Development of novel nucleoside analogues for use against drug resistant strains of HIV-1

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Nucleoside analogue inhibitors of the reverse transcriptase (RT) enzyme of HIV-1 were the first class of compounds to be used in anti-HIV-1 therapy and are a cornerstone in highly active antiretroviral therapy. Despite the number of inhibitors of HIV-1 RT available for clinical use at the present time and the effectiveness of these compounds in combination regimens, long-term exposure of patients to these drugs often results in the development of viral resistance or long-term toxicity. For this reason, efforts to identify new agents with activity against drug-resistant strains of HIV-1 and with a toxicity profile that allows for individual patient tolerance of the drugs are still warranted.

AZT (zidovudine), the first approved drug for the treatment of HIV-1 infections[1] is a member of the 2′,3′-dideoxy class of nucleoside analogues. AZT, along with other members of this class of compounds shown in Fig. 1, including d4T (stavudine)[2], ddI (Ref. 3) , ddC (zalcitabine)[4], the heterosubstituted (−)-enantiomer of 2′-deoxy-3′-thiacytidine, 3TC (epivir or lamivudine)[5] (B. Belleau *et al. 5th International Conference on AIDS*, 4–9 June 1989, Montreal, Quebec, Canada, abstract T.C.O.1), and, more recently, the carbocyclic analogue abacavir (1592U89)[6,7], continue to represent a major chemotherapeutic approach towards the management of HIV-1 infections. All approved nucleoside analogue inhibitors of reverse transcriptase (RT) (NRTIs) are 2′,3′-dideoxyl derivatives of the natural nucleotide substrates of DNA polymerases and are all thought to inhibit RT activity in a similar fashion[8–12]. That is, following intracellular conversion to their 5′-triphosphate derivative (nucleotide), they compete with natural nucleotides for binding to RT and, subsequently, cause chain termination through incorporation into the nascent DNA strand (Fig. 2). Chain termination is caused by the lack of an hydroxyl motif at the 3′ carbon of the pentose ring that is necessary to form a 3′–5′ phosphodiester bond with the next nucleoside substrate in the elongating DNA strand. The efficacy of a nucleoside analogue is dependent on several factors, including its oral bioavailability, cellular uptake, the intracellular anabolism to its triphosphate derivative, the ability to compete with natural nucleotides as a substrate for RT, and the degree of drug resistance developed by the virus[13,14].

Currently, appearance of drug resistant viruses is an inevitable consequence of prolonged exposure of HIV-1 to antiretroviral therapy. This is believed to be caused both by a high turnover of HIV-1 in patients[15,16] and by low fidelity of the viral RT (Ref. 17). To achieve efficient inhibition of HIV-1 replication in patients, and to delay or prevent appearance of drug resistant viruses, drug combinations, which include NRTIs, non-nucleoside RT inhibitors (NNRTIs) and/or protease inhibitors (PIs), have been used effectively in treating HIV-1 infection[18,19]. However, recent studies show that HIV-1 can become resistant to multiple drugs in patients

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 1359-6446/00/$ – see front matter PII: S1359-6446(00)01558-0

