Antiviral Agents Acting as DNA or RNA Chain Terminators

E. De Clercq and J. Neyts

Contents

Abstract Nucleoside or nucleotide analogue inhibitors of viral replication almost act as chain terminators during DNA (DNA- and retroviruses) or RNA (RNA viruses) synthesis. Following intracellular phosphorylation, by viral and/or cellular kinases, the 5′-triphosphate metabolites (or 2′-diphosphate metabolites in the case of acyclic nucleoside phosphonate analogues) compete with the natural substrate in the DNA or RNA polymerization reaction. Obligatory chain terminators (e.g., acyclovir) do not offer the 3′-hydroxyl function at the riboside moiety of the molecule. Nucleoside analogues that possess a hydroxyl function at a position equivalent of the 3′-hydroxyl position may act as chain terminators if this hydroxyl group is conformationally constrained (e.g., ganciclovir) or sterically hindered to enter into a phosphodiester linkage with the incoming nucleotide. In case that the 3′-hydroxylgroup is correctly positioned, chain elongation may be hampered through steric hindrance from neighboring substituents (e.g., 2′-*C*-methyl or 4′-azido nucleoside inhibitors of HCV replication). Here, we review the molecular mechanism of action and the clinical applications of the nucleosides and nucleotides acting as chain terminators. A further discussion of clinical applications in combination therapy can be found in Chap. 12.

E. De Clercq (✉)
Rega Institute for Medical Research, K.U.Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium
erik.declercq@rega.kuleuven.be

H.-G. Kräusslich, R. Bartenschlager (eds.), *Antiviral Strategies,* Handbook of Experimental Pharmacology 189,

Abbreviations

TACV	Triacyclic analogue of acyclovir
TGCV	Triacyclic analogue of ganciclovir
ANP	Acyclic nucleoside phosphonate
TK	Thymidine kinase
HPMPA	(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine
ACV	Acyclovir
GCV	Ganciclovir
TDF	Tenofovir disoproxil fumarate
HPMPC	Cidofovir, (S)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine
PMPA	Tenofovir, (*R*)-9-(2-phosphonylmethoxypropyl)adenine
PMEA	Adefovir, 9-(2-phosphonylmethoxyethyl)adenine
HDP	Hexadecyloxypropyl
ODP	Octadecyloxyethyl
ddN	2′,3′-Dideoxynucleoside
NRTIs	Nucleoside reverse transcriptase inhibitors
NtRTIs	Nucleotide reverse transcriptase inhibitors
NNRTIs	Nonnucleoside reverse transcriptase inhibitors
NRRIs	Nucleoside RNA replicase inhibitors
NNRRIs	Nonnucleoside RNA replicase inhibitors

1 Introduction

Most of the nucleoside (or nucleotide) analogues used as antiviral agents act by terminating elongation of the nucleic acid chain during DNA (DNA viruses and retroviruses) or RNA (RNA viruses) synthesis. To act as DNA or RNA chain terminators, these compounds have to be converted to their 5′-triphosphate form before they compete with the natural substrates (dNTPs for DNA synthesis and NTPs for RNA synthesis) in the DNA or RNA polymerization reaction. When incorporated in their 5′-monophosphate form, after removal of the pyrophosphate group, at the 3′-end of the growing nucleic acid chain, they terminate chain elongation. In essence, they do so if they do not offer the 3′-hydroxyl function at the (2′-deoxy)riboside moiety, which is required for attachment of the incoming nucleotide. The nucleotide analogues that act in this fashion can be termed obligatory chain terminators [typical examples are acyclovir (triphosphate) and adefovir (diphosphate)].

However, nucleotide analogues possessing an hydroxyl function at a position equivalent to the 3′-hydroxyl position may nevertheless act as chain terminator if this hydroxyl group is conformationally constrained [as in ganciclovir (triphosphate) or cidofovir (diphosphate)] or sterically hindered to enter into a phosphodiester linkage with the incoming nucleotide. But even if the 3′-hydroxyl group would be correctly positioned, further chain elongation may still be hampered through steric hindrance from neighboring substituents such as, for example, from the

2′-*C*-methyl group or 4′-*C*-azido group in nucleoside analogues targeting the hepatitis C virus (HCV) RNA polymerase NS5B.

Depending on their activity spectrum and structural characteristics, chain terminators can be divided into four categories: (1) acyclic (or carbocyclic) nucleoside analogues (prototype: acyclovir), which are primarily active against herpesviruses [HSV (herpes simplex virus), VZV (varicella-zoster virus), and CMV (cytomegalovirus)] (Fig. 1); (2) acyclic nucleoside phosphonates (prototypes: cidofovir, adefovir, and tenofovir), which are targeted at DNA viruses (cidofovir), hepadnaviruses (adefovir), and retroviruses (tenofovir) (Fig. 2); (3) 2′,3′-dideoxynucleoside analogues, which are active against retroviruses [immunodeficiency virus (HIV)] as well as hepadnaviruses [hepatitis B virus (HBV)] (Fig. 3); and (4) the anti-HBV (Fig. 4a) and anti-HCV agents (Fig. 4b). These different categories of chain terminators will be reviewed, essentially from two viewpoints: first, their clinical use, and second, their mechanism of action.

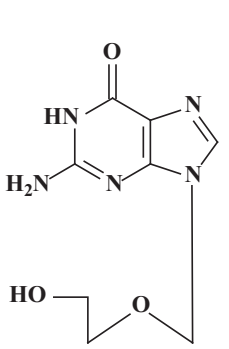
2 Acyclic (or Carbocyclic) Nucleoside Analogues

Of the acyclic nucleoside analogues (Fig. 1), acyclovir and its prodrug valaciclovir, ganciclovir and its prodrug valganciclovir, penciclovir and its prodrug famciclovir have been formally licensed for clinical use. Except for (val)ganciclovir, which can lead to bone marrow suppression (i.e., neutropenia), the acyclic nucleoside analogues are generally well tolerated with few, if any, side effects.

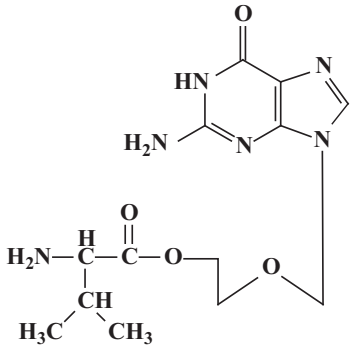
Acyclovir and valaciclovir (Fig. 1) are used in the treatment of mucosal, cutaneous and systemic HSV-1 and HSV-2 infections (including herpetic keratitis, herpetic encephalitis, genital herpes, neonatal herpes, and herpes labialis), and VZV infections (including varicella and herpes zoster). Acyclovir is administered orally at doses of 1 g $(5 \times 200\,\text{mg})$ per day for genital herpes, up to 4 g $(5 \times 800\,\text{mg})$ per day for herpes zoster; or topically as a 3% ophthalmic cream for herpetic keratitis or 5% cream for herpes labialis; or intravenously at 30 mg/kg $(3 \times 10\,\text{mg/kg})$ per day for herpetic encephalitis or other severe infections with herpesviruses. Valaciclovir is administered orally at 1 g $(2 \times 500\,\text{mg})$ per day for genital herpes and up to 3 g $(3 \times 1\,\text{g})$ per day for herpes zoster.

Ganciclovir and valganciclovir (Fig. 1) are used in the treatment of CMV infections (e.g., CMV retinitis) in immunosuppressed (e.g., AIDS) patients. Ganciclovir is administered intravenously at 10 mg/kg $(2 \times 5\,\text{mg/kg})$ per day for induction therapy. Valganciclovir is administered orally at 900 mg $(2 \times 450\,\text{mg})$ per day for maintenance therapy. Both valaciclovir and valganciclovir can also be used as prophylaxis of CMV infections via the oral route in transplant recipients.

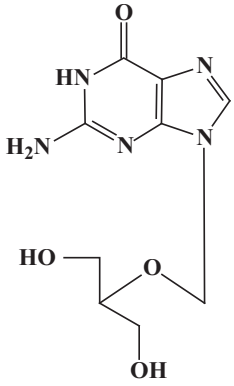
Penciclovir (Fig. 1) can be used topically (as a 1% cream) in the treatment of superficial mucocutaneous HSV infections. Its prodrug famciclovir (Fig. 1) is administered orally at 750 mg $(3 \times 250\,\text{mg})$ or 1,500 mg $(3 \times 500\,\text{mg})$ per day in the treatment of HSV-1 or HSV-2 infections and VZV infections (i.e., herpes zoster).



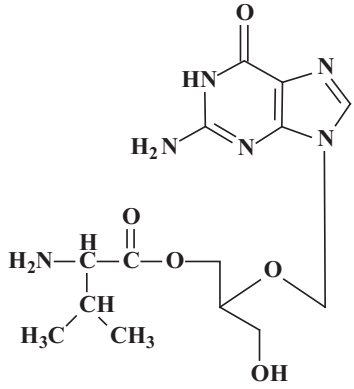
Acyclovir (ACV)
Zovirax®



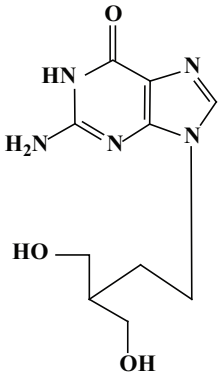
Valaciclovir (VACV)
Zelitrex®, Valtrex®



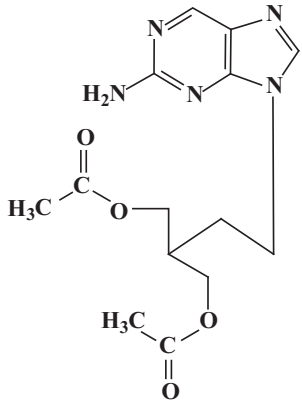
Ganciclovir
Cymevene®, Cytovene®



Valganciclovir
Valcyte®

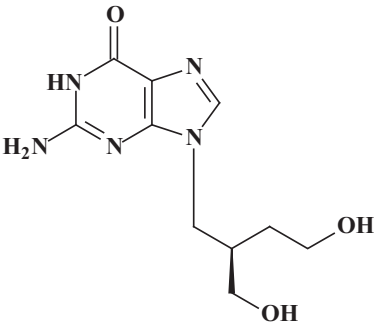


Penciclovir (PCV)
Denavir®, Vectavir®

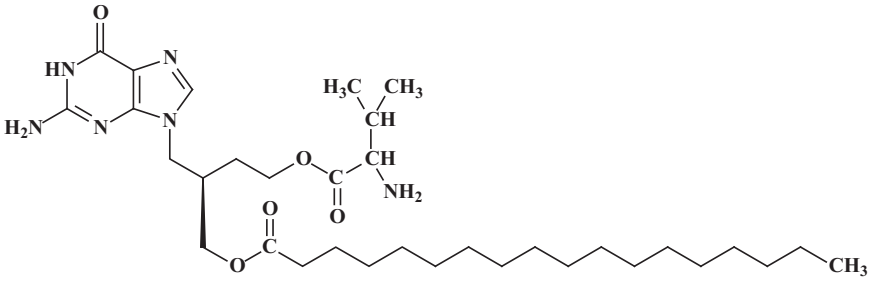


Famciclovir (FCV)
Famvir®

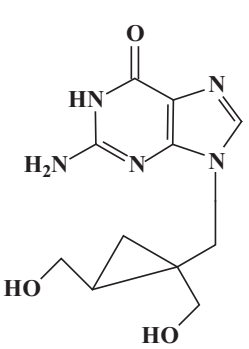
Fig. 1 Structural formulae of acyclic nucleoside analogues (anti-herpesvirus agents)



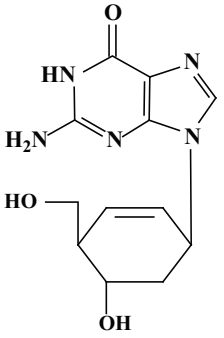
H2G
2HM-HBG



H2G prodrug
MIV-606
Valomaciclovir stearate



A-5021



Cyclohexenyl G

Fig. 1 (continued)

