cellular esterases, which release the monophosphate of the compound (PMPA). Acyclic phosphonates are promising leads for the discovery of new antiviral drugs (De Clercq 2007).

Phosphorylation of nucleoside reverse transcriptase inhibitors

To be incorporated into DNA by HIV-1 RT, NRTIs have to be phosphorylated by cellular enzymes. With the exception of tenofovir, all FDA-approved chain terminators are administrated as unphosphorylated nucleosides and must be triphosphorylated after their penetration into the host cell (Balzarini et al. 1989; Furman et al. 1986). Tenofovir, which is a monophosphate analog, only needs to be biphosphorylated, avoiding the usually rate-limiting first phosphorylation. However, in the case of AZT, the second phosphorylation is rate limiting, whereas the first one is extremely rapid. The phosphorylation rate of AZT by thymidine kinase is similar to that of thymidine (Lavie et al. 1997a, b; Schneider et al. 1998). Therefore, all NRTIs can be considered as prodrugs. Their activation and phosphorylation pathways are summarized in Scheme 1 (Anderson et al. 2004b). The same enzyme, 5′-nucleoside diphosphate kinase (5′NDPK), performs the last phosphorylation step of all NRTIs, while the enzymes involved in the two first phosphorylation steps are specific to each base (Scheme 1).

Several NRTIs undergo base modification between the phosphorylation steps. Didanosine (ddI) is first converted into ddI-monophosphate (ddI-MP) by 5′-nucleotidase (5′ NT), then transformed into ddAMP by adenylosuccinate synthetase and adenylosuccinate lyase (Johnson and Fridland 1989; Scheme 1). This NRTI is more active in resting cells than AZT due to the better phosphorylation of ddI, which is directly linked to the activity of 5′NT in those cells (Gao et al. 1993). ABC is transformed into ABC-MP by adenosine phosphotransferase (APT), then converted into the guanosine analog CBVMP by adenosine monophosphate deaminase (AMPD; Ray et al. 2002). The first activation step of tenofovir is the hydrolysis of its diester moiety by cellular esterases (Van Gelder et al. 2000). The first ester is hydrolyzed in three steps, the first one being catalyzed by a carboxylesterase whereas the following two are spontaneous. The second ester is hydrolyzed by a phosphodiesterase and releases the monophosphate, which will be further phos-

Scheme 1 Activation and phosphorylation of NRTIs



5'NDPK : 5'-nucleoside diphosphate kinase
5'NT : 5'-nucleotidase
AMPD : Adenosine monophosphate deaminase
AMPK : Adenosine monophosphate kinase
APT : Adenosine phosphotransferase
dCK : Deoxycytidine kinase
dCMPK : Deoxycytidinemonophosphate kinase
dP : Diphosphate
gK : Guanylate kinase
MP : Monophospahte
TP : Triphosphate

phorylated by the same pathway as other adenosine analogs (Scheme 1).

Resistance to nucleoside reverse transcriptase inhibitors

Shortly after FDA approved AZT for clinical use, reduced sensitivity to this drug was detected in patients after monotherapy more than a six-month period (Larder et al. 1989). Indeed, the emergence and selection of mutations conferring resistance to anti-HIV-1 drugs is a major concern in AIDS treatment (http://hivdb.stanford.edu/ and http://resdb.lanl.gov/Resist_DB/default.htm). The rapid emergence of resistance mutations is due to the low fidelity of HIV-1 RT (which is enhanced by the lack of intrinsic proofreading activity; Preston et al. 1988; Roberts et al. 1988), the high level of HIV-1 replication (Perelson et al. 1996), and the high rate of RT-mediated recombination (Kellam and Larder 1995; Moutouh et al. 1996). However, the low fidelity of HIV-1 RT is also essential for the efficiency of NRTIs, as it favors their incorporation into DNA.

There are two main biochemical mechanisms of resistance to NRTIs (Deval et al. 2004; Goldschmidt and Marquet 2004). The first one increases discrimination of RT towards modified nucleosides (Huang et al. 1998; Larder and Stammers 1999; Sarafianos et al. 1999). Two

factors govern the NRTI incorporation efficiency (and therefore its chain terminating potency): (1) the competition of the modified nucleotide with natural dNTPs for binding to the RT active site and (2) the incorporation rate of NRTIs into the DNA. Some resistance mutations interfere with both factors, independently or simultaneously: i.e., they affect K_m or/and k_{pol} of NRTIs. Three main mutations in HIV-1 RT confer improved discrimination to NRTIs. Mutations M184I/V are frequently associated with FTC, 3TC, and ABC resistance. They are also associated with some ddI and ddC resistant viruses. They confer high resistance to ddI, ddC, 3TC, and FTC and intermediate resistance to ABC (Vivet-Boudou et al. 2006). L74V provides high resistance to ddC, but it is not frequently observed in ddC-experienced patients. It is one of the main mutations associated with intermediate resistance to ABC and ddI. K65R is mainly associated with resistance to TDF, ABC, ddC, and ddI, conferring intermediate resistance. It also provides strong resistance to 3TC and FTC, albeit being less frequently observed with these NRTIs.