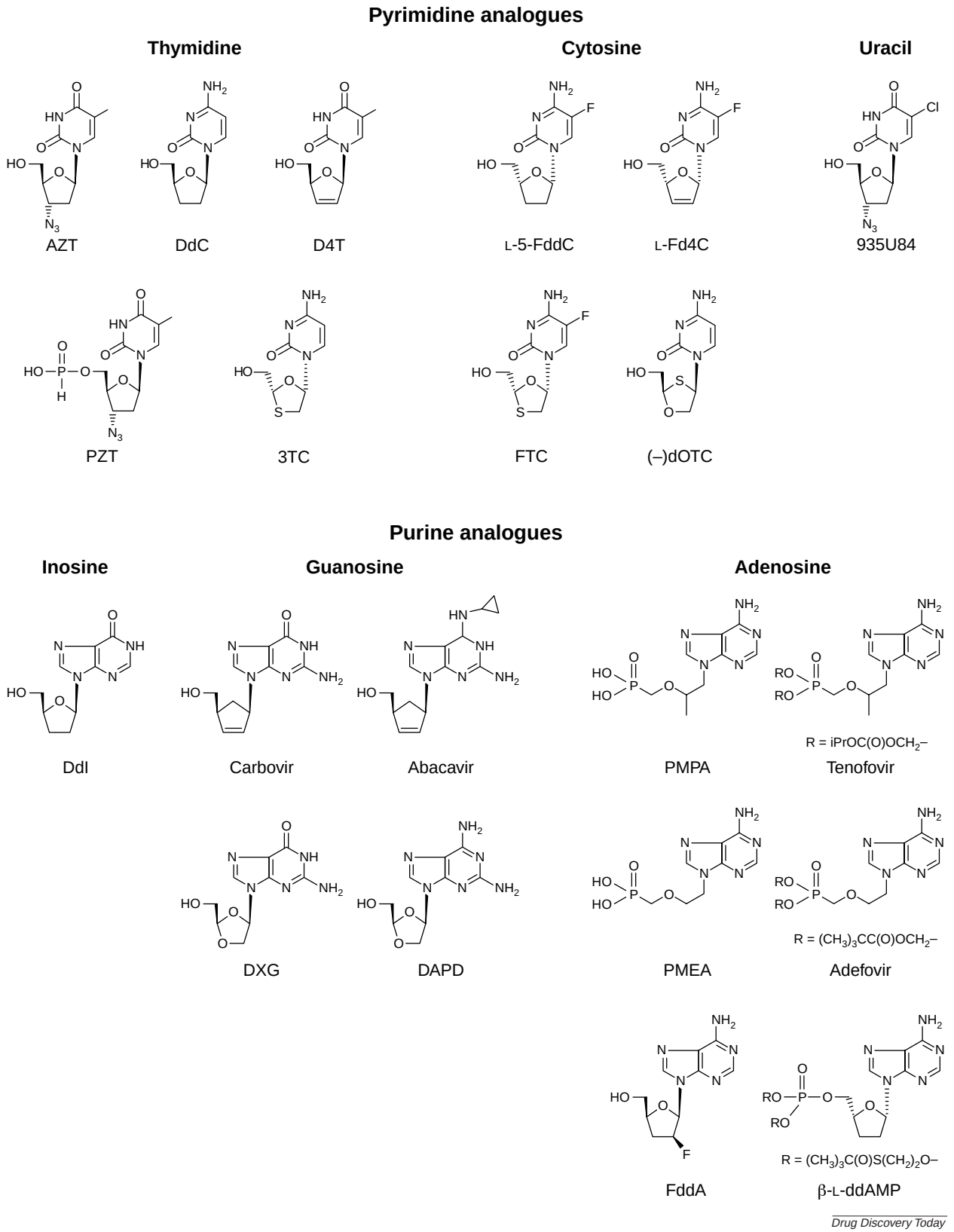

Figure 1. *Nucleoside analogue inhibitors of HIV-1.*

undergoing combination therapy, although resistance takes longer to develop than in a single drug regime[20,21]. Drug resistant viruses have been well documented in patients undergoing either monotherapy or combination therapy with two drugs that include AZT, ddI, ddC and 3TC (Refs 22–25). A single amino acid substitution within the RT enzyme is sufficient to cause resistance *in vitro*, as in the case of the M184V mutation and 3TC, although a combination of mutations is required to confer high-level resistance to AZT (Refs 23,26–30 and Table 1). Of particular concern is the fact that mutant viruses might be transmitted during *de novo* infections[14,31]; therefore, it is of utmost importance to identify new agents that are active against these drug resistant strains of HIV-1 and are well tolerated by individuals living with HIV-1.

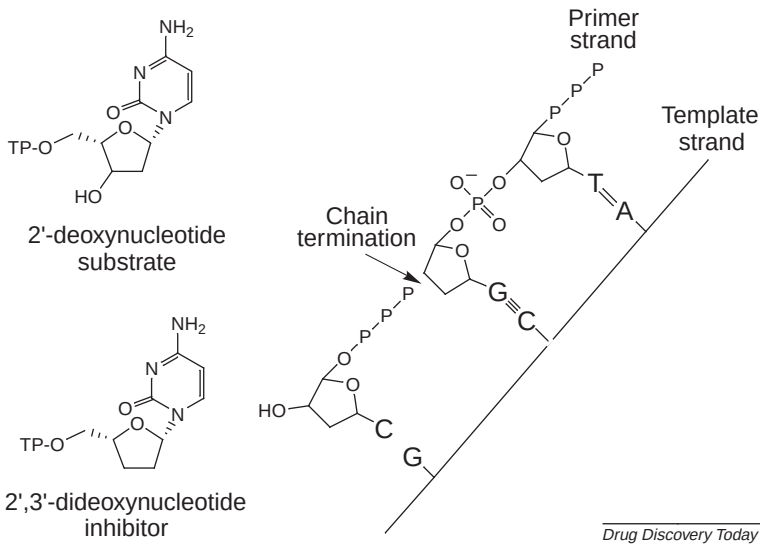

Figure 2. *Schematic representation of the mechanism by which 2′,3′-dideoxy and acyclic nucleoside analogues inhibit DNA synthesis. Following intracellular conversion to their 5′-triphosphate derivative, nucleoside analogues compete with natural nucleotides for binding to reverse transcriptase. Chain termination, which occurs on incorporation of the nucleoside analogue into the nascent, elongating primer strand of DNA, is caused by the lack of an hydroxyl motif at the 3′ carbon of the sugar ring that is required to form a 3′–5′ phosphodiester bond with the next nucleoside substrate in the elongating DNA strand.*

Drugs in clinical development

The most important aspect of any new anti-HIV-1 agent, including NRTIs, is its ability to inhibit drug resistant strains of HIV. In addition, because multi-drug regimens form the existing paradigm for HIV-1 therapy, any new agent will inevitably be used in combination with other drugs and, therefore, not only has to be well tolerated, but needs to have minimal negative drug-interaction effects. At the present time, coviracil (FTC or emtricitabine), DAPD, tenofovir (the prodrug of PMPA), and phosphazid (AZT-5′-*H*-phosphonate) are in clinical trials and the D-enantiomer of dOTC is in preclinical development. Unfortunately, clinical development of adefovir, the prodrug of 9-(2-phosphonylmethoxyethyl) adenine and FddA has been terminated. Whether any of the compounds in the advanced development stage reach the marketplace remains to be seen.