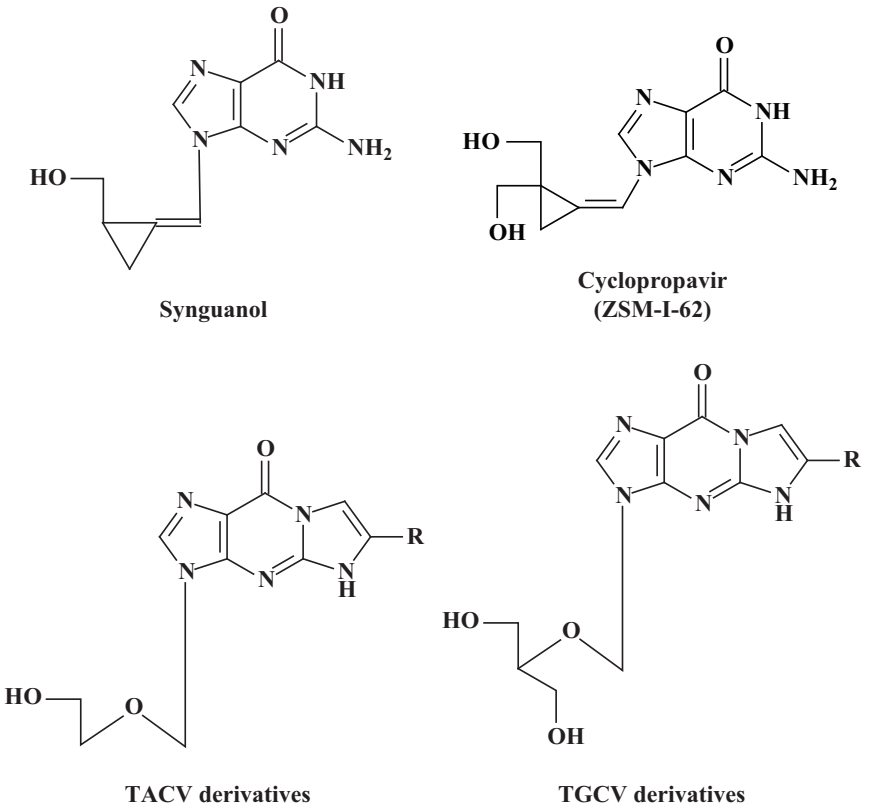


Fig. 1 (continued)

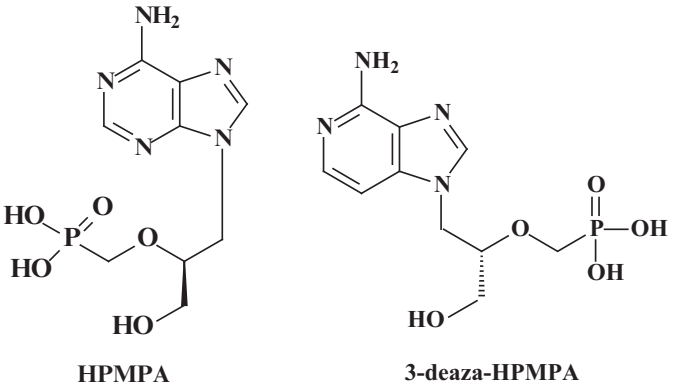
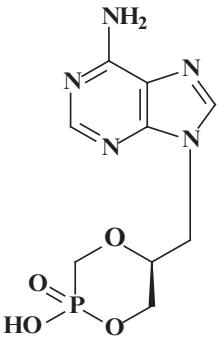
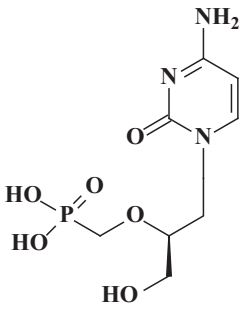


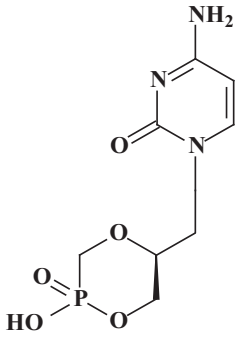
Fig. 2 Structural formulae of acyclic nucleoside phosphonates (anti-DNA virus and/or -retrovirus agents)



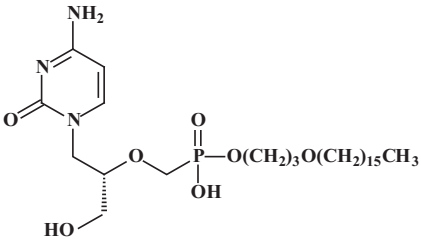
cHPMPA



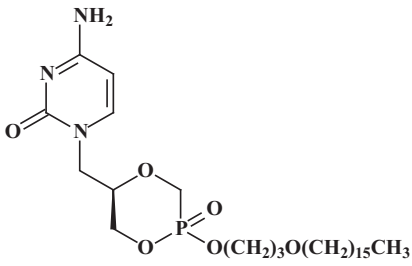
HPMPC
CDV



cHPMPC
cCDV

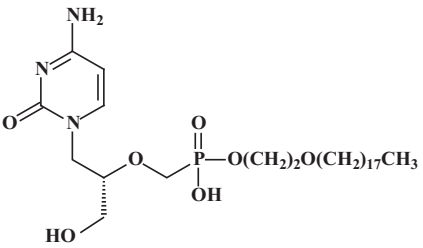


HDP-CDV
(CMX001)

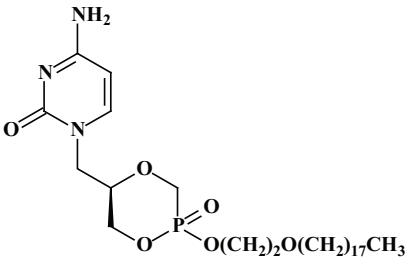


HDP-cCDV

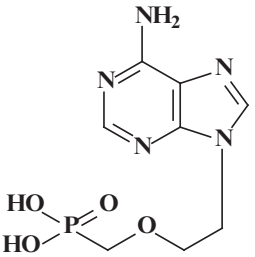
Fig. 2 (continued)



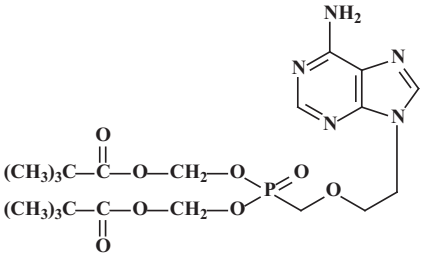
ODE-CDV



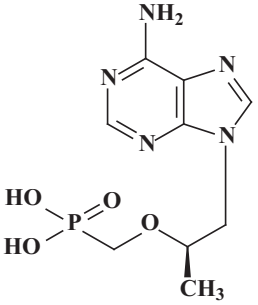
ODE-cCDV



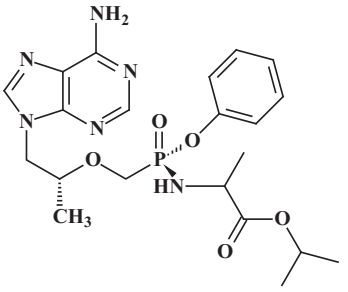
PMEA
Adefovir



Bis(POM)PMEA
Adefovir dipivoxil

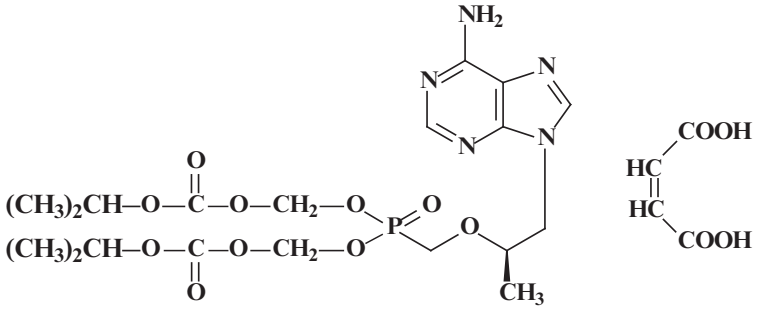


PMPA
Tenofovir

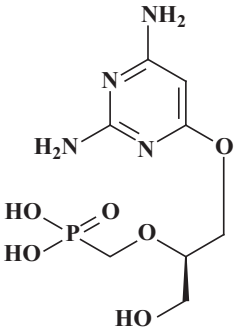


GS 7340

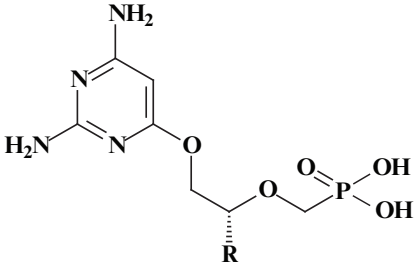
Fig. 2 (continued)



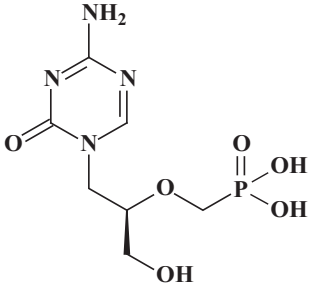
Bis(POC)PMPA fumarate
Tenofovir disoproxil fumarate



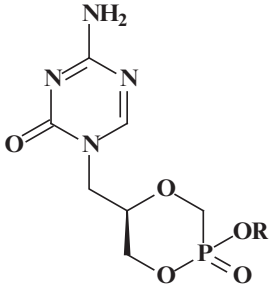
HPMPO-DAPy



R = CH3 : PMPO-DAPy
R = H : PMEO-DAPy

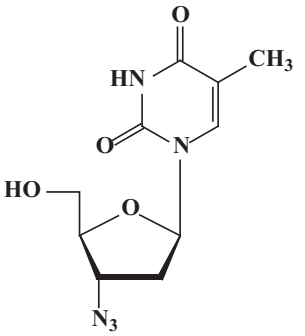


5-aza-HPMPC

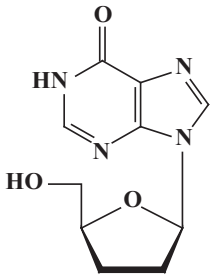


R = H: 5-aza-cHPMPC
R = Alkoxyalkyl: 5-Aza-cHPMPC prodrugs

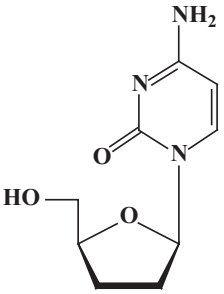
Fig. 2 (continued)



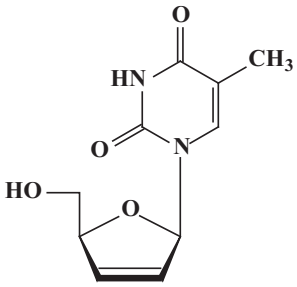
Zidovudine
3'-Azido-2',3'-dideoxythymidine,
azidothymidine (AZT)
Retrovir®



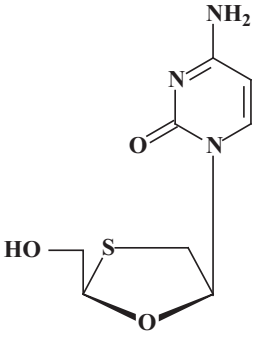
Didanosine
2',3'-Dideoxyinosine (ddI)
Videx®, Videx® EC



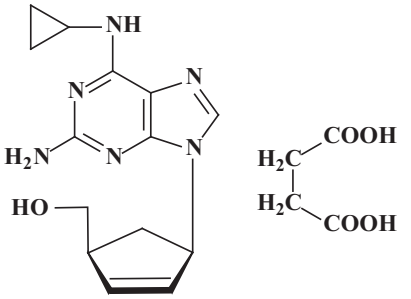
Zalcitabine
2',3'-Dideoxycytidine (ddC)
Hivid®



Stavudine
2',3'-Didehydro-2',3'-dideoxythymidine
(d4T)
Zerit®

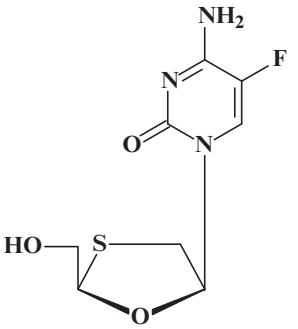


Lamivudine
2',3'-Dideoxy-3'-thiacytidine
3TC
Epivir®

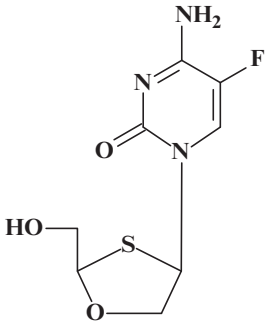


Abacavir
(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-
9*H*-purin-9-yl]-
2-cyclopentene-1-methanol succinate
(ABC)
Ziagen®