The second resistance pathway consists of excising the blocking nucleotide by a hydrolytic mechanism (Arion et al. 1998, 2000; Meyer et al. 1998, 1999). Removal of the last incorporated nucleotide releases an unblocked DNA chain and the analog triphosphate (Hsieh et al. 1993; Reardon 1993). AZT-resistant RT transfers the chain terminator to an adenosine triphosphate (ATP) in a reaction named ATP-lysis, whose mechanism is similar to that of pyrophosphorolysis (Lennerstrand et al. 2001; Meyer et al. 2000, 2002; Smith et al. 2005), the reverse reaction of polymerization. This resistance mechanism was first observed with AZT and d4T and RTs bearing all or subsets of the so-called thymidine-associated resistance mutations (TAMs: M41L, D67N, K70R, L210W, T215Y/F, and K219Q). Remarkably, these mutations strongly enhance ATP-lysis but have little or no effect on pyrophosphorolysis. In the absence of the next incoming dNTP, AZT-resistant RT is able to excise most NRTIs with some efficiency (White et al. 2004), but in most cases, primer unblocking is strongly inhibited by the next incoming dNTP. Indeed, excision of the incorporated NRTI is only possible when the 3′ end of the primer is in the N site of RT, whereas by binding to the N site, dNTPs lock the primer terminus in the P site, thus forming a dead-end complex, which can neither be extended nor unblocked. The excision efficiency of NRTIs is inversely related to the stability of this dead-end complex (Goldschmidt and Marquet 2004).

Importantly, mutations M184V (Gotte et al. 2000; Larder et al. 1995), L74V (Miranda et al. 2005; St Clair et al. 1991), and K65R (Parikh et al. 2006; White et al. 2006) resensitize TAM-containing RT to AZT. The incompatibility of some resistance mutations is a key element in the long-term efficiency of anti-HIV-1/AIDS regimen containing several NRTIs.

Despite the efficiency of multidrug regimen, multidrug resistant viruses are frequently observed in the clinic. As far as NRTIs are concerned, mutations A62V, V75I, F77L, F116Y, and Q151M (usually termed the Q151M complex) in RT confer resistance to all NRTIs, except tenofovir and 3TC (Vivet-Boudou et al. 2006). Resistance is due to a k_{pol} effect, i.e. a decreased polymerization rate of the NRTIs, including AZT (Deval et al. 2002). On the other hand, when associated with TAMs, an insertion of two amino acids (SA, SG, or more frequently SS) between position 69 and 70 of HIV-1 RT confer resistance to all known NRTIs (Cases-Gonzalez et al. 2007; Menendez-Arias et al. 2006; Prado et al. 2004; White et al. 2004). The insertion decreases the stability of the DEC, and favors unblocking of the primer by the TAMs (Goldschmidt and Marquet 2004).

Nucleoside reverse transcriptase inhibitor toxicity

The toxicity of NRTIs is another challenge in antiretroviral therapy. Several adverse effects have been linked to the use of NRTIs, including headaches, gastrointestinal intolerance, nausea, lactic acidosis, and pancreatitis among others. Some of these side effects may induce serious and fatal complications, as described for some severe cases of lactic acidosis (Sundar et al. 1997).

Most NRTI toxic effects are linked to damages to mitochondria, revealed by abnormal mitochondrial structure and mitochondrial DNA (mtDNA) depletion (Kakuda 2000). NRTIs do not all have the same impact on mitochondria and do not all produce the same side effects (Feng et al. 2004; Kakuda 2000). Mitochondrial damage induced by emtricitabine and tenofovir is milder than the one caused by other NRTIs (Birkus et al. 2002; Feng et al. 2004; Vidal et al. 2006). Mitochondrial toxicity often occurs in the liver and muscle tissues and is closely correlated to the pharmacology of NRTIs. One of the proposed mechanisms is depletion of mtDNA, as NRTI triphosphates are incorporated by DNA polymerase γ (Johnson et al. 2001; Kakuda 2000; Lee et al. 2003). Indeed, there is a good correlation between mitochondrial toxicity and the efficiency of NRTI incorporation by DNA polymerase (Lee et al. 2003; Moyle 2005).