FTC (coviracil)

Coviracil, or FTC (Triangle Pharmaceuticals, Durham, NC, USA), is the 5-fluoro derivative of 3TC (Fig. 1). This compound, like 3TC, is a potent and selective inhibitor of both HIV-1 and the hepatitis B virus (HBV)[32–34]. FTC is reported to be 4–10-fold more effective in tissue culture assays than 3TC (Ref. 35). Phase I clinical data indicate that FTC is well tolerated with no serious or severe adverse events attributed to the drug, and information from Phase I/II trials shows that, with a daily dose of 200 mg or more, significant reductions in viral load (1.72–1.92 log units) are achieved over a 14 day period (J. Kahn *et al. 12th World AIDS Conference*, 28 June–3 July 1998, Geneva, Switzerland, poster 12208). In a Phase I/II trial that directly compared FTC with 3TC, Delehanty *et al.* (*6th Conference on Retroviruses and Opportunistic Infections*, 31 January–4 February 1999, Chicago, IL, USA, abstract 6) reported that over a 12-day period, FTC (25 and 100 mg once daily) had similar antiviral activity to 3TC (150 mg twice daily).

If the safety data for FTC are favorable, it is likely that this drug will be used in a similar way to 3TC, for both HIV-1 and HBV infections. Unfortunately, the similarity between these compounds is evident as both nucleosides elicit the mutation leading to M184V in the viral RT gene (Table 1). For this reason, the potential use of FTC, with respect to the development of viral resistance and patient tolerance, is limited. In addition, the putative advantage of FTC over 3TC, namely, increased antiviral potency when administered in combination with approved anti-HIV-1 agents, remains to be verified[35,36]. Therefore, at this time, the once daily regimen possible with FTC but not 3TC means that the main advantage of FTC might be a drug cost, marketing or quality of life issue.

Until recently it was believed that, of the NRTIs in clinical development, FTC was probably the most

Table 1. Mutations within the HIV-1 reverse transcriptase gene associated with viral resistance to pyrimidine analogues[a]

Compound[b]	Status[c]	Key mutation[d]	Fold resistance[e]	Cross resistance	Comments	Sponsor
AZT	M	M41L	4		Effect of T215Y is partially reversed by L74V, M184V/I or the NNRTI mutations L100I and Y181C	GlaxoWellcome
		D67N		PZT[f]		
		M41L/T215Y	60–70			
		K67N/K70R/T215Y/K219Q	120			
		M41L/K67N/K70R/T215Y	180			
PZT	C	D67N	10–15			
d4T	M	V75T	7	ddI, ddC, FTC	V75T was observed with d4T selection *in vitro*, rarely seen in patients receiving d4T	Bristol-Myers Squibb
ddC	M	K65R	4–10	ddI, DAPD, tenofovir, adefovir	K65R is a common mutation in the purine nucleoside group	Roche Pharmaceuticals
		T69D	5			
		L74V	5–10			
		V75T	5			
		M184V	2–5	ddI, 3TC, FTC		
		Y215C	4			
3TC	M	M184V	>100	FTC, ddI, ddC	M184V is thought to suppress effects of AZT resistance mutations	GlaxoWellcome
FTC	C(H)	M184V	>100	3TC, ddI, ddC		Triangle Pharmaceuticals
(−)dOTC	P	V75I	<2		<2 fold change in potency when tested in cell culture assays using primary cells	BioChem Pharma
		M184V	<2	3TC, FTC, ddI, ddC		
935U83	?	None			No resistant virus generated in culture	GlaxoWellcome

[a]For a more extensive review of nucleoside-related mutations, see Refs 21–31 and 66.

[b]Prodrugs are grouped together with their parent nucleoside or nucleotide.

[c]The status for each compound is either commercially available (marketed, M), in clinical development (C), clinical development on hold (H), clinical development discontinued (D), in the preclinical or research stage of development (P) or unknown (?).

[d]This list of mutations is designed to highlight only those mutations for which a significant quantity of viral resistance is associated.

[e]The change in resistance listed is approximate based on various studies performed in different laboratories using different methodologies.

[f]PZT has been reported to be resistant to most mutations causing high-level resistance to AZT (Ref. 48).

Abbreviations: 3TC, epivir/lamivudine; AZT, 3′-azido-3′-deoxythymidine; d4T, 2′,3′-didehypdro-2′,3′-dideoxythymidine; DAPD, (−)-β-D-2,6-diaminopurine dioxolane; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; FTC, coviracil/emtricitabine; PZT, phosphazid.

advanced (in Phase III clinical trials). However, on 7 April 2000, Triangle Pharmaceuticals announced a hold on study FTC-302, pending discussions with the South African Medicines Control Panel. The FDA indicated that, as a result of the factors considered in issuing the clinical hold, study FTC-302 might not provide adequate support as part of an NDA submission. How this development will effect the eventual commercialization of FTC is not clear at this time.