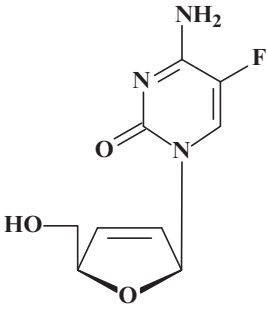
Fig. 3 Structural formulae of 2′,3′-dideoxynucleoside analogues (anti-HIV) agents



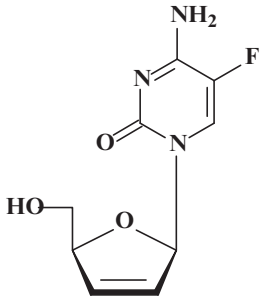
(-)-FTC
Emtricitabine
(-)-β-L-3'-thia-2',3'-dideoxy-5-fluorocytidine
Emtriva®



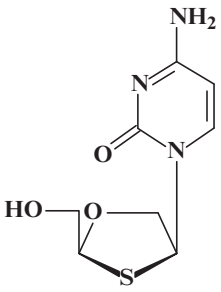
Racemic (±)FTC (FdOTC)
Racivir®



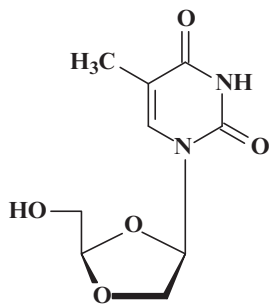
ACH-126443 (β-L-Fd4C)
Elvucitabine



DPC-817 (β-D-Fd4C)
Reverset® (RVT)
Dexelvucitabine

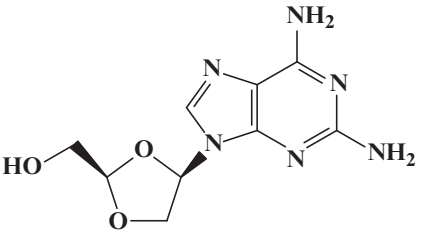


SPD-754, AVX-754 ((-)-dOTC)
Apricitabine

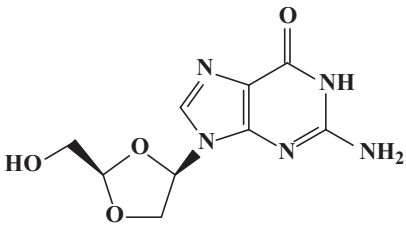


1-(β-D-dioxolane)thymine
DOT

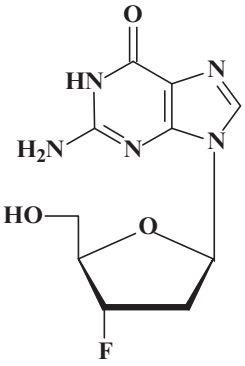
Fig. 3 (continued)



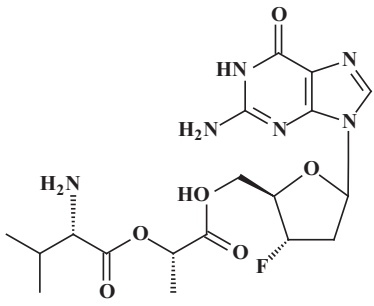
DAPD
Amdoxovir



DXG

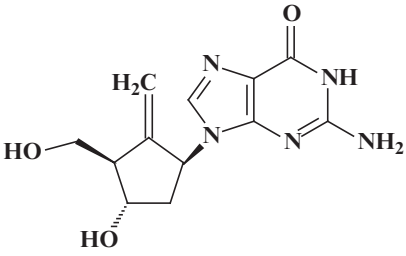


FLG
3'-Fluoro-2',3'-dideoxyguanosine

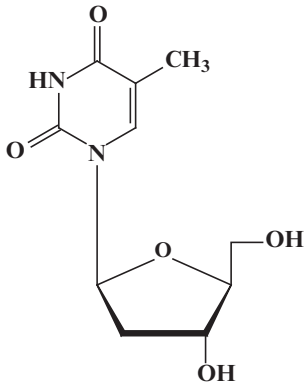


MIV-210
[FLG (3'-fluoro-2',3'-dideoxyguanosine) prodrug]

Fig. 3 (continued)

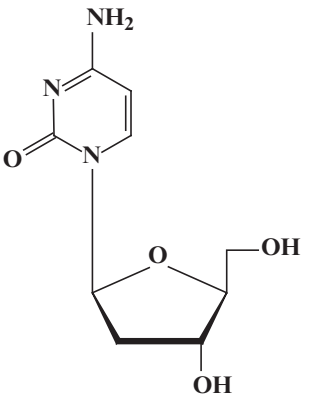


Entecavir
Baraclude®

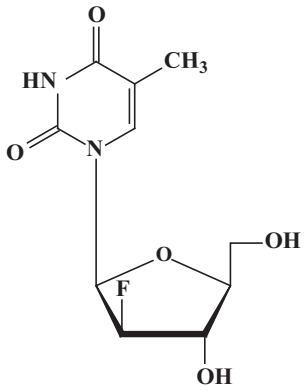


L-dT
β-L-thymidine
Telbivudine
Tyzeka®, Sebivo®

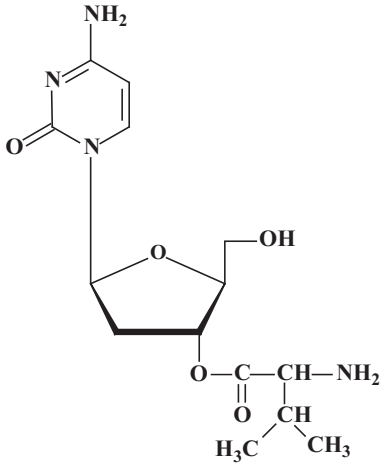
Fig. 4a Structural formulae of anti-hepatitis B agents



L-dC
β-L-2'-deoxycytidine



L-FMAU
Clevudine

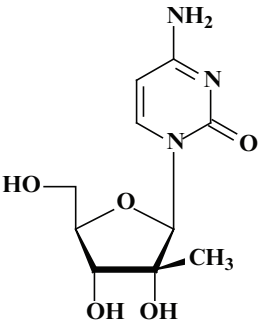


3'-Val-L-dC
3'-Valine ester of β-L-2'-deoxycytidine
Valtorcitabine

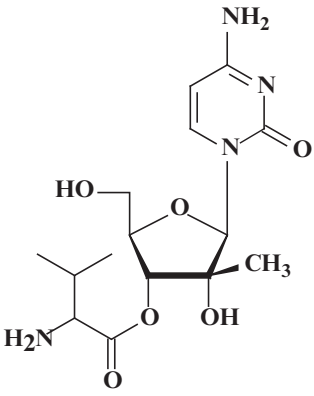
Fig. 4a (continued)

With acyclovir, valaciclovir, ganciclovir, valganciclovir, and famciclovir being on the market (De Clercq 2004a), the only other acyclic guanosine analogue under clinical development for the potential treatment of HSV and VZV infections is MIV-606 (valomaciclovir stearate), the oral prodrug of H2G (Fig. 1) (De Clercq and Field 2006). This compound is being pursued primarily for the treatment of herpes zoster.

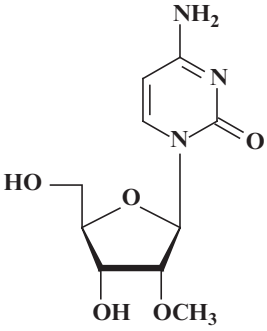
Carbocyclic guanosine analogues have been described to inhibit HSV-1, HSV-2, VZV, CMV, and/or other herpesviruses [viz. HHV-6 (human herpesvirus type 6)],



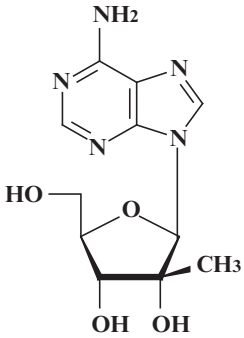
2'-*C*-methylcytidine



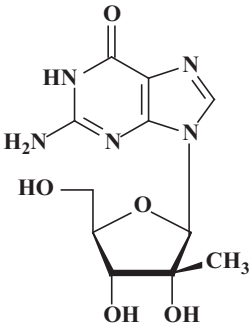
NM283
3'-Valine ester of 2'-*C*-methylcytidine
Valopicitabine



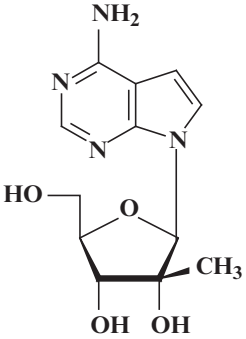
2'-*O*-methylcytidine



2'-*C*-methyladenosine

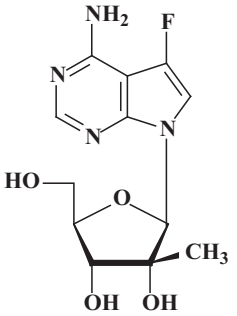


2'-*C*-methylguanosine

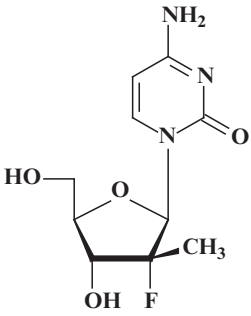


7-deaza-2'-*C*-methyladenosine

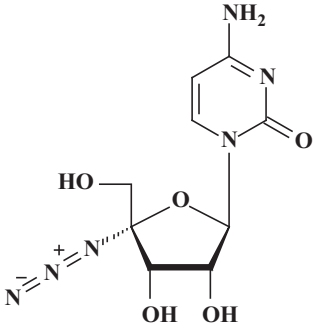
Fig. 4b Structural formulae of anti-hepatitis C agents



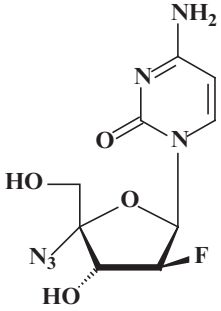
7-Deaza-7-fluoro-2'-*C*-methyladenosine



2'-Deoxy-2'-fluoro-2'-*C*-methylcytidine
PSI-6130



4'-Azidocytidine
(R1479)



RO-0622

Fig. 4b (continued)

including A-5021, cyclohexenylguanine, synguanol (De Clercq et al. 2001), and cyclopropavir (Zhou et al. 2004; Kern et al. 2005) (Fig. 1). The latter compound, cyclopropavir, has been reported to be very effective in reducing mortality in mice infected with murine CMV (Kern et al. 2004).

The acyclic guanosine analogues acyclovir (ACV) and ganciclovir (GCV) have been further modified by introduction of a 1,N^2-ethene-1,2-diyl bridge to form their tricyclic analogues TACV and TGCV (Fig. 1) (Golankiewicz et al. 1994, 2001; Ostrowski et al. 2006). These tricyclic analogues exhibited marked activity against HSV-1, HSV-2, and VZV, some of their six-substituted derivatives being intrinsically fluorescent.