Nucleoside reverse transcriptase inhibitors in development

In addition to the FDA-approved NRTI drugs, several modified nucleosides are in clinical and preclinical development. The main reasons for continuing the search for new NRTIs (and inhibitors in general) directed against HIV-1 are to decrease toxicity, augment efficiency against resistant

viruses, and simplify anti-HIV-1 regimen. Several reviews have inventoried those new NRTIs (De Clercq 2004; Otto 2004; Vivet-Boudou et al. 2006). Figure 2 represents eight of the most promising chain terminators in development. In this paper, we focus on the inhibitors currently in clinical trials, but two compounds in preclinical development that account for a slightly different approach to traditional chain terminators will also be discussed.

Apricitabine [(−)-2′-deoxy-3′-oxa-4′-thiocytidine and formerly AVX754 and SPD754] is a deoxycytidine analog undergoing phase II clinical tests. The structural originality of this molecule lies in its "oxa-thio" ribose structure. This chain terminator showed in vitro activity against wild-type, AZT-, and 3TC-resistant HIV-1 strains (de Muys et al. 1999; Richard et al. 2000). It also showed additive antiviral activity in combination with AZT, d4T, or FTC, albeit having less potency than these NRTIs in vitro when used alone (Gu et al. 2006). In fact, clinical trials of apricitabine showed much better results than the in vitro tests (Cahn et al. 2006; Gu et al. 2006). These results reflect the very good pharmacokinetic profile of this compound. In vitro experiments showed that resistance to apricitabine appeared slowly compared to 3TC and was associated with mutations K65R, V75I, and M184V. Apricitabine did not select any particular resistance mutation during a 10-day monotherapy (Cahn et al. 2006; Gu et al. 2006) and showed very low toxicity, causing no damage to mtDNA (Cahn et al. 2006; Gu et al. 2006). This observation is in keeping with the high selectivity of apricitabine for HIV-1 RT, as compared to human DNA polymerase γ (de Muys et al. 1999).

Racivir® [(+/−) 2′,3′-dideoxy-3′-thia-5-fluorocytidine, RCV or (+/-)FTC] is a racemic mixture of the two β enantiomers of FTC and is in phase II clinical trials. It has shown significant anti-HIV (and anti-HBV) activity in vitro (Schinazi et al. 1992). The potency of the "L" nucleoside alone is higher than that of the "D" enantiomer (up to 155