DAPD

Several groups have reported the synthesis of pyrimidine- and purine-derived nucleoside analogues containing a dioxolane sugar derivative, with an oxygen atom at the 3′-position of the sugar ring. From this class of compounds, the guanine analogues DXG and DAPD (Triangle Pharmaceuticals; Fig. 1) have been reported to inhibit HIV-1 *in vitro*[37,38]. Following oral administration of DAPD to woodchucks or rhesus monkeys, plasma concentrations of DXG

were significantly higher than of DAPD (Refs 39,40). This information, together with the fact that the triphosphate (TP) anabolite of DXG (DXG-TP) but not of DAPD (DAPD-TP), is an effective inhibitor of the RT enzyme of HIV-1, strongly suggests that DAPD is a prodrug of DXG. A comparative analysis of the *in vitro* profile of DXG and DAPD is reported by both Gu *et al.*[40], and by J. Mewshaw *et al.* (*39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 26–29 September 1999, San Francisco, CA, USA, poster 924). In studies *in vitro*, DXG is more potent than DAPD, most likely because of a slow rate of conversion of DAPD into DXG under tissue culture conditions. Both compounds have a negligible effect on cell proliferation over short periods of time and both are active against a wide variety of viral isolates tested in different cell systems. DXG and DAPD maintain their potency against all drug resistant strains of HIV-1 tested, except for those viruses that express K65R or L74V mutant RT (Table 2). In these cases, there is an approximate fivefold decrease in potency for both nucleoside analogues. Both research groups also report on the hypersensitivity of viruses containing mutations associated with NNRTIs to DXG.

Recently, the preclinical safety evaluation of DAPD has been reported (J. Mewshaw *et al. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 26–29 September 1999, San Francisco, CA, USA, poster 924) and, based on the encouraging body of data on the activity, toxicity and pharmacokinetics of DAPD, Triangle Pharmaceuticals has initiated a Phase I/II dose-escalation study. The stated strategy is to use this compound in antiviral therapy-naive or -experienced (including drug resistant) patients. The initial results from a 14 day monotherapy study with DAPD were presented by D.D. Richman *et al.* (*7th Conference on Retroviruses and Opportunistic Infections*. 30 January–2 February 2000, San Francisco, CA, USA, abstract 668). This on-going study is designed to examine the anti-HIV-1 activity, tolerability and pharmacokinetics of escalating doses of DAPD. Data presented included the first four doses in treatment-naive HIV patients. The median decrease in viral load was −0.45,

Table 2. Mutations within the HIV-1 reverse transcriptase gene associated with viral resistance to purine analogues[a]

Compound[b]	Status[c]	Key mutation[d]	Fold resistance[e]	Cross resistance	Comments	Sponsor
ddI	M	K65R	4–10	ddC, tenofovir, adefovir, DAPD	Suppresses some resistance to AZT	Bristol-Myers Squibb
		L74V	5–10			
		M184V	2–5	3TC, FTC		
Carbovir/abacavir	M	K65R	3	ddC, ddI, tenofovir, adefovir, DAPD		GlaxoWellcome
		L74V	4	DAPD		
		Y115F	2			
		M184V	2–5	3TC, FTC		
DXG/DAPD	C	K65R	5–8	ddC, ddI, tenofovir, adefovir		Triangle Pharmaceuticals
		L74V	5–10	Abacavir		
PMPA/tenofovir	C	K65R	8–9	ddC, ddI, DAPD, adefovir		Gilead Sciences
PMEA/adefovir	D	K65R	12–15	ddC, ddI, DAPD, tenofovir		Gilead Sciences
		K70E	9			
FddA	D	P119S	4			US Bioscience

[a]For a more extensive review of nucleoside-related mutations, see Refs 21–31 and 66.

[b]Prodrugs are grouped together with their parent nucleoside or nucleotide.

[c]The status for each compound is either commercially available (marketed, M), in clinical development (C), clinical development on hold (H), clinical development discontinued (D), in the preclinical or research stage of development (P) or unknown (?).

[d]This list of mutations is designed to highlight only those mutations for which a significant quantity of viral resistance is associated.

[e]The change in resistance listed is approximate based on various studies performed in different laboratories using different methodologies.

[f]PZT has been reported to be resistant to most mutations causing high-level resistance to AZT (Ref. 48).

Abbreviations: 3TC, epivir/lamivudine; AZT, 3′-azido-3′-deoxythymidine; DAPD, (−)-β-D-2,6-diaminopurine dioxolane; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; DXG, (−)-β-D-1′,3′-dioxolane guanosine; FTC, coviracil/emtricitabine; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; PMPA, 9-(2-phosphonylmethoxypropyl)adenine.

–1.0, –1.19 and –1.5 log units, in patients receiving doses of 25, 100, 200 or 300 mg twice daily, respectively. The viral suppression observed at each dose suggests a dose–response relationship. In addition, DAPD was well tolerated at all doses, with no significant or consistent adverse effects during the dosing period. This is encouraging for the progression of DAPD in clinical trials. Because none of the currently approved anti-HIV-1 nucleoside analogues contain a dioxolane sugar motif, DXG and its putative prodrug analogue DAPD represent a novel class of nucleosides with potential utility in anti-HIV-1 therapy.