All acyclic and carbocyclic guanosine analogues depicted in Fig. 1 follow the same *modus operandi* as exemplified for acyclovir (ACV) in Fig. 5, in that they need three phosphorylations to be converted to their active metabolite, the triphosphate form, which then interacts with the target enzyme, the viral DNA polymerase, as a chain terminator (De Clercq 2002). In its DNA chain-terminating

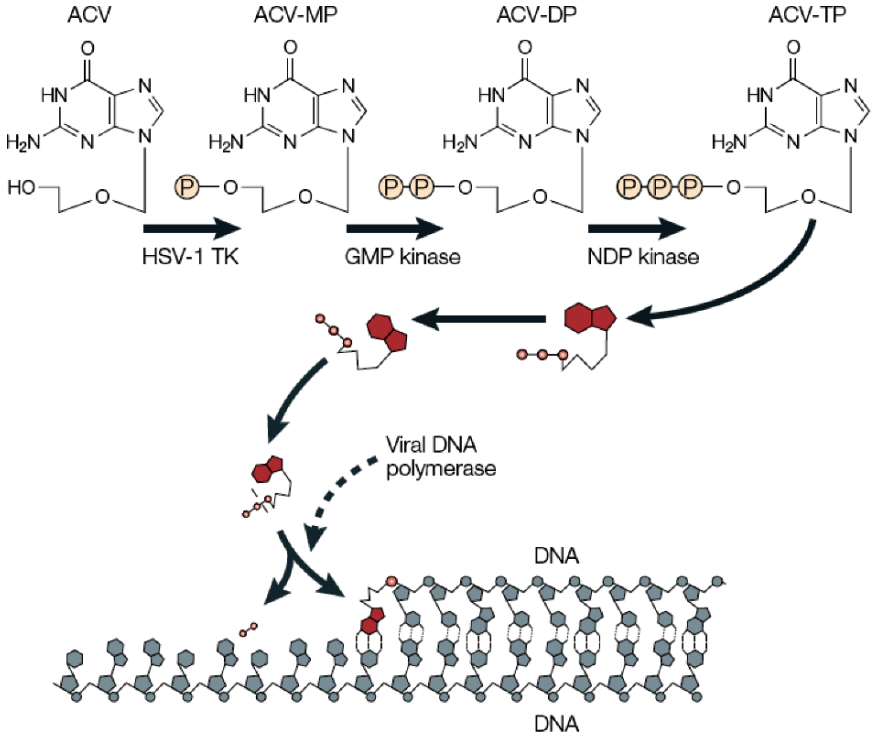


Fig. 5 Mechanism of antiviral action of acyclovir (De Clercq 2002)

action, acyclovir triphosphate (ACV-TP) directly competes with the natural substrate (dGTP). To be converted to ACV-TP, ACV is successively phosphorylated by the HSV- or VZV-encoded thymidine kinase, and two cell-derived kinases, guanosine 5′-monophosphate kinase (GMP kinase) and nucleoside 5′-diphosphate kinase (NDP kinase).

The first phosphorylation carried out by the virus-induced thymidine kinase (TK) (Elion et al. 1977) is crucial for the specific (and selective) activity of acyclovir and all the acyclic (and carbocyclic) analogues derived thereof (Fig. 1). Herpesviruses which do not encode their own TK or which lost their capacity (e.g., TK-deficient HSV-1, HSV-2, or VZV variants) no longer respond to the antiviral action of acyclovir or other acyclic guanosine analogues and vice versa; human tumor cells that have been transduced by the HSV TK become exquisitely sensitive to the cytostatic action of these acyclic guanosine analogues (i.e., ganciclovir) (Degrève et al. 1999; De Clercq et al. 2001).

In this context, it should be noted that the specific antiviral activity of ganciclovir against HSV (which is more potent than that of acyclovir) can be fully explained by the compound being specifically recognized as substrate by the HSV-encoded TK. For CMV, however, which does not encode a virus-specified TK, the activity of ganciclovir depends on the phosphorylation by a virus-encoded protein kinase, which

specifically recognizes ganciclovir as substrate for conversion to its monophosphate (Sullivan et al. 1992). The latter then follows the same pathway as illustrated for acyclovir in Fig. 5.

3 Acyclic Nucleoside Phosphonates

The acyclic nucleoside phosphonates (ANPs) can be considered as nucleotide rather than nucleoside analogues, in that, besides the purine or pyrimidine base, they contain an (acyclic) sugar moiety to which a phosphonate is attached. In these nucleotide analogues (Fig. 2), the phosphoric ester grouping $(=\mathrm{P}--\mathrm{O}--\mathrm{C}--)$ is transformed to its isomeric phosphonomethyl ether $(=\mathrm{P}--\mathrm{C}--\mathrm{O}--)$, which is able to withstand attack by cytoplasmic enzymes (De Clercq and Holý 2005). This phosphonate group contributes not only to the prolonged antiviral activity of this class of compounds and their relative resilience to antiviral drug resistance development, but is also associated with dose-limiting nephrotoxicity.

The prototype member of the ANPs is (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) (Fig. 2), first described for its broad-spectrum anti-DNA virus activity in 1986 (De Clercq et al. 1986). Then followed by the description of various other acyclic nucleoside phosphonates in 1987 (De Clercq et al. 1987). At present three acyclic nucleoside phosphonates have been licensed for clinical use: cidofovir, adefovir, and tenofovir (Fig. 2).

Cidofovir (Fig. 2) has been formally approved for the treatment of CMV retinitis in AIDS patients, where it is administered intravenously at a dose not exceeding 5 mg/kg once weekly during the first two weeks (and every other week thereafter). Cidofovir is also used "off label" for the treatment of human papilloma virus (HPV) infections (i.e., cutaneous warts, anogenital warts, laryngeal and pharyngeal papilloma), polyomavirus [i.e., progressive (i.e., multifocal leukoencephalopathy (PML)], adenovirus, herpesvirus, and poxvirus (i.e., molluscum contagiosum) infections, where it can be administered intravenously (at a dose of $\leq 5\,\mathrm{mg/kg}$ once weekly or every other week) or topically as a 1% gel or cream (De Clercq and Holý 2005). Especially in immunosuppressed patients (i.e., transplant recipients), local treatment of HPV-associated lesions has often yielded spectacular results (Bonatti et al. 2007).

Adefovir in its prodrug form, adefovir dipivoxil, is indicated in the treatment of chronic HBV infections (chronic hepatitis B), where, if administered orally as a single dose of 10 mg per day, HBV DNA load is reduced significantly $(>3\log_{10})$ over a 1- or 2-year period (Hadziyannis et al. 2005).

Tenofovir in its prodrug form tenofovir, disoproxil fumarate (TDF), is indicated in the treatment of HIV infections (AIDS). It is administered as a single oral dose of 300 mg per day. When combined with emtricitabine and efavirenz, TDF has proven to be more efficacious than the standard combination therapy of combivir (azidothymidine plus lamivudine) and efavirenz (Gallant et al. 2006) and less prone to cause adverse side effects (Pozniak et al. 2006; De Clercq 2007b).

For the treatment of AIDS, TDF is now available in three commercial preparations, as such (Viread®), in combination with emtricitabine (Truvada®) and in combination with emtricitabine and efavirenz (Atripla®). The latter represents a three-drugs-in-once combination pill, which has become available for the treatment of AIDS since July 2006. The different milestones that marked the development and ultimate commercialization of Atripla® (in 2006) since the original identification of adefovir as an antiretroviral agent (in 1986) have been previously reviewed (De Clercq 2006, 2007a–c).

In addition to cidofovir, adefovir, and tenofovir, several new acyclic nucleoside phosphonates have been recently accredited with marked antiviral effects: that is, 6-[2-phosphonomethoxyalkoxy)-2,4-diaminopyrimidines (HPMPO-DAPy, PMEO-DAPy, and PMPA-DAPy) (Fig. 2) (De Clercq et al. 2005; Balzarini et al. 2007; De Clercq 2007d) and triazine analogues of cidofovir (5-aza-HPMPC, 5-aza-cHPMPC (Fig. 2) (Krečmerová et al. 2007a, b). Further studies are needed to establish whether HPMPO-DAPy, PMEO-DAPy, PMPA-DAPy, 5-aza-cHPMPC, or alkyloxyalkyl prodrugs thereof are advantageous in terms of activity spectrum, efficacy, safety, or pharmacokinetics as compared to the first-generation ANPs cidofovir (HPMPC), adefovir (PMEA), or tenofovir (PMPA).

To increase the oral bioavailability of adefovir and tenofovir, the prodrugs adefovir dipivoxil and tenofovir disoproxil as well as alkoxyalkyl esters of cidofovir, that is, hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE) of HPMPC and HPMPA (Fig. 2), have been developed. These lipid ANP esters show indeed markedly increased oral bioavailability (Painter and Hostetler 2004) as well as enhanced in vitro and in vivo antiviral activity against a wide range of viruses [i.e., adenovirus (Hartline et al. 2005); polyoma-, herpes-, and poxviruses (Kern et al. 2002; Quenelle et al. 2004; Buller et al. 2004; Beadle et al. 2006; Quenelle et al. 2007; Hostetler et al. 2007; Dal Pozzo et al. 2007; Lebeau et al. 2007)]. Quite surprisingly, alkoxyalkyl esters of acyclic nucleoside phosphonates, that is, HDP-HPMPA and ODE-HPMPA, known for their well-established broad-spectrum activity against various DNA viruses and retroviruses, have recently been associated with anti-HCV replicon activity (Wyles et al. 2008), an observation that needs further scrutiny.

Appropriately designed prodrugs, for example, phosphonoamidates (Lee et al. 2005), may allow acyclic nucleoside phosphonates such as tenofovir to be specifically targeted at tissues, that is, lymphatic tissue, where the virus (i.e., HIV) replicates. This principle has been recently extended to another nucleotide analogue, GS-9148 (Cihlar et al. 2008) and its phosphonoamidate prodrug, GS-9131 (Ray et al. 2008).

After the ANPs (i.e., cidofovir, adefovir, and tenofovir) have been released (intra- or extracellularly) from their prodrugs through the intervention of intra- or extracellular esterases, they need only two phosphorylation steps to be converted to their active metabolites (i.e., HPMPCpp, PMEApp, and PMPApp), which will then compete with the natural substrates (dCTP for HPMPCpp, and dATP for PMEApp and PMPApp) for incorporation into the viral DNA (Fig. 6a).

If incorporated into the DNA chain, PMEApp and PMPApp obligatorily act as chain terminators (Fig. 6bb), following incorporation of a single molecule (PMEA

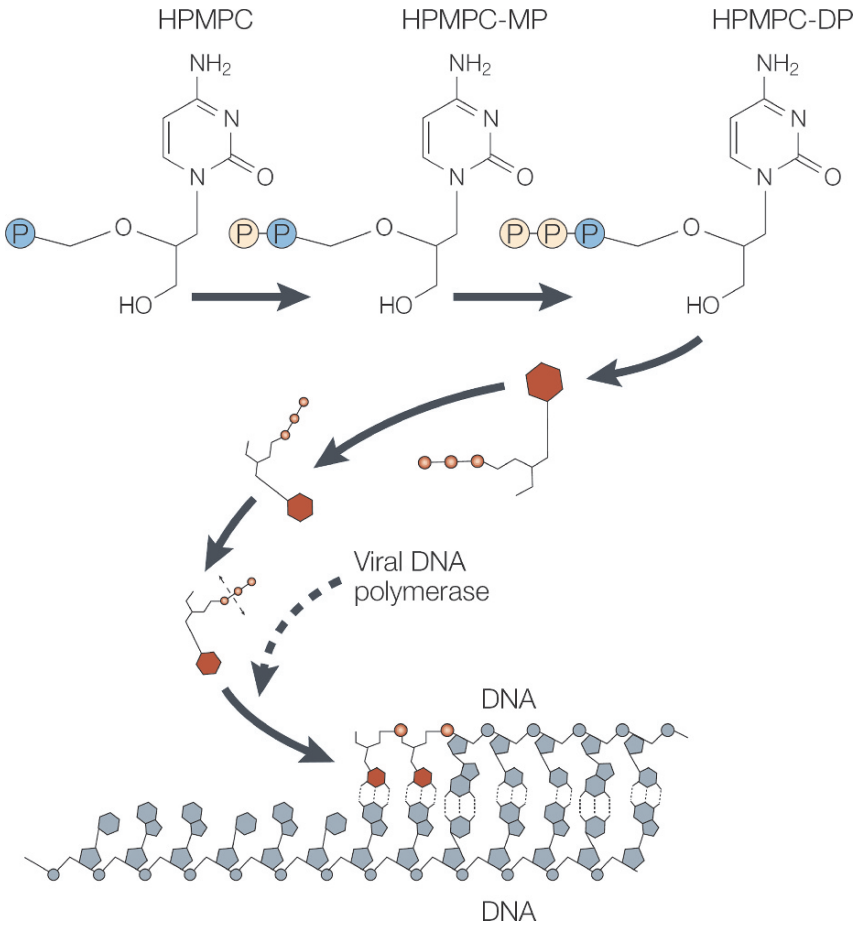


Fig. 6a Mechanism of antiviral action of cidofovir

or PMPA) at the 3′-end (De Clercq and Holý 2005). HPMPC requires two consecutive ("tandem") incorporations to efficiently terminate DNA elongation (Fig. 6ba), as has been demonstrated in the case of CMV DNA synthesis (Xiong et al. 1997). In the case of the vaccinia viral DNA polymerase, HPMPC has been shown to terminate DNA elongation after it is incorporated at the penultimate position, thus allowing one more (regular) nucleotide to be incorporated at the 3′-end (Magee et al. 2005). Recent findings indicated, however, that both HPMPA and HPMPC can be faithfully incorporated into the template strand, thereby inhibiting *trans*-lesion DNA synthesis (Magee et al. 2008).