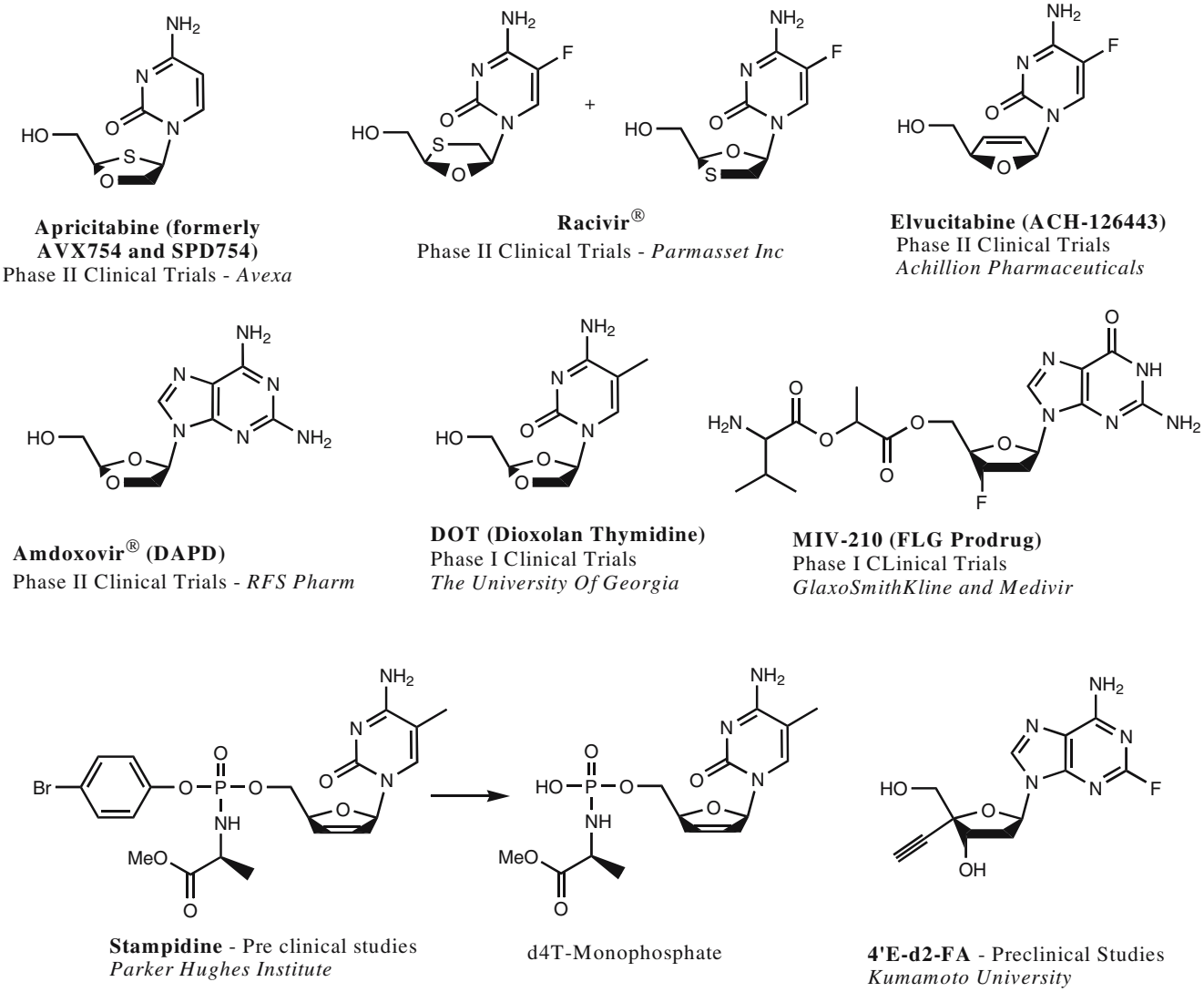


Fig. 2 NRTIs in development

times based on EC_{50} values). The racemate inhibits HIV replication with an average efficacy close to the "L" compound (FTC; Schinazi et al. 1992). In preclinical studies, racivir retained higher activity against HIV containing the M184V resistance mutation than FTC. Racivir has an excellent bioavailability, and its pharmacokinetic profile supports a once-a-day dosing (Herzmann et al. 2005). This NRTI has also shown promising antiviral activity when used in combination with d4T and Efavirenz (Herzmann et al. 2005).

Elvucitabine (ACH-126,443, L-d4FC, 2′-3′-didehydro-2′,3′-dideoxy-β-L-5-fluorocytidine) is a deoxycytidine analog substituted with a fluorine atom on position 5. The 2′-deoxyribose ring is devoid of 3′OH and bears a double bond between carbons 2′ and 3′ (Fig. 2). It has demonstrated potent in vitro activity against HIV (Lin et al. 1987), 10- to 20-fold superior to FTC. It also exhibited very good oral bioavailability (Dutschman et al. 1998). This compound showed a synergistic activity with d4T and AZT and an additive activity with ddI and ddC (Dutschman et al. 1998). In vitro studies showed that elvucitabine selected resistance mutations M184I and M184V (Hammond et al. 2005). In phase II trials, elvucitabine showed potent antiviral activity against 3TC and other NRTI resistant viruses (Vivet-Boudou et al. 2006). However, clinical trials of elvucitabine are on hold, as it showed bone marrow suppression in several patients, with CD4+ cell numbers dropping. Noticeably, the development of reverset, the D-enantiomer of elvucitabine, was also discontinued earlier in 2006 due to pancreatic inflammation issues. Further dosing studies will be required to evaluate the utility of elvucitabine in HIV infection treatments.

Amdoxovir [diaminopurine dioxolane, (2R, 4R)-(2,6-diaminopurin-9-yl)-1,3-dioxolane-2-methanol, (−)-β-D-2,6-diaminopurine dioxolane or DAPD], a modified purine nucleoside with a dioxolane ring instead of the usual ribose, reached phase II clinical trials. This compound was designed to be a more soluble and bioavailable prodrug of (−)-β-D-dioxolane-guanine (DXG). This prodrug is deaminated by adenosine deaminase to the dioxolane guanosine nucleoside. The latter is then triphosphorylated to give the active chain terminating nucleotide (Furman et al. 2001; Gu et al. 1999; Kim et al. 1993). Although the activity of amdoxovir in vitro is less potent than that of AZT, it retained activity against AZT resistant species, and its good bioavailability makes it a good drug candidate (Gu et al. 1999). In fact DAPD showed common patterns of resistance with some dideoxynucleosides and no cross-resistance with AZT. It, therefore, should be used in combination with the latter drug (Bazmi et al. 2000; Jeffrey et al. 2003). The first toxicological studies showed that DAPD is well tolerated. However, lens opacities were observed on five patients. Clinical studies have therefore been put on hold by original developers, Gilead Sciences in 2004. RFS Pharma is continuing the development of DAPD. The drug is undergoing phase II clinical trials and is tested against HIV and HBV infections.