Tenofovir [bis(POC)-PMPA]

Tenofovir is a lipophilic ester prodrug of PMPA (Gilead Sciences, Foster City, CA, USA; Fig. 1) designed to increase the oral bioavailability of PMPA. PMPA is a nucleotide analogue that belongs to a class of compounds developed from work by A. Holy who, in 1978, reported the synthesis and broad-spectrum antiviral activity of DHPA (Ref. 41). PMPA is an acyclic phosphonate nucleotide that has potent antiviral activity against both HIV-1 and HBV (Ref. 42). The phosphonate construction used in this compound, and others described later, mimics the monophosphate entity of a natural nucleotide and, therefore, bypasses the first enzymatic step in the anabolism to the active nucleotide TP. One of the most exciting observations in the preclinical analysis of PMPA is the highly effective pre- or post-exposure prophylaxis of simian immunodeficiency virus (SIV) infection in rhesus monkeys[43]. In these experiments, the establishment of SIV in the monkeys is prevented if the drug is administered 48 h before, or 4 or 24 h after, inoculation with the virus[43]. PMPA is also effective against chronic SIV infection in cynomologus monkeys[44]. Studies *in vitro* show that PMPA is an effective inhibitor of HIV-1 with an IC_{50} (concentration required to reduce virus replication by 50% in culture assays) in the range 0.2–0.6 μM, depending on the cell system and virus strain used. In addition, PMPA is non-toxic with CC_{50} values (concentration required to inhibit cell growth by 50%) of approximately 1 mM (Ref. 45). Equally important is the fact that PMPA maintains its potency when tested against a variety of HIV-1 variants that are resistant to AZT, ddI, ddC 3TC, FddA, ddG (dideoxyguanosine), d4T, various NNRTIs and PIs (Ref. 45). In all cases except for the K65R mutant of HIV-1, the decrease in potency observed against these virus strains is in the order of 2–3-fold, which might not be clinically relevant. However, when viruses with a RT containing the K65R mutation is used, the change in potency was approximately eightfold[45] (Table 2). Interestingly, the overall potency of the lipophilic ester prodrug, tenofovir, in culture assays was approximately 100-fold greater than for the parent compound, PMPA, which might be due to better cellular penetration. As with PMPA, the only mutation that elicited large changes in the potency of tenofovir was K65R (Table 1, Ref. 45). Preclinical toxicity studies performed using intravenous administration of PMPA demonstrate the safety of PMPA at doses in the clinically effective range. Nephrotoxicity, characterized by proximal convoluted tubule degeneration, is the principal toxicity observed following infusion of PMPA at very high doses (75 mg kg^{-1} day^{-1})[46]. PMPA has also been administered intravenously (1 or 3 mg kg^{-1} day^{-1}) in a Phase I/II clinical trial to determine the safety, pharmacokinetics and antiretroviral activity of this compound in humans[46]. In this short-term study, PMPA was found to be safe, well tolerated and effective in reducing levels of HIV RNA in the plasma. After seven consecutive days of intravenous PMPA dosing, the median reduction in plasma HIV-1 RNA (measured as copies ml^{-1}) was –0.6 and –1.1 log units in the 1 and 3 mg kg^{-1} day^{-1} groups, respectively[46]. The positive results of this study coincided with the synthesis, *in vitro* evaluation and clinical testing of the orally bioavailable, lipid ester prodrug, tenofovir (S.G. Deeks *et al. Fifth Conference on Retroviruses and Opportunistic Infections*. 1–5 February 1998, Chicago, IL, USA, abstract 772/LB-8). More recently, Schooley *et al.* (*39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 26–29 September 1999, San Francisco, CA, USA, abstract LB-19) reported interim results from a Phase III study lasting 48 weeks. At 24 weeks, once daily dosing of tenofovir (300, 150 or 75 mg) was well tolerated and demonstrated significant dose-related antiretroviral activity.

Phosphazid (PZT)

To address concerns about drug toxicity in patients undergoing AZT therapy, in 1991 Tarussova *et al.*[47] reported the synthesis and anti-HIV-1 evaluation *in vitro* of 5′-hydrogenphosphonates of 3′-azido-2′,3′-dideoxynucleotides. In this study, the authors reported an IC_{50} of 0.072 μM for phosphazid (PZT) the 5′-hydrogen phosphonate derivative of AZT (Fig. 1). This is approximately tenfold less potent than AZT (IC_{50} 0.005 μM) in assays in culture. However, as PZT is much less toxic than AZT (CC_{50} values of 2.5 mM and 210 μM for PZT and AZT, respectively), the overall selectivity index (SI) for PZT (CC_{50}/IC_{50}) is superior to that of AZT (Ref. 47). Building on these studies, Machado *et al.*[48] confirmed that, for assays performed in peripheral blood mononuclear cells using HIV-1_{IIIB} or HIV-1_{HXB2}, the SI was better for PZT than for AZT. Machado *et al.*[48] also evaluated the activity of PZT against AZT-resistant strains of HIV-1 and found that, in general, the level of virus resistance was similar for PZT and AZT. However, virus selected by

continuous exposure to PZT contained only one mutation, an aspartate-to-asparagine change at amino acid residue 67 (D67N), which caused a 10–15-fold reduction in the potency of PZT (Table 1). Although this mutation is also associated with resistance to AZT, it must be stressed that this was the only mutation observed over the course of the experiment[48]. Therefore, the D67N mutation could represent a distinct resistance profile for PZT.