As has been demonstrated for tenofovir, when incorporated at the 3′-end of reverse transcriptase (RT)-driven DNA chain allows the PMPA residue to adopt multiple conformations [in contrast with the more rigid conformation of the 2′,3′-dideoxynucleosides (see infra) (Tuske et al. 2004). This greater flexibility in conformation may impede development of resistance to tenofovir.

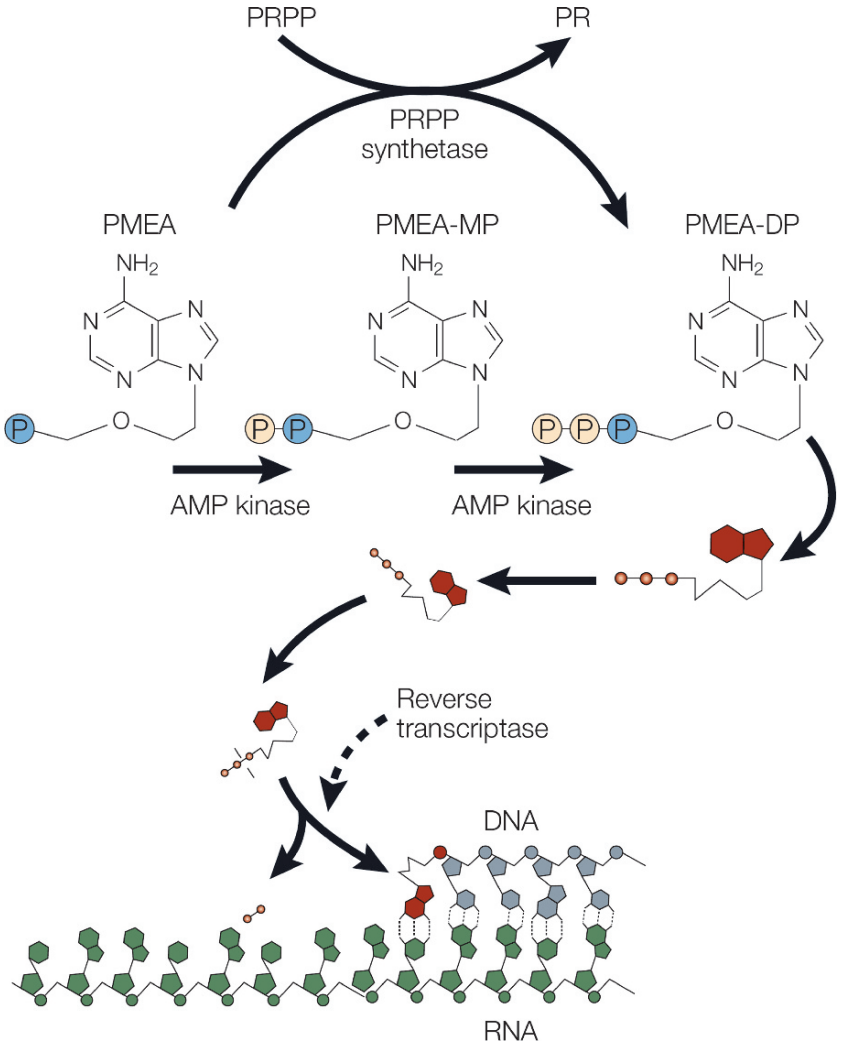


Fig. 6b Mechanism of antiviral action of adefovir

4 Dideoxynucleoside Analogues

The 2′,3′-dideoxynucleoside (ddN) analogues (Fig. 3) encompass a vast group of compounds that have been found active against HIV and HBV, although they have been primarily pursued for the treatment of HIV infections (AIDS). They are targeted at the HIV-associated reverse transcriptase (RT) and therefore also referred to as nucleoside reverse transcriptase inhibitors (NRTIs). They have to be distinguished from the nucleotide reverse transcriptase inhibitors (NtRTIs) such as adefovir (PMEA) and tenofovir (PMPA) (see above) which, like the NRTIs, act as chain

terminators, and the NNRTIs (nonnucleoside reverse transcriptase inhibitors), which directly bind to, and thereby inactivate, the functioning of the HIV-1 RT.

At present there are seven NRTIs, which have been formally approved for the treatment of AIDS: 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine), 2′,3′-dideoxyinosine (ddI, didanosine), 2′,3′-dideoxycytidine (ddC, zalcitabine), 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T, stavudine), (−)-L-3′-thia-2′,3′-dideoxycytidine (3TC, lamivudine), cyclopentenyl N^6-cyclopropylaminopurine (abacavir, ABC), and (−)-L-5-fluoro-3′-thia-2′,3′-dideoxycytidine ((−)FTC, emtricitabine) (De Clercq 2004a) (Fig. 3).

Depending on the nature of the compound, the ddN analogues have been associated with varying toxic side effects such as bone marrow suppression (AZT), pancreatitis (ddI), hypersensitivity reactions (ABC), and neurologic complications consequently to mitochondrial toxicity (ddC), while others, such as 3TC and (-)FTC, have few, if any, side effects.

These compounds are used in the treatment of HIV-1 and HIV-2 infections (lamivudine is also used in the treatment of HBV infections). All the NRTIs are administered orally; AZT at a dose of 600 mg (2 × 300 mg) per day, ddI at a dose of 400 mg (2 × 200 mg) per day, ddC at a dose of 2.25 mg (3 × 0.75 mg) per day, d4T at a dose of 80 mg (2 × 40 mg) per day, 3TC at a dose of 300 mg (2 × 150 mg) per day (for the treatment of HBV infections at a daily dose of 100 mg), ABC at a dose of 600 mg (2 × 300 mg) per day, and (−)FTC at a dose of 200 mg per day. Commercially available are also fixed dose combinations of AZT (300 mg) with 3TC (150 mg) (Combivir®, 2 pills daily) and of AZT (300 mg) with 3TC (150 mg) and ABC (300 mg) (Trizivir®, 2 pills daily), and, as mentioned earlier, of (−)FTC (200 mg) with TDF (300 mg) (Truvada®, 1 pill daily), and of (−)FTC (200 mg) with TDF (300 mg) and the NNRTI efavirenz (600 mg) (Atripla®, 1 pill daily).

All NRTIs, as exemplified for AZT (Fig. 7), act in a similar fashion: following their uptake by the cells, they are phosphorylated successively to their 5′-monophosphate, 5′-diphosphate, and 5′-triphosphate form (De Clercq 2002). Unlike the first phosphorylation step in the metabolic pathway of the acyclic guanosine analogues (see above), which is carried out by a virus-encoded enzyme (thymidine kinase), the first as well as the subsequent phosphorylations of the 2′,3′-dideoxynucleosides are carried out by cellular enzymes, that is, a 2′-deoxynucleoside (e.g., dThd) kinase, a 2′-deoxynucleotide (e.g., dTMP) kinase, and a (2′-deoxy)nucleoside 5′-diphosphate (NDP) kinase.

After the ddNs have been converted to their 5′-triphosphate form (ddN-TP), they shut off RNA-dependent DNA synthesis as obligate chain terminators: AZT-TP and d4T-TP in competition with dTTP; ddC-TP, 3TC-TP and (−)FTC-TP in competition with dCTP; ddA-TP (originating from ddI via the following pathway: ddI → ddIMP → succinyl − ddAMP → ddAMP → ddADP → ddATP) in competition with dATP; and carbovir-TP (formed from ABC upon its phosphorylation to ABC-MP, deamination to carbovir-MP, and phosphorylation to carbovir-DP and -TP) in competition with dGTP. Thus, for all natural substrates of DNA synthesis, competing ddN analogues have been developed that eventually act as chain terminators of the HIV RT-driven DNA polymerization (De Clercq 2004b).

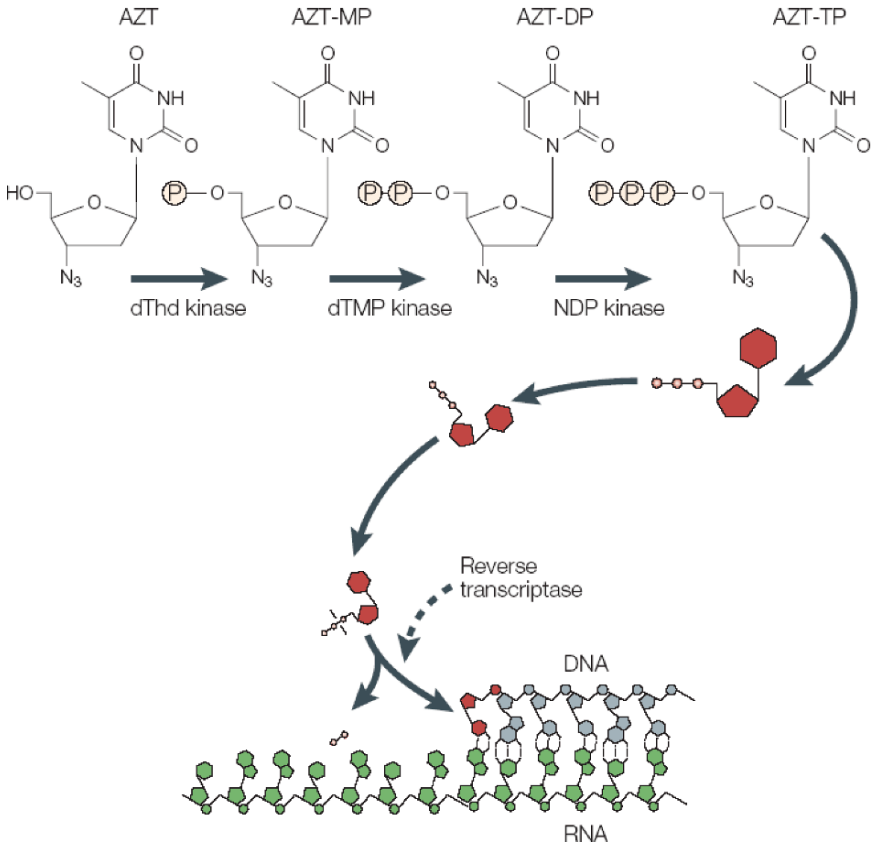


Fig. 7 Mechanism of antiviral action of AZT

In addition to the seven ddNs (or NRTIs) that have been formally approved for the treatment of HIV infection, several other NRTIs acting as chain terminators are still (or have been) under (pre)clinical development: that is, amdoxovir, racivir, dexelvucitabine [previously called reverset (D-d4FC)], elvucitabine (L-d4FC), and D-DOT (dioxolane thymine) (Fig. 3), and starting from D-d4FC and L-d4FC, D- and L-2′,3′-didehydro-2′,3′-dideoxy-3′-fluoro-carbocyclic nucleosides have been synthesized (Wang et al. 2005, 2007).

In principle, resistance to ddNs can be attributed to reduced binding affinity of the ddN-TP to the HIV RT or increased excision velocity of the chain terminating ddN-MP from the 3′-end (see chapter by Nijhuis et al, this volume, for a discussion of resistance mechanisms). The new ddN analogues (i.e., D-d4FC and D-DOT) were designed to circumvent the resistance problem. As compared to other ddN analogues, D-DOT is more resilient to TAM (thymidine analogue-associated mutations), and, also as compared to AZT-MP, DOT-MP is less efficiently removed from the 3′ -end through ATP-mediated pyrophosphorolysis (Lennerstrand et al.

2007). The potent activity of D-DOT against AZT- and 3TC-resistant HIV-1 strains together with its excellent pharmacokinetic profile in rhesus monkeys suggest that further development of D-DOT towards HIV-1 chemotherapy is warranted (Asif et al. 2007).