Another dioxolane-based NRTI is in development. Dioxolane thymidine [(2R, 4R)-4-(thymidin-1-yl)-1,3-dioxolane-2-methanol or (−)-β-D-thymidine dioxolane or DOT] has made it into phase I clinical trials. It shows excellent bioavailability in monkeys and rats (close to 100%; Chu et al. 2005). Most interestingly, DOT shows antiviral activity against all common resistant HIV strains. It is the first thymidine-based nucleoside to show such activity (Kim et al. 1992). The intra cyclic 3′ oxygen seems to play an important role in the activity of DOT and DAPD against resistant strains of HIV. A series of phosphates and phosphoramidates DOT prodrugs were prepared and showed enhanced antiviral activity (Liang et al. 2006).

MIV-210, a prodrug of β-D-2′-3′-dideoxy-3′-fluoro-guanosine (FLG), is in phase I clinical trials. Its originality lies in the 3′-fluorine mimicking the natural 3′OH of unmodified nucleosides. This prodrug afforded high bioavailability and high FLG levels in blood plasma. In vitro, MIV-210 demonstrated activity against HIV strains resistant to all approved NRTIs. The prodrug MIV-210 has four- to fivefold better oral bioavailability than FLG (De Clercq and Field 2006).

Prodrugs designed to enhance cellular penetration or bioavailability or to liberate monophosphate analogs, thus bypassing the first phosphorylation step of nucleosides, constitute an important field in the search for novel NRTIs (Balzarini et al. 1989). Tenofovir, the approved prodrug of PMPA-MP, nicely illustrates this last point. Stampidine (Fig. 2), a stavudine prodrug, also shunts the limiting first phosphorylation step and is highly effective (Venkatachalam et al. 1998). It showed exceptional antiviral activity against HIV-1, including AZT-resistant strains, with mean EC_{50} values 1,000-fold lower than those of stavudine (Uckun et al. 2002). Stampidine is quickly transformed in the liver cytosol into alaninyl-stavudine-MP and stavudine. Ala-stavudine-MP is the major product, and stavudine formation was only observed in plasma, not in cells (Chen et al. 2002).

Other promising NRTIs in development include the 4′-ethynyl nucleosides (Kodama et al. 2001; Ohrui et al. 2000). The originality of these NRTIs is that although they do not lack the 3′-hydroxyl group, they still act as chain terminators: The presence of the ethynyl group inhibits the reactivity of the 3′OH by forming a neopentyle structure (Ohrui 2006). The lead compound of this new class of inhibitors is 4′-E-d2-FA (2′-deoxy-4′-C-ethynyl-2-fluoroadenosine; Fig. 2), a fluroadenosine-deoxynucleoside. Many of the 4′-ethynyl nucleosides appeared to be toxic, but 4′-E-d2-FA displayed highly potent activity against all

HIV-1 strains, very favorable toxicity profiles, and stability in plasma (Ohrui et al. 2006).

Non-nucleoside reverse transcriptase inhibitors

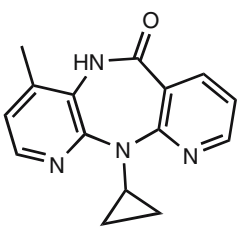
FDA-approved non-nucleoside reverse transcriptase inhibitors

Unlike NRTIs, NNRTIs do not require cellular activation to inhibit HIV-1 RT. They are not incorporated into nascent viral DNA, are noncompetitive inhibitors, and bind into a hydrophobic "pocket" in the p66 subunit of HIV-1 RT located close to (but distinct from) the NRTI binding site (Kohlstaedt et al. 1992; Pata et al. 2004). NNRTI binding distorts the nearby RT polymerase active site, thus affecting the chemical step of polymerization (De Clercq 1998; Spence et al. 1995). Crystal structures of HIV-1 RT/NNRTI complexes include nevirapine (Ren et al. 1995), delavirdine (Esnouf et al. 1997), and efavirenz (Ren et al. 2000), the three FDA-approved NNRTIs (Fig. 3). The NNRTI binding pocket does not exist in the unliganded RT and is formed upon binding by the side chains of aromatic (including Y181 and Y188) and hydrophobic amino-acid residues (Das et al. 2005; Rodgers et al. 1995). NNRTIs develop extensive hydrophobic interactions and π–π interactions, and some of them even form hydrogen bonds with an amino acid (K101). Although they all bind in a similar manner to RT (despite having various structures), the extent of RT/NNRTI interactions is different. NNRTIs are highly specific for HIV-1 and do not inhibit HIV-2 or any other retrovirus. This specificity is also a drawback, as it allows the virus to rapidly select resistant mutants. NNRTI-resistance mutations affect the binding of the inhibitors to their binding pocket. These mutations alter the size, shape, or polarity of the NNRTI binding pocket or affect the access of NNRTIs to this site (Das et al. 2005).