The results of a Phase I, open-label, 12 week, dose-escalation study was reported in 1998 (Ref. 49). In this trial, dosing regimens of 200 or 400 mg twice daily were compared with the same doses given three times daily. Mean decreases in HIV-1 RNA were –0.31, –0.38, –0.7 and –0.78 log copies ml^{-1} in the four respective treatment groups. These decreases were sustained for the duration of the study and no major toxicity was noted. More recent information on this compound is not available.

Drugs in preclinical development

(–)dOTC

The dOTC (BioChem Pharma, Laval, Quebec, Canada) class of molecules are novel 4′-thio dideoxynucleoside analogues containing an oxygen heteroatom at the 3′-position of the sugar moiety (Fig. 1). The chemical synthesis and anti-HIV-1 properties of the racemate (+/−)dOTC or dOTC, as well as the individual enantiomers of dOTC [(+)dOTC and (−)dOTC] against HIV-1 in cell lines and primary cells have been reported[50]. This class of 2,4-disubstituted 1,3-oxathiolane nucleosides is a hybrid of the 4′-thio and isonucleoside families of compounds. It is isomeric to the 2,5-disubstituted 1,3-oxathiolanes by transposition of the heteroatoms in the sugar moiety of 3TC. The individual enantiomers of dOTC are relatively equipotent inhibitors of $HIV\text{-}1_{IIIB}$, however, the β-L enantiomer [(+)dOTC] is more toxic in a number of cell lines compared with the β-D enantiomer [(−)dOTC; Ref. 50]. Expanding on these observations, De Muys *et al.*[50] reported the selectivity of dOTC and its enantiomers for HIV-1 RT over the cellular polymerases α, β and γ. In these experiments, the TP derivative of (+)dOTC, [(+)dOTC-TP], was approximately 6–7-fold more potent than (−)dOTC-TP against RT. However, intracellular metabolism studies showed that the accumulation of (−)dOTC-TP within cells was approximately tenfold greater than that of (+)dOTC-TP (Ref. 50). In cell culture studies, the potency of both the racemate and (−)dOTC were minimally changed when tested against clinical isolates resistant to 3TC, AZT, or both, while changes in potency observed for (+)dOTC for these same viruses ranged from five- to ten-fold[50] (Table 1). Building on the activity of dOTC against 3TC-resistant virus *in vitro*, Stoddart *et al.*[51] describe the ability of dOTC to inhibit HIV-1 expressing M183V mutant RT *in vivo,* using a severe combined immunodeficient (SCID) murine model. In addition, in cell culture studies, phenotypic resistance to (−)dOTC develops slowly (>12 passages)[50,52] (Table 1), while after 6 passages virus with an isoleucine to valine change at position 184 (I184V) emerged in the presence of (+)dOTC (J. Bedard *et al. 12th International Conference on Antiviral Research*. 21–26 March 1999, Jerusalem, Israel, abstract 44).

Short-term evaluation of animal toxicity in rats and monkeys supports the investigational new drug (IND) submission for the racemate, dOTC. Human pharmacokinetic and efficacy data obtained from a Phase I/II study were reported in 1999. The efficacy results suggest that dOTC is a potent antiviral agent with mean log reduction in viral load greater than one log unit, even at the lowest concentration tested (200 mg twice daily for seven days; R. Wood *et al. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 26–29 September 1999, San Francisco, CA, USA, abstract 503). Unfortunately, results from long-term animal toxicity studies have raised concerns about dOTC. In order to address those, BioChem Pharma has decided to continue with the development of the (−)dOTC. As mentioned previously, this enantiomer is equipotent to the racemate, active against 3TC- and AZT-resistant strains of HIV-1 and virus resistance to (−)dOTC is slow to develop in culture[50,52].

Discontinued clinical trials

FddA (lodenosine)

The purine nucleoside lodenosine or FddA (US Bioscience, West Conshohocken, PA, USA; Fig. 1) was first reported by Marquez *et al.*[53] in 1987. FddA lacks potency in tissue culture assays against wild-type strains of HIV-1; however when tested against ddI- or AZT-resistant strains of virus, the potency is only minimally changed (2–3-fold change in IC_{50})[54]. The amino acid substitution most commonly associated with the change in phenotype to FddA is a switch from proline to serine at amino acid 119 (P119S) of the RT (Ref. 55). A 2–3-fold reduction in potency of FddA is also observed when tested against virus containing either a K65R or L74V substitution (Table 2). This information, combined with a good toxicity profile *in vitro*, the observed efficacy *in vivo* and data following combination with existing anti-HIV-1 agents, supported the clinical development of this compound[54–56].

In 1999, Young *et al.* (*39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 26–29 September 1999, San Francisco, CA, USA, abstract 1975) reported Phase II results from a multicenter, randomized trial of FddA in combination with stavudine (d4T) and indinavir.