5 Nucleoside analogues active against HBV or HCV

All the nucleoside (and nucleotide) analogues that have entered the clinic for the treatment of HBV infections (i.e., nucleoside analogues: lamivudine, entecavir, telbivudine; nucleotide analogues adefovir and tenofovir) are fairly well tolerated without side effects that would preclude their long-term usage. The nucleoside analogues in (pre)clinical development for the treatment of HCV infections are not yet sufficiently advanced to assess their tolerability and/or safety.

5.1 Anti-HBV Agents

In addition to the NRTI lamivudine (3TC) and the NtRTI adefovir dipivoxil and tenofovir disoproxil fumarate (which has been recently licensed for the treatment of chronic hepatitis B), two other nucleoside analogues, that is, entecavir and L-dT (telbivudine) (Fig. 4aa), have been licensed for the treatment of HBV infections. Two other compounds 3′-Val-L-dC (valtorcitabine) and L-FMAU (clevudine) (Fig. 4aa) are in clinical development for the treatment of HBV infections, and yet two other compounds, that is, racivir and elvucitabine (Fig. 3), yield potential for the treatment of both HBV and HIV infections.

Thus, entecavir (Zoulim 2006) and telbivudine (Ruiz-Sancho et al. 2007) represent new treatment options for chronic hepatitis B, although, surprisingly (Hirsch 2007), entecavir led to the emergence of HIV-1 variants with the lamivudine-resistant mutation M184V in patients with HIV-1 and HBV coinfection (McMahon et al. 2007). This argues against the use of entecavir in persons coinfected with HIV-1 and HBV, who are not receiving fully suppressive anti-HIV drug regimens.

Entecavir, telbivudine, clevudine, and the other nucleoside analogues (Fig. 4aa) need to be phosphorylated to their 5′-triphosphate form to be antivirally active (Fig. 8). This again implies three phosphorylation steps based successively on a nucleoside kinase, nucleoside 5′-monophosphate kinase, and nucleoside 5′-diphosphate kinase. These reactions have been characterized only in a few cases, that is, thymidylate kinase in the metabolism of clevudine (Hu et al. 2005).

The 5′-triphosphate metabolite of entecavir has been shown to accumulate intracellularly at concentrations that are inhibitory to 3TC-resistant HBV DNA polymerase (Levine et al. 2002). This would imply that entecavir should be active against HBV infections that have become resistant to treatment with lamivudine. Yet, it should be taken into account that treatment with lamivudine leads to the same

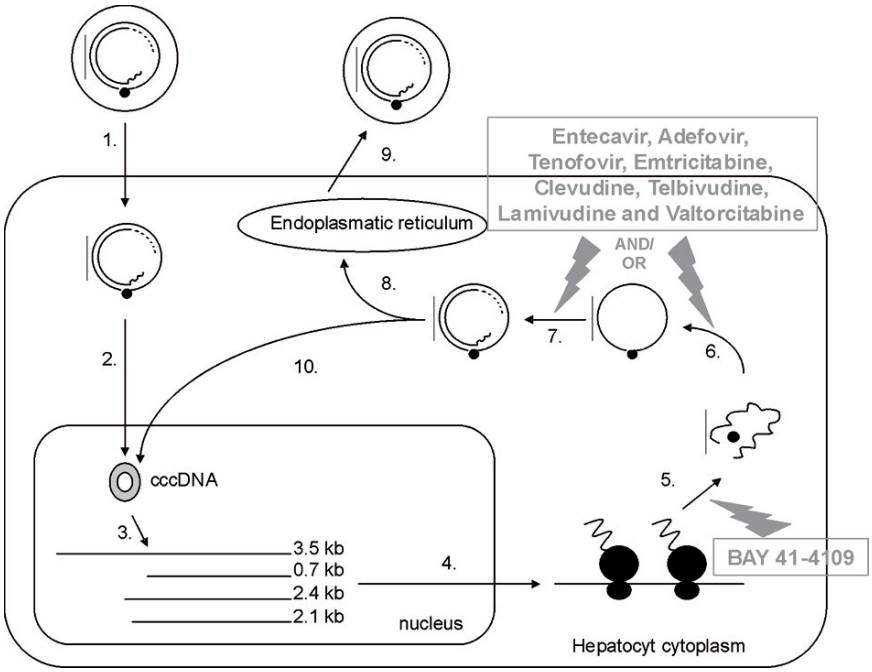


Fig. 8 HBV replication cycle and site of action of several anti-HBV agents

resistance mutations (M204I/V) that may also appear following entecavir treatment (Locarnini et al. 2004). Adefovir resistance, however, is based on mutations (A181V, N236T) that do not give cross-resistance with either lamivudine or entecavir (Locarnini et al. 2004).

The mechanism of action of entecavir, L-dT, L-dC, and L-FMAU at the HBV DNA polymerase level remains to be established. Since these compounds contain a 3′-hydroxyl group, they should, theoretically, be able to permit DNA chain elongation. If, nevertheless, they act as chain terminators, they may do so through an altered conformation of the sugar ring, resulting in an inadequate positioning of the 3′-hydroxyl group prohibiting DNA chain elongation.

5.2 Anti-HCV Agents

In analogy with the designation of NRTIs and NNRTIs for the "nucleoside" and "nonnucleoside" type of reverse transcriptase (RT) inhibitors to target HIV, the corresponding inhibitors to target HCV may be termed NRRIs (for nucleoside RNA replicase inhibitors) and NNRRIs (for nonnucleoside RNA replicase inhibitors).

The NRRI 2′-*C*-methylcytidine (Fig. 4ab) has in its oral prodrug form (NM 283, valopicitabine) acceded into phase II clinical trials in chronic HCV-infected patients.

Preclinical data suggest antiviral synergy for the combination of NM 283 with interferon (IFN) (Afdhal et al. 2004). In vitro ribavirin [a nucleoside analogue that acts as an IMP dehydrogenase inhibitor (not a chain terminator)] was shown to antagonize the anti-HCV activity of 2-*C*-methylcytidine (Coelmont et al. 2006). Treatment with NM 283 at optimal dosing produced consistent HCV RNA reduction averaging >1.2 $\log_{10}$ in a difficult-to-treat cohort, that is, patients that had previously failed pegylated IFN and ribavirin combination therapy (Toniutto et al. 2007). Valopicitabine combined with standard therapy of pegylated interferon and ribavirin was shown to clear HCV in 72% of patients who completed 12 weeks of treatment (www.idenix.com).

Following 2′-*C*-methylcytidine, various other nucleoside analogues targeting the HCV NS5B polymerase have been reported to inhibit HCV replication in vitro (HCV replicon system): 2′-*O*-methylcytidine (Carroll et al. 2003), 2′-*C*-methyladenosine (Carroll et al. 2003; Tomassini et al. 2005), 2′-*C*-methylguanosine (Migliaccio et al. 2003; Eldrup et al. 2004), 7-deaza-2′-*C*-methyladenosine (Olsen et al. 2004), 7-deaza-7-fluoro-2′-*C*-methyladenosine (Carroll et al. 2007), 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (PSI-6130) (Stuyver et al. 2006), 4′-azidocytidine (R1479) (Klumpp et al. 2006) (and an oral prodrug thereof, termed R1626) (Klumpp et al. 2007) (Fig. 4ab).

How do these NRRIs interact with their final target, the HCV RNA replicase? They are phosphorylated to their 5′-triphosphate form, and then inhibit the HCV replicase. As they possess a 3′-hydroxyl function, they may not be considered as obligate chain terminators, but they may act as virtual chain terminators, viz. by steric hindrance exerted by the neighboring 2′-*C*-methyl and/or 4′-C-azido groups. Similar to their NRTI and NNRTI counterparts in the case of HIV reverse transcriptase, the NRRIs (2′-*C*-methylnucleosides) interact, upon their phosphorylation to the corresponding 5′-triphosphates, with a region of the HCV RNA replicase (or NS5B RNA-dependent RNA polymerase) that is clearly distinct from the site(s) of interaction of the NNRRIs (Tomei et al. 2005).

For the 2′-*C*-methyladenosine triphosphate, significantly higher concentrations were detected than that for 2′-*O*-methylcytidine triphosphate, which is consistent with the greater potency of 2′-*C*-methyladenosine over 2′-*O*-methylcytidine in the HCV replicon assay (Carroll et al. 2003). The relative inactivity of 2′-*O*-methylcytidine in inhibiting HCV replication could be ascribed to its poor intracellular conversion to the 5′-triphosphate; its activity could be restored when using a monophosphate prodrug (Tomassini et al. 2005). The 2′-*C*-methyl ribonucleosides 2′-*C*-methyladenosine and 2′-*C*-methylguanosine were identified as potent inhibitors of HCV RNA replication and the corresponding triphosphates were found to be potent inhibitors of the HCV NS5B-mediated RNA synthesis (Eldrup et al. 2004). The 2′-*C*-methyl ribonucleosides were shown to be efficient chain-terminating inhibitors of HCV genome replication (Migliaccio et al. 2003). Characterization of drug-resistant HCV replicons defined a single S282T mutation within the active site of the viral RNA polymerase that conferred loss of sensitivity to 2′-*C*-methyl ribonucleosides in both replicon and isolated polymerase assays (Migliaccio et al. 2003). It has been suggested that the 2′-*C*-methyl entity

is able to sterically block the next incoming ribonucleotide 5′-triphosphate (NTP) (Migliaccio et al. 2003). Additional modifications, as, for example, the 7-deaza modification, may further disrupt the alignment of the 3′-OH for nucleophilic attack on the α-phosphate of this incoming NTP, so as to more efficiently terminate chain elongation (Olsen et al. 2004).

The 2′-*C*-methyl-substituted ribonucleosides 2′-*C*-methyladenosine and -guanosine were also found to inhibit the replication of flaviviruses other than HCV, such as bovine viral diarrhea virus (BVDV), yellow fever virus, and West Nile virus (Migliaccio et al. 2003). Other 2′-*C*-methylribonucleosides such as β-D-2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (PSI-6130), however, showed little if any activity against BVDV, West Nile virus, or yellow fever virus (Stuyver et al. 2006).

PSI-6130, upon phosphorylation to its 5′-triphosphate, is incorporated (as PSI-6130-MP) as a nonobligate chain terminator (Murakami et al. 2008) into RNA catalyzed by purified RNA-dependent RNA polymerase (NS5B). PSI-6130 is metabolized intracellularly to the 5′-triphosphate of β-D-2′-deoxy-2′-fluoro-2′-*C*-methyluridine (PSI-6206) (Ma et al. 2007), but, as compared to the 5′-triphosphate of PSI-6130, the 5′-triphosphate of PSI-6206 is less efficient an inhibitor of HCV RNA-dependent RNA polymerase than PSI-6130 5′-triphosphate (Murakami et al. 2007).

R1479 (4′-azidocytidine) inhibits HCV replication in the replicon system with similar potency compared with 2′-*C*-methylcytidine (Klumpp et al. 2006). R1479 5′-triphosphate elicits similar potency compared with 3′-dCTP in inhibiting the NS5B-dependent RNA synthesis. R1479-TP was incorporated into nascent RNA by the HCV polymerase and reduced further elongation with similar efficiency compared with 3′-dCTP, an obligate chain terminator. The S282T mutation in the NS5B polymerase, which has been shown to confer resistance to 2′-*C*-methylnucleosides (see above), did not confer cross-resistance to R1479 (Klumpp et al. 2006). In vitro studies mapped resistance to R1479 to amino acid substitutions S96T and S96T/N142T of NS5B. These mutations, in turn, did not confer resistance to 2′-*C*-methylcytidine (Le Pogam et al. 2006). These findings would argue for combination therapy of R1479 with 2′-*C*-methylribonucleosides.

HCV polymerase that carried the S282T mutation did no longer incorporate 2′-*C*-methyl-CTP during the initiation step of RNA synthesis (Dutartre et al. 2006). The presence of the S282T mutation induces a general reduction (5–20-fold) in terms of polymerase efficiency (Dutartre et al. 2006), which may translate to decreased viral fitness (Ludmerer et al. 2005).

Nonobligate chain terminators, such as 2′-*C*-methylated nucleosides, following incorporation in their 5′-monophosphate (nucleotide) form, can be excised again through pyrophosphorolytic excision in the presence of pyrophosphate (PPi) (Deval et al. 2007). Previous studies have suggested mechanisms of action of removal of (obligate) chain terminators by both BVDV RNA-dependent RNA polymerase (D'Abramo et al. 2004) and HIV-1 reverse transcriptase (Götte 2006).