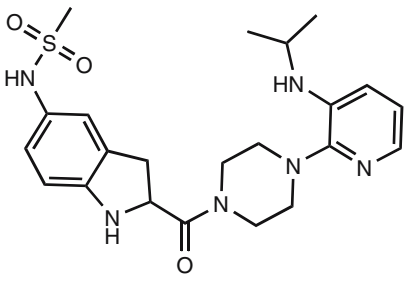
The first approved NNRTI drug was nevirapine (Fig. 3), in 1996 (Merluzzi et al. 1990). Its EC_{50} values are in the nanomolar range, and it acts synergistically with chain terminators (Richman et al. 1991). In the patients after monotherapy with nevirapine, mutations appeared after only 1 week of treatment (Richman et al. 1994). In addition to mutations at amino-acid residues 103,106, and 181 that had been identified by selection in vitro, mutations at residues 108, 188, and 190 were also found in patient isolates (Richman et al. 1994; Spence et al. 1996). The most frequent mutations conferring high resistance to nevirapine are K103N, Y181C, and G190A (Milinkovic and Martinez 2004). The loss of aromatic ring interactions between the inhibitor and the enzyme linked to mutation Y181C increases the dissociation rate of the NNRTI/enzyme complex (Spence et al. 1996). However, administrating nevirapine with other HIV-1 inhibitors such as AZT can prevent mutation Y181C. At the same time, when introduced in an AZT-resistant strain, mutation Y181C suppresses resistance to AZT (Byrnes et al. 1994; Larder 1992; Richman et al. 1994).

Noticeably, treatments consisting of a single dose of nevirapine are used in developing countries to limit mother-to-child HIV-1 transmission. They significantly reduce transmission, but as they do not provide maximal suppression of viral replication, they favor emergence of resistance mutations. Studies have reported resistance frequencies ranging from 25 to 69% in mothers receiving single-dose nevirapine alone (Giaquinto et al. 2006).

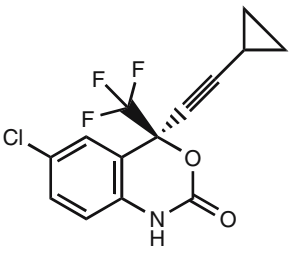
Delavirdine (Fig. 3), approved by FDA in 1997, is bulkier than nevirapine. Due to its size, it establishes more contacts with RT, in particular, hydrogen bonds with K103 and extended hydrophobic interactions with P236 (Esnouf et al. 1997). The most frequent mutations in patients developing virologic failure while receiving delavirdine are K103N (in >50% of patients), Y181C, and P236L (Balzarini 2004). Interestingly, mutation P236L, which confers 50-fold resistance to delavirdine, resensitizes HIV-1 to nevirapine (Dueweke et al. 1993). On the other hand, mutations G190A/S and P225H, which confer resistance to nevirapine and efavirenz, produce hypersensitivity to delavirdine (Balzarini 2004).



Nevirapine - Viramune®
Boehringer Ingelheim - 1996



Delavirdine - Rescriptor®
Pfizer - 1997



Efavirenz - Sustiva®
Bristol Myers-Squibb - 1998

Fig. 3 FDA-approved NNRTIs for the treatment of HIV

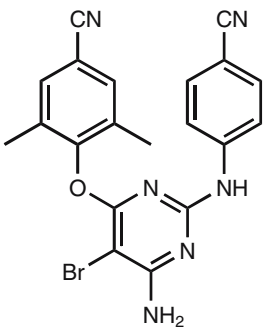
Efavirenz is currently the last approved NNRTI for the inhibition of HIV-1 replication. It shows excellent bioavailability and an interesting inhibition profile towards the virus (Pelemans et al. 1997). The most common resistance mutations in viruses of patients receiving efavirenz are K103N (in >50% patients), G190S/A/E, and Y188L (Balzarini 2004). Mutation K103N, which confers high-level resistance to the three FDA-approved NNRTIs, affects the kinetics of inhibitor binding by stabilizing the unbound state of HIV-1 RT (Hsiou et al. 2001). A recent study suggests that efavirenz, TMC 120 (formerly in development), and TMC 125 (in development) also reduce production of viral particles by enhancing intracellular multimerization of Gag-Pol precursors through the embedded RT sequence, thus inducing premature processing of Gag and Gag-Pol polyproteins (Figueiredo et al. 2006). This effect was not observed with nevirapine and delavirdine.