Efficacy results of the interim (12-week) analysis showed that, although not statistically significant, the slope and the magnitude of the mean decline in plasma HIV-1 RNA was somewhat greater for the FddA-containing arms (at 100, 200 or 300 mg twice daily) than for the control arm containing 3TC. In addition, safety results indicated that FddA was well tolerated. The overall conclusion was that all treatment arms would continue and the data reassessed at weeks 24 and 48. Therefore, it was unexpected when, on 14 October 1999, the company announced the suspension of FddA clinical trials, pending review of additional scientific information regarding serious adverse events (including deaths) seen during the Phase II studies.

Adefovir (Bis Pom PMEA)

Adefovir is a lipophilic ester prodrug of PMEA (Gilead Sciences; Fig. 1; for review see Ref. 42). The development of compounds from the PMEA and PMPA class of molecules, as mentioned previously, emerged from the work of A. Holy[41]. The antiviral potential of PMEA has been demonstrated both *in vitro* and *in vivo*[42,57]. However, PMEA itself has low oral bioavailability (7.8% in rats and 4.0% in cynomologous monkeys)[42,57], hence the conversion to its lipophilic ester prodrug, adefovir (Table 2).

Clinical trials of adefovir began in the mid-1990s with Phase I/II results reported in 1997 (Ref. 58). In these early studies, adefovir alone was shown to reduce viral load in HIV-1-infected patients, which led to its advancement into more rigorous testing[58]. In 1999, M.D. Miller *et al.* (*2nd International Workshop on Salvage Therapy for HIV Infection*. 19–21 May 1999, Toronto, Canada, poster 4) described results from a Phase II/III study on the effect of adefovir on genotypically and phenotypically defined 3TC- or AZT/3TC-resistant HIV-1. Interim analysis at week 24 showed that adefovir was effective in patients with M184V-mutant HIV, suggesting that it might be effective as a salvage therapy. Later in 1999, J. Gallant *et al.* (*39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 26–29 September 1999, San Francisco, CA, USA, abstract 1976) described the efficacy of adefovir and efavirenz in salvage regimens, the effect of pre-existing RT mutations on the response to adefovir, and the use of adefovir at two dose levels (120 mg or 60 mg daily) in combination with NRTIs and PIs. At the same conference, E.S. Ho *et al.* (abstract 1286) showed the results of a study designed to investigate the mechanism by which adefovir induces renal toxicity. In this study, the investigators identified the human renal organic anion transporter 1 as the target of the cytotoxic effects of adefovir on renal tubules. This is reminiscent of the nephrotoxicity reported for another acyclic phosphonate, cidofovir, which was advanced for the treatment of HCMV infections[59]. Apparently, concerns over the safety of individuals receiving the 60 mg dose of adefovir were too great to allow the FDA advisory committee to recommend accelerated approval. For this reason, Gilead Sciences announced the termination of its HIV development program for adefovir at 60 mg. Adefovir is still progressing (30, 10 and 5 mg daily doses) in clinical trials for HBV indications.

Discovery pipeline

In light of the rate at which promising compounds drop out of drug development programs, HIV-1-infected individuals need new drugs that are well tolerated, economical and active against drug resistant strains of the virus. The list of compounds in preclinical or clinical development described previously might look impressive. However, on closer inspection, it is likely that only a few of them will (i) be approved for human use, and (ii) actually address the safety and efficacy needs of people living with HIV-1. Thus, it is important for continued research into novel agents, including nucleosides, to fill the armamentarium of anti-HIV-1 drugs. It is evident from the volume of literature that many approaches are being used to develop new anti-HIV-1 nucleosides. If there is any pattern to the research efforts, it might be in the emphasis on prodrug formulations of existing nucleosides and the use of L-nucleosides to inhibit HIV-1 RT specifically.

Prodrugs

Technically, all nucleosides whose active species is a TP anabolite are prodrugs. However, in this section, 'prodrug' is used to describe one or more of the vast array of strategies employed to enhance the activity *in vivo*, or reduce the toxicity, of a nucleoside before it is converted to its TP form. The rationale for the selection of a particular prodrug strategy lies in the particular deficiency displayed by the parent nucleoside, such as poor oral bioavailability, CNS penetration, cellular uptake or the rate-limiting enzymatic step in the conversion to the TP species. For example, the dioxolane purine nucleoside analogue DXG has low oral bioavailability. This problem is partially overcome by the conversion of the guanine base to its 2,6-diamino purine derivative (DAPD). DAPD, as mentioned previously, is rapidly converted to DXG in the bloodstream[39,40]. Similarly, part of the reason for the synthesis of abacavir, the 6-cyclopropylamino derivative of the guanine analogue carbovir (Fig. 1), is to increase oral bioavailability[6]. In the case of adefovir and tenofovir, the phosphonate group helps to overcome problems with intracellular metabolism and the bis(POM) or bis(POC) residues increase the oral bioavailability of these phosphonate prodrugs.