6 Perspective

Chain terminators acting at the DNA level such as the acyclic guanosine analogues (following phosphorylation to their triphosphate form) have gained wide acceptance as antiviral drugs, that is, (val)acyclovir in the treatment of HSV infections and (val)ganciclovir in the treatment of CMV infections (in immunosuppressed patients). The acyclic nucleoside phosphonates (or nucleotide analogues), also acting at the DNA level (following phosphorylation to their diphosphate form), have proven to be key antiviral drugs for the treatment of HIV infections (tenofovir) and HBV infections (adefovir and tenofovir). The 2′,3′-dideoxynucleoside analogues, which following their phosphorylation to the 5′-triphosphate form, act as chain terminators of the HIV reverse transcription reaction, are inherent part of various antiviral drug cocktails used in the treatment of AIDS. Whether RNA chain terminators may ultimately evolve to useful drugs in the treatment of HCV infections is anticipated, but not yet demonstrated. An additional, albeit yet to be ascertained, application for the RNA chain terminators is their potential use for the treatment of flavivirus infections (other than HCV) and RNA virus infections other than flavi-, such as a picornavirus infections.

Acknowledgments This is part of the activities of the VIRGIL European Network of Excellence on Antiviral Drug Resistance supported by a grant (LSHM-CT-2004-503359) from the Priority 1 "Life Sciences, Genomics and Biotechnology for Health" and is supported by a grant from the FWO (no. G.0267.04).

References

Afdhal N, Godofsky E, Dienstag J, Rustgi V, Schick L, McEniry D, Zhou X-J, Chao G, Fang C, Fielman B, Myers M, Brown N (2004) Final phase I/II trial results for NM283, a new polymerase inhibitor for hepatitis C: antiviral efficacy and tolerance in patients with HCV-1 infection, including previous interferon failures. Hepatology 40:726A

Asif G, Hurwitz SJ, Obikhod A, Delinsky D, Narayanasamy J, Chu CK, McClure HM, Schinazi RF (2007) Pharmacokinetics of the anti-HIV agent 1-(β-D-dioxolane)thymine in Rhesus monkeys. Antimicrob Agents Chemother 51:2424–2429

Balzarini J, Schols D, Van Laethem K, De Clercq E, Hocková D, Masojidkova M, Holý A (2007) Pronounced *in vitro* and *in vivo* antiretroviral activity of 5-substituted 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidines. J Antimicrob Chemother 59:80–86

Beadle JR, Wan WB, Ciesla SL, Keith KA, Hartline C, Kern ER, Hostetler KY (2006) Synthesis and antiviral evaluation of alkoxyalkyl derivatives of 9-(S)-(3-hydroxy-2-phosphonomethoxypropyl)adenine against cytomegalovirus and orthopoxviruses. J Med Chem 49:2010–2015

Bonatti H, Aigner F, De Clercq E, Boesmueller C, Widschwendner A, Larcher C, Margreiter R, Schneeberger S (2007) Local administration of cidofovir for human papilloma virus associated skin lesions in transplant recipients. Transpl Int 20:238–246

Buller RM, Owens G, Schriewer J, Melman L, Beadle JR, Hostetler KY (2004) Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. Virology 318:474–481

Carroll SS, Tomassini JE, Bosserman M, Getty K, Stahlhut MW, Eldrup AB, Bhat B, Hall D, Simcoe AL, LaFemina R, Rutkowski CA, Wolanski B, Yang Z, Migliaccio G, De Francesco R, Kuo LC, MacCoss M, Olsen DB (2003) Inhibition of hepatitis C virus RNA replication by 2′-modified nucleoside analogs. J Biol Chem 278:11979–11984

Carroll S, Koeplinger K, Burlein C, Stahlhut M, Vavrek M, MacCoss M, Hazuda D, Olsen D (2007) Nucleoside inhibitors of hepatitis C virus RNA polymerase: improved potency and liver targeting with 7-deaza-7-fluoro-2′-C-methyladenosine. In: Late breaker presentations. 20th international conference on antiviral research, Palm Springs, CA, USA, April 29–May 3, 2007, LB-01

Cihlar T, Ray AS, Boojamra CG, Zhang L, Hui H, Laflamme G, Vela JE, Grant D, Chen J, Myrick F, White KL, Gao Y, Lin KY, Douglas JL, Parkin NT, Carey A, Pakdaman R, Mackman RL (2008) Design and profiling of GS-9148, a novel nucleotide analog active against nucleoside-resistant variants of human immunodeficiency virus type 1, and its orally bioavailable phosphonoamidate prodrug, GS-9131. Antimicrob Agents Chemother 52:655–665

Coelmont L, Paeshuyse J, Windisch MP, De Clercq E, Bartenschlager R, Neyts J (2006) Ribavirin antagonizes the in vitro anti-hepatitis C virus activity of 2′-*C*-methylcytidine, the active component of valopicitabine. Antimicrob Agents Chemother 50:3444–3446

D'Abramo CM, Cellai L, Götte M (2004) Excision of incorporated nucleotide analogue chain-terminators can diminish their inhibitory effects on viral RNA-dependent RNA polymerases. J Mol Biol 337:1–14

Dal Pozzo F, Andrei G, Lebeau I, Beadle JR, Hostetler KY, De Clercq E, Snoeck R (2007) In vitro evaluation of the anti-orf virus activity of alkoxyalkyl esters of CDV, cCDV and (S)-HPMPA. Antiviral Res 75:52–57

De Clercq E (2002) Strategies in the design of antiviral drugs. Nat Rev Drug Discov 1:13–25

De Clercq E (2004a) Antiviral drugs in current clinical use. J Clin Virol 30:115–133

De Clercq E (2004b) Antivirals and antiviral strategies. Nat Rev Microbiol 2:704–720

De Clercq E (2006) From adefovir to Atripla™ via tenofovir, Viread™ and Truvada™. Future Virol 1:709–715

De Clercq E (2007a) Acyclic nucleoside phosphonates: past, present and future. Bridging chemistry to HIV, HBV, HCV, HPV, adeno-, herpes-, and poxvirus infections: the phosphonate bridge. Biochem Pharmacol 73:911–922

De Clercq E (2007b) Anti-HIV drugs. Verh K Acad Geneesk Belg 44:81–104

De Clercq E (2007c) The design of drugs for HIV and HCV. Nat Rev Drug Discov 6:1001–1018

De Clercq E (2007d) The acyclic nucleoside phosphonates from inception to clinical use: historical perspective. Antiviral Res 75:1–13

De Clercq E, Holý A (2005) Acyclic nucleoside phoshonates: a key class of antiviral drugs. Nat Rev Drug Discov 4:928–940

De Clercq E, Field HJ (2006) Antiviral prodrugs – the development of successful prodrug strategies for antiviral chemotherapy. Brit J Pharmacol 147:1–11

De Clercq E, Holý A, Rosenberg I, Sakuma T, Balzarini J, Maudgal PC (1986) A novel selective broad-spectrum anti-DNA virus agent. Nature 323:464–467

De Clercq E, Sakuma T, Baba M, Pauwels R, Balzarini J, Rosenberg I, Holý A (1987) Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. Antiviral Res 8:261–272

De Clercq E, Andrei G, Snoeck R, De Bolle L, Naesens L, Degrève B, Balzarini J, Zhang Y, Schols D, Leyssen P, Ying C, Neyts J (2001) Acyclic/carbocyclic guanosine analogues as anti-herpesvirus agents. Nucleosides Nucleotides Nucleic Acids 20:271–285

De Clercq E, Andrei G, Balzarini J, Leyssen P, Naesens L, Neyts J, Pannecouque C, Snoeck R, Ying C, Hocková D, Holý A (2005) Antiviral potential of a new generation of acyclic nucleoside phosphonates, the 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines. Nucleosides Nucleotides Nucleic Acids 24:331–341

Degrève B, De Clercq E, Balzarini J (1999) Bystander effect of purine nucleoside analogues in HSV-1 tk suicide gene therapy is superior to that of pyrimidine nucleoside analogues. Gene Ther 6:162–170

Deval J, Powdrill MH, D'Abramo CM, Cellai L, Götte M (2007) Pyrophosphorolytic excision of nonobligate chain terminators by hepatitis C virus NS5B polymerase. Antimicrob Agents Chemother 51:2920–2928

Dutartre H, Bussetta C, Boretto J, Canard B (2006) General catalytic deficiency of hepatitis C virus RNA polymerase with an S28T mutation and mutually exclusive resistance towards 2′-modified nucleotide analogues. Antimicrob Agents Chemother 50:4161–4169

Eldrup AB, Allerson CR, Bennett CF, Bera S, Bhat B, Bhat N, Bosserman MR, Brooks J, Burlein C, Carroll SS, Cook PD, Getty KL, MacCoss M, McMasters DR, Olsen DB, Prakash TP, Prhavc M, Song Q, Tomassini JE, Xia J (2004) Structure-activity relationship of purine ribonucleosides for inhibition of hepatitis C virus RNA-dependent RNA polymerase. J Med Chem 47:2283–2295

Elion GB, Furman PA, Fyfe JA, de Miranda P, Beauchamp L, Schaeffer HJ (1977) Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc Natl Acad Sci USA 74:5716–5720

Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, Lu B, McColl D, Chuck S, Enejosa J, Toole JJ, Cheng AK (2006) Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 354:251–260

Golankiewicz B, Ostrowski T, Andrei G, Snoeck R, De Clercq E (1994) Tricyclic analogues of acyclovir and ganciclovir. Influence of substituents in the heterocyclic moiety on the antiviral activity. J Med Chem 37:3187–3190

Golankiewicz B, Ostrowski T, Goslinski T, Januszczyk P, Zeidler J, Baranowski D, De Clercq E (2001) Fluorescent tricyclic analogues of acyclovir and ganciclovir. A structure-antiviral activity study. J Med Chem 44:4284–4287

Götte M (2006) Effects of nucleotides and nucleotide analogue inhibitors of HIV-1 reverse transcriptase in a ratchet model of polymerase translocation. Curr Pharm Des 12:1867–1877

Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart CL (2005) Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N Engl J Med 352:2673–2681

Hartline CB, Gustin KM, Wan WB, Ciesla SL, Beadle JR, Hostetler KY, Kern ER (2005) Ether lipid-ester prodrugs of acyclic nucleoside phosphonates: activity against adenovirus replication in vitro. J Infect Dis 191:396–399

Hirsch MS (2007) Entecavir surprise. N Engl J Med 356:2641–2643

Hostetler KY, Beadle JR, Trahan J, Aldern KA, Owens G, Schriewer J, Melman L, Buller RM (2007) Oral 1-O-octadecyl-2-O-benzyl-*sn*-glycero-3-cidofovir targets the lung and is effective against a lethal respiratory challenge with ectromelia virus in mice. Antiviral Res 73:212–218

Hu R, Li L, Degrève B, Dutschman GE, Lam W, Cheng YC (2005) Behavior of thymidylate kinase toward monophosphate metabolites and its role in the metabolism of 1-(2′-deoxy-2′-fluoro-beta-L-arabinofuranosyl)-5-methyluracil (Clevudine) and 2′,3′-didehydro-2′,3′-dideoxythymidine in cells. Antimicrob Agents Chemother 49:2044–2049

Kern ER, Hartline C, Harden E, Keith K, Rodriguez N, Beadle JR, Hostetler KY (2002) Enhanced inhibition of orthopoxvirus replication in vitro by alkoxyalkyl esters of cidofovir and cyclic cidofovir. Antimicrob Agents Chemother 46:991–995

Kern ER, Bidanset DJ, Hartline CB, Yan Z, Zemlicka J, Quenelle DC (2004) Oral activity of a methylenecyclopropane analog, cyclopropavir, in animal models for cytomegalovirus infections. Antimicrob Agents Chemother 48:4745–4753

Kern ER, Kushner NL, Hartline CB, Williams-Aziz SL, Harden EA, Zhou S, Zemlicka J, Prichard MN (2005) In vitro activity and mechanism of action of methylenecyclopropane analogs of nucleosides against herpesvirus replication. Antimicrob Agents Chemother 49:1039–1045