As with all antiretroviral agents, NNRTIs have side effects, but they are relatively moderate with few individuals discontinuing in clinical studies as a result of adverse drug events. The majority of adverse events with NNRTIs occur within the first month and are predictable and manageable without therapy interruption (Moyle 2001). The most frequent adverse effect is rash, which is observed with all FDA-approved NNRTIs. Rare cases of Stevens–Johnson syndromes have also been reported. Elevations in liver function tests have also been associated with these drugs. Besides, dysphoria, mood changes, depression, insomnia, somnolence, anxiety, vivid dreams, impaired concentration, and dizziness have been observed upon treatment with efavirenz (Weller and Williams 2001).

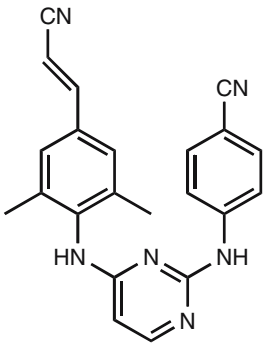
Non-nucleoside reverse transcriptase inhibitors in development

The drawback of the high specificity of NNRTIs is the rapid emergence of resistance mutations: Mutations conferring resistance to nevirapine are selected as quickly as 1 week after the beginning of treatment compared to more than 4 weeks for NRTIs. The common inhibition mechanism causes problems of cross-resistance, and research on novel NNRTIs is focused on finding molecules that are active against RT resistant towards the FDA-approved drugs (Fig. 4).

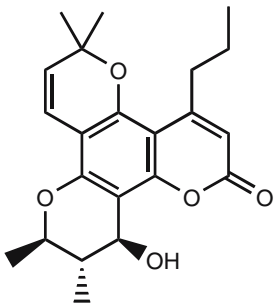
The most advanced new NNRTI, etravirine [TMC 125, 4-((6-amino-5-bromo-2-((4-cyanophenyl)amino)-4-pyrimidinyl)oxy)-3,5-dimethylbenzonitrile], is in phase III clinical trials. It shows potent activity against HIV-1, with EC_{50} values comparable to those of previously approved NNRTIs (Andries et al. 2004). In vitro tests have shown that unlike



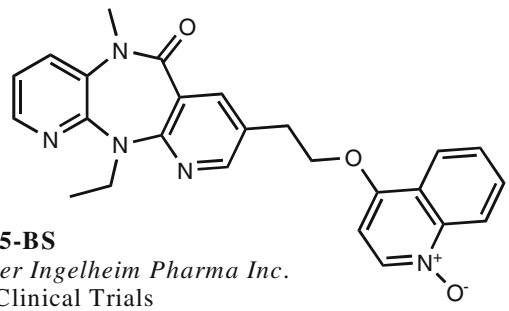
TMC125-Etravirine
Tibotec/Johnson&Johnson
Phase III Clinical Trials



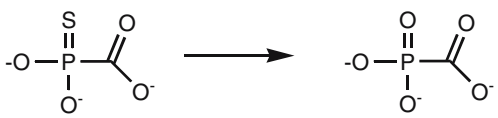
TMC 278-Rilpivirine
Tibotec/Johnson&Johnson
Phase II Clinical Trials



(+)-Calanolide A
Sarawak MediChem Pharmaceuticals
Phase II Clinical Trials



BILR 355-BS
Boehringer Ingelheim Pharma Inc.
Phase II Clinical Trials



Thiovir *Adventrx pharmaceuticals* Preclinical Trials Foscarnet

Fig. 4 NNRTIs in development

for FDA-approved NNRTIs, more than one mutation is necessary to allow the virus to develop resistance to etravirine. TMC125 has a unique profile of activity against NNRTI-resistant virus and possesses a high genetic barrier to the development of resistance in vitro (Vingerhoets et al. 2005). Recently, studies conducted on infected patients demonstrated that etravirine retained activity against multi-resistant HIV-1 strains (http://www.retroconference.org/2006/Abstracts/27455.HTM).

Rilpivirine [TMC 278, 4-((4-5(4-((1E)-2-Cyanoethyl)-2,6-dimethylphenyl)amino)-2-pyrimydinyl)amino)benzonitrile)], also developed by Tibotec/Johnson&Johnson, is structurally related to TMC-125 and is in phase II clinical trials. It showed excellent EC_{50} values against different HIV-1 strains, including those highly resistant to nevirapine and efavirenz. It showed good bioavailability and caused no significant side effects on rats and dogs. Clinical trials showed that rilpivirine has potent activity and induces no viral genotype or phenotype changes during the trial period (7 days). Only gastrointestinal disorder was identified as a side effect in 60% of the subjects (Goebel et al. 2006). Rilpivirine is being developed as a once-daily dosing component of a combination therapy.