As mentioned, there are numerous ways to modify the parent nucleoside or nucleotide to enhance its efficacy *in vivo*. These include the addition of the lipophilic esters bis(POM) or bis(POC), phosphoramidate derivatives of nucleosides and *S*-(2-hydroxyethylsulfidyl)-2-thioethyl (DTE) or *S*-acyl-2-thioethyl (SATE) esters (for review, see Ref. 60). The importance of prodrug variations of nucleosides or nucleotides, with respect to drug resistance or toxicity issues, is not readily obvious. However, without some of the chemical prodrug formulations available, compounds such as DAPD, tenofovir and adefovir would not have entered clinical trials. Prodrug formulations allow expansion of the range of available nucleosides for testing against HIV-1 *in vivo*. For example, J-L. Imbach *et al.* (*11th International Conference on Antiviral Research*. 5–10 April 1998, San Diego, CA, USA, abstract 3) reported that β-L-ddA (β-L-2′,3′-dideoxyadenosine) does not inhibit HIV-1 effectively in cell culture assays, whereas its TP anabolite does inhibit HIV-1 RT *in vitro*, suggesting that the conversion of β-L-ddA to its TP derivative does not occur intracellularly. Therefore, Imbach and colleagues synthesized β-L-ddAMP-bis-(tbutylSATE) to overcome the delivery, uptake and anabolism issues of β-L-ddA (Fig. 1). The results were extremely encouraging, as potent anti-HIV-1 activity in culture was observed (EC_{50} of 0.002 μM), while TP levels within the cells remained above the K_i for RT for up to 48 h. This extremely potent SATE derivate of β-L-ddAMP was thought to be entering clinical trials as part of the Novirio Pharmaceuticals (Cambridge, MA, USA) portfolio; however, no new information has been released about this compound in the past year.

Novel nucleosides

With the low success rate of converting active nucleosides into approved drugs, it is difficult to identify where the next new agent will come from. For example, in 1989, 5-chloro-2′,3′-dideoxy-3′-fluorouridine was reported as the most selective anti-HIV-1 agent in a series of 2′- and 3′-fluorinated nucleoside analogues[60]. In 1996, the safety and pharmacokinetics of this same molecule (935U83, Fig. 1) were reported by GlaxoWellcome (Greenford, Middlesex, UK)[61]. Now, four years later, there has been no new news about this compound.

Many laboratories continue to work on novel nucleoside inhibitors of HIV-1 RT, with much effort spent on the biological evaluation of nucleoside analogues with the unnatural β-L(−) configuration. This work is encouraged by relaxed enantioselectivity of HIV-1 RT for the unnatural sugar configuration[62]. For example, Dutschman *et al.*[63] recently described the intracellular metabolism and combination anti-HIV-1 activity of L(−)Fd4C (Fig. 1). This compound was reported to have no toxic effect on mitochondrial DNA synthesis at concentrations up to 10 μM, and to act synergistically with d4T and AZT in culture studies[63]. Similarly, Lee *et al.*[64] reported the synthesis of 2′-fluoro-2′,3′-unsaturated L-nucleosides. From this series, the cytosine, 5-fluorocytosine, adenine and 2-fluoroadenine derivatives had encouraging activity against HIV-1 in culture assays. Finally, at the most recent meeting of the International Society for Antiviral Research, G. Gosselin *et al.* (*13th International Conference on Antiviral Research*. 16–21 April 2000, Baltimore, MD, USA, abstract 1) reported encouraging results on the stereospecific synthesis and anti-HIV-1 activity of several L-nucleosides. The most interesting compound from this study, with respect to potency (EC_{50} = 0.032 μM) and SI (2900), was L-5-FddC (Fig. 1), for which the activity and selectivity data are previously reported[65]. From the continuum of work on L-5-FddC, it is possible that this compound is heading into the clinical development pipeline.

Summary

Nucleoside analogues were the first class of compounds approved for combating HIV-1 (as well as other viral) infections and they continue to have a major role in this regard. Unfortunately, the chronic nature of HIV-1 infection, along with the ability of the virus to mutate under selective pressure sets a tough standard for new antiretroviral agents: the drug must be well tolerated and effective against drug resistant strains of HIV-1. There are no clear strategies for identification of novel antiretroviral nucleosides; rather, an empirical approach is still the norm, and there are no set criteria, from a preclinical perspective, which can predict clinical success. None of the compounds reported in this text as being in clinical or preclinical development is guaranteed to reach commercialization. Even FTC, which appeared to be well on the way towards successful completion of its clinical development program, is now undergoing re-evaluation. However, apart from the discovery of a prophylactic or therapeutic vaccine, combination therapy will continue to dominate the HIV treatment paradigm in the near future, and as the need is so great, the difficulty of this task must not daunt efforts to produce new, effective anti-HIV-1 agents. Fortunately, efforts are underway to identify not only new NRTIs (as described here) but also new NNRTIs and PIs. In addition, in the past 15 years, the growth of our understanding of the biology and pathogenesis of HIV-1 infection has led to the identification and validation of additional molecular targets to be used in the search for effective anti-viral agents. From a therapeutic perspective, the next major step forward in anti-HIV-1 therapy will

occur when compounds directed against some of these newer molecular targets, as well as new NRTIs, NNRTIs or PIs, active against drug resistant virus, pass through clinical trials and are included in the existing combination regimens.

Acknowledgements

We would like to thank Zhengxian Gu for help in producing Figure 2 and Andree Briseboise and Rachel Unger for help in preparation of this manuscript.

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