Calanolide A is an HIV inhibitor extracted from a tropical rainforest tree (*Calophyllum lanigerum*; Kashman et al. 1992). Interestingly, calanolide A inhibits viruses bearing mutation Y181C, which are resistant to most NNRTIs, with tenfold enhanced potency as compared to wild-type virus (Currens et al. 1996). Enhanced inhibition has also been observed on viruses resistant to AZT (Buckheit et al. 1999). Although calanolide A is less potent against wild-type HIV-1 than approved drugs, it remains interesting because of its ability to inhibit resistant strains of the virus. Clinical studies on calanolide A demonstrated a good pharmacokinetic profile and a favorable toxicity profile. Very few adverse effects were observed and correlated with treatment dosing (Creagh et al. 2001). Calanolide A can be chemically synthesized and extracted from different plants (Flavin et al. 1996; Sekino et al. 2004; Sunthitikawinsakul et al. 2003).

BILR 355-BS is a new drug developed by Boehringer Ingelheim. It shows potent activity against wild-type and NNRTI-resistant viruses with EC_{50} values ranging from 0.25 nM (wild type) to 13 nM (http://www.retroconference.org/2005/cd/Abstracts/25580.htm). The effect of BILR 355-BS is boosted by co-administration of ritonavir (an HIV-1 protease inhibitor that also inhibits cytochrome P450 isoenzyme CYP3A4), as plasma concentrations of the drug were increased by three- to fivefold (Sobieszczyk et al. 2005). No safety concerns were observed on the patients after BILR 355-BS treatment. The main resistance mutation selected in vitro by BILR 355 BS was V106A, and only minimal cross-resistance was observed with nevirapine and delavirdine. Moreover, replication capacity of mutant V106A is greatly reduced, possibly explaining the low prevalence of this mutation among resistant strains of HIV (http://thebody.org/confs/icaac2005/hoffman3.html).

Foscarnet has long been known for inhibiting HIV (Sandstrom et al. 1985; Vrang and Oberg 1986). However, because of its very low bioavailability and due to nephrotoxicity, it was never approved for clinical use. Foscarnet has been used for treating cytomegalovirus retinitis in AIDS patients. In fact, foscarnet inhibits several viral polymerases (Crumpacker 1992; Reno et al. 1978) including HIV-1 RT. This pyrophosphate analog interferes with the translocation of RT and therefore prevents deoxynucleotide to be added to the elongating DNA (Marchand et al. 2007). Foscarnet has also proven to be a potential solution for salvage therapy, in the case of patients with no treatment options left, as nucleotide excision mutations showed hypersusceptibility to foscarnet (Mathiesen et al. 2004).

Thiovir is a foscarnet analog, with one sulfur atom replacing an oxygen, and shows much improved oral bioavalability. Compared to foscarnet, it has a lower toxicity and a better efficiency. The unique mechanism of action of thiovir makes it a very interesting antiviral, as it selects for different resistance mutations than approved NRTIs and NNRTIs, resulting in no cross-resistance and, even better, in restoring the virus susceptibility to AZT (http://www.iasociety.org/abstract/show.asp?abstract_id=2177410).

Conclusions

In the beginning of the AIDS pandemic, people had an average asymptomatic period of about 10 years after infection by HIV-1 and only survived 1 to 2 years after they manifested AIDS. HAART dramatically improved the life expectancy of people living with HIV-1.

The first line treatment usually combines one NNRTI or one protease inhibitor with a couple of NRTIs. Sometimes, several drugs are combined in a single pill, as in Atripla® (efavirenz/tenofovir/FTC), Combivir® (AZT/3TC), Epzicom® (abacavir/3TC), Trizivir® (AZT/3TC/abacavir), and Truvada® (tenofovir/FTC). Indeed, combining drugs and thus decreasing the number of pills is a major goal in the fight against AIDS, as it simplifies the patients' daily life, increases their adherence to treatments, and thus decreases the risk of selecting drug resistant viral strains.

Drug-resistant viruses, toxicity, and limited bioavailability of current drugs also highlight the need for continuing the search for novel anti-HIV-1 drugs. Prodrug design has definitely proven to be an essential research field, as it can be applied to all NRTIs and can lead to dramatic activity enhancement by improving drug delivery and phosphorylation.

Although the first RT inhibitor has been approved by FDA 20 years ago, it is still possible to target RT and inhibit HIV-1 replication by new mechanisms. Recently, Jochmans et al. (2006) have described indolopyridones that inhibtit HIV-1 reverse transcription by blocking the post-translocated state of RT, preventing the binding of the next incoming nucleotide. Novel nucleoside analogs causing "delayed polymerization arrest" (Boyer et al. 2005) or "lethal mutagenesis" (Anderson et al. 2004a) are other promising leads for finding novel ways to inhibit HIV replication. These approaches have yet to be validated in cell-based assays, but one compound (KP-1461) is currently into clinical trials and is assumed to inhibit HIV replication via lethal mutagenesis.

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