Klumpp K, Lévêque V, Le Pogam S, Ma H, Jiang W-R, Kang H, Granycome C, Singer M, Laxton C, Hang JQ, Sarma K, Smith DB, Heindl D, Hobbs CJ, Merrett JH, Symons J, Cammack N, Martin JA, Devos R, Nájera I (2006) The novel nucleoside analog R1479 (4′-azidocytidine) is a potent inhibitor of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture. J Biol Chem 281:3793–3799

Klumpp K, Smith D, Brandl M, Alfredson T, Sarma K, Smith M, Nájera I, Jiang W-R, Le Pogam S, Lévêque V, Ma H, Tu Y, Chan R, Chen C-W, Wu X, Birudaraj R, Swallow S, Martin JA, Cammack N, Berns H, Fettner S, Ipe D, Mannino M, O'Mara E, Washington C, Roberts S, Cooksley G, Dore G, Shaw D, Blue DR Jr, Zahm F, Hill G (2007) Design and characterization of R1626, a prodrug of the HCV replication inhibitor R1479 (4′-azidocytidine) with enhanced oral bioavailability. Antiviral Res 74:A35 (abstract no 21)

Krečmerová M, Holý A, Pískala A, Masojídková M, Andrei G, Naesens L, Neyts J, Balzarini J, De Clercq E, Snoeck R (2007a) Antiviral activity of triazine analogues of 1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (cidofovir) and related compounds. J Med Chem 50:1069–1077

Krečmerová M, Holý A, Pohl R, Masojídková M, Andrei G, Naesens L, Neyts J, Balzarini J, De Clercq E, Snoeck R (2007b) Ester prodrugs of cyclic 1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine: synthesis and antiviral activity. J Med Chem 50:5765–5772

Le Pogam S, Jiang W-R, Lévêque V, Rajyaguru S, Ma H, Kang H, Jiang S, Singer M, Ali S, Klumpp K, Smith D, Symons J, Cammack N, Nájera I (2006) In vitro selected Con1 subgenomic replicons resistant to 2′-C-methyl-cytidine or to R1479 show lack of cross resistance. Virology 351:349–359

Lebeau I, Andrei G, Krečmerová M, De Clercq E, Holý A, Snoeck R (2007) Inhibitory activities of three classes of acyclic nucleoside phosphonates against murine polyomavirus and primate simian virus 40 strains. Antimicrob Agents Chemother 51:2268–2273

Lee WA, He GX, Eisenberg E, Cihlar T, Swaminathan S, Mulato A, Cundy KC (2005) Selective intracellular activation of a novel prodrug of the human immunodeficiency virus reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. Antimicrob Agents Chemother 49:1898–1906

Lennerstrand J, Chu CK, Schinazi RF (2007) Biochemical studies on the mechanism of human immunodeficiency virus type 1 reverse transcriptase resistance to 1-(β-D-dioxolane)thymine triphosphate. Antimicrob Agents Chemother 51:2078–2084

Levine S, Hernandez D, Yamanaka G, Zhang S, Rose R, Weinheimer S, Colonno RJ (2002) Efficacies of entecavir against lamivudine-resistant hepatitis B virus replication and recombinant polymerases in vitro. Antimicrob Agents Chemother 46:2525–2532

Locarnini S, Hatzakis A, Heathcote J, Keeffe EB, Liang TJ, Mutimer D, Pawlotsky JM, Zoulim F (2004) Management of antiviral resistance in patients with chronic hepatitis B. Antiviral Ther 9:679–693

Ludmerer SW, Graham DJ, Boots E, Murray EM, Simcoe A, Markel EJ, Grobler JA, Flores OA, Olsen DB, Hazuda DJ, LaFemina RL (2005) Replication fitness and NS5B drug sensitivity of diverse hepatitis C virus isolates characterized by using a transient replication assay. Antimicrob Agents Chemother 49:2059–2069

Ma H, Jiang WR, Robledo N, Leveque V, Ali S, Lara-Jaime T, Masjedizadeh M, Smith DB, Cammack N, Klumpp K, Symons J (2007) Characterization of the metabolic activation of hepatitis C virus nucleoside inhibitor beta-D-2′-deoxy-2′-fluoro-2′-C-methylcytidine (PSI-6130) and identification of a novel active 5′-triphosphate species. J Biol Chem 282:29812–29820

Magee WC, Hostetler KY, Evans DH (2005) Mechanism of inhibition of vaccinia virus DNA polymerase by cidofovir diphosphate. Antimicrob Agents Chemother 49:3153–3162

Magee WC, Aldern KA, Hostetler KY, Evans DH (2008) Cidofovir and (S)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenine are highly effective inhibitors of vaccinia virus DNA polymerase when incorporated into the template strand. Antimicrob Agents Chemother 52:586–597

McMahon MA, Jilek BL, Brennan TP, Shen L, Zhou Y, Wind-Rotolo M, Xing S, Bhat S, Hale B, Hegarty R, Chong CR, Liu JQ, Siliciano RF, Thio CL (2007) The HBV drug entecavir – effects on HIV-1 replication and resistance. N Engl J Med Chem 356:2614–2621

Migliaccio G, Tomassini JE, Carroll SS, Tomei L, Altamura S, Bhat B, Bartholomew L, Bosserman MR, Ceccacci A, Colwell LF, Cortese R, De Francesco R, Eldrup AB, Getty KL, Hou XS, LaFemina RL, Ludmerer SW, MacCoss M, McMasters DR, Stahlhut MW, Olsen DB, Hazuda

DJ, Flores OA (2003) Characterization of resistance to non-obligate chain-terminating ribonucleoside analogs that inhibit hepatitis C virus replication in vitro. J Biol Chem 278:49164–49170

Murakami E, Bao H, Ramesh M, McBrayer TR, Whitaker T, Micolochick Steuer HM, Schinazi RF, Stuyver LJ, Obikhod A, Otto MJ, Furman PA (2007) Mechanism of activation of beta-D-2′-deoxy-2′-fluoro-2′-c-methylcytidine and inhibition of hepatitis C virus NS5B RNA polymerase. Antimicrob Agents Chemother 51:503–509

Murakami E, Niu C, Bao H, Micolochick Steuer HM, Whitaker T, Nachman T, Sofia MA, Wang P, Otto MJ, Furman PA (2008) The mechanism of action of beta-D-2′-deoxy-2′-fluoro-2′-C-methylcytidine involves a second metabolic pathway leading to beta-D-2′-deoxy-2′-fluoro-2′-C-methyluridine 5′-triphosphate, a potent inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. Antimicrob Agents Chemother 52:458–464

Olsen DB, Eldrup AB, Bartholomew L, Bhat B, Bosserman MR, Ceccacci A, Colwell LF, Fay JF, Flores OA, Getty KL, Grobler JA, LaFemina RL, Markel EJ, Migliaccio G, Prhavc M, Stahlhut MW, Tomassini JE, MacCoss M, Hazuda DJ, Carroll SS (2004) A 7-deaza-adenosine analog is a potent and selective inhibitor of hepatitis C virus replication with excellent pharmacokinetic properties. Antimicrob Agents Chemother 48:3944–3953

Ostrowski T, Golankiewicz B, De Clercq E, Balzarini J (2006) Synthesis and biological activity of tricyclic analogues of 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine. Bioorg Med Chem 14:3535–3542

Painter GR, Hostetler KY (2004) Design and development of oral drugs for the prophylaxis and treatment of smallpox infection. Trends Biotechnol 22:423–427

Pozniak AL, Gallant JE, DeJesus E, Arribas JR, Gazzard B, Campo RE, Chen SS, McColl D, Enejosa J, Toole JJ, Cheng AK (2006) Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naive patients: virologic, immunologic, and morphologic changes – a 96-week analysis. J Acquir Immune Defic Syndr 43:535–540

Quenelle DC, Collins DJ, Wan WB, Beadle JR, Hostetler KY, Kern ER (2004) Oral treatment of cowpox and vaccinia virus infections in mice with ether lipid esters of cidofovir. Antimicrob Agents Chemother 48:404–412

Quenelle DC, Collins DJ, Herror BP, Keith KA, Trahan J, Beadle JR, Hostetler KY, Kern ER (2007) Effect of oral treatment with hexadecyloxypropyl-[(S)-9-(3-hydroxy-2- phosphonylmethoxypropyl)adenine] [(S)-HPMPA] or octadecyloxyethyl-(S)-HPMPA on cowpox or vaccinia virus infections in mice. Antimicrob Agents Chemother 51:3940–3947

Ray AS, Vela JE, Boojamra CG, Zhang L, Hui H, Callebaut C, Stray K, Lin KY, Gao Y, Mackman RL, Cihlar T (2008) Intracellular metabolism of the nucleotide prodrug GS-9131, a potent anti-human immunodeficiency virus agent. Antimicrob Agents Chemother 52:648–654

Ruiz-Sancho A, Sheldon J, Soriano V (2007) Telbivudine: a new option for the treatment of chronic hepatitis B. Expert Opin Biol Ther 7:751–761

Stuyver LJ, McBrayer TR, Tharnish PM, Clark J, Hollecker L, Lostia S, Nachman T, Grier J, Bennett MA, Xie M-Y, Schinazi RF, Morrey JD, Julander JL, Furman PA, Otto MJ (2006) Inhibition of hepatitis C replicon RNA synthesis by β-D-2′-deoxy-2′-fluoro-2′-C-methylcytidine: a specific inhibitor of hepatitis C virus replication. Antiviral Chem Chemother 17:79–87

Sullivan V, Talarico CL, Stanat SC, Davis M, Coen DM, Biron KK (1992) A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature 359:85

Tomassini JE, Getty K, Stahlhut MW, Shim S, Bhat B, Eldrup AB, Prakash TP, Carroll SS, Flores O, MacCoss M, McMasters DR, Migliaccio G, Olsen DB (2005) Inhibitory effect of 2′-substituted nucleosides on hepatitis C virus replication correlates with metabolic properties in replicon cells. Antimicrob Agents Chemother 49:2050–2058

Tomei L, Altamura S, Paonessa G, De Francesco R, Migliaccio G (2005) HCV antiviral resistance: the impact of *in vitro* studies on the development of antiviral agents targeting the viral NS5B polymerase. Antiviral Chem Chemother 16:225–245

Toniutto P, Fabris C, Bitetto D, Fornasiere E, Rapetti R, Pirisi M (2007) Valopicitabine dihydrochloride: a specific polymerase inhibitor of hepatitis C virus. Curr Opin Investig Drugs 8:150–158

Tuske S, Sarafianos SG, Clark AD, Ding J, Naeger LK, White KL, Miller MD, Gibbs CS, Boyer PL, Clark P, Wang G, Gaffney BL, Jones RA, Jerina DM, Hughes SH, Arnold E (2004) Structures of HIV-1 RT-DNA complexes before and after incorporation of the anti-AIDS drug tenofovir. Nat Struct Mol Biol 11:469–474

Wang J, Jin Y, Rapp KL, Bennett M, Schinazi RF, Chu CK (2005) Synthesis, antiviral activity, and mechanism of drug resistance of D- and L-2′,3′-didehydro-2′,3′-dideoxy-2′-fluorocarbocyclic nucleosides. J Med Chem 48:3736–3748

Wang J, Jin Y, Rapp KL, Schinazi RF, Chu CK (2007) D- and L-2′,3′-didehydro-2′,3′-dideoxy-3′-fluoro-carbocyclic nucleosides: synthesis, anti-HIV activity and mechanism of resistance. J Med Chem 50:1828–1839

Wyles DL, Schooley RT, Kaihara KA, Beadle JR, Hostetler KY (2008) Anti-hepatitis C virus replicon activity of alkoxyalkyl esters of (S)-HPMPA and other acyclic nucleoside phosphonates. In: Abstracts of the 21st international conference on antiviral research, Montreal, QC, Canada, 13–17 April 2008. Antiviral Res 78:A21, no 15

Xiong X, Smith JL, Chen MS (1997) Effect of incorporation of cidofovir into DNA by human cytomegalovirus DNA polymerase on DNA elongation. Antimicrob Agents Chemother 41:594–599

Zhou S, Breitenbach JM, Borysko KZ, Drach JC, Kern ER, Gullen E, Cheng YC, Zemlicka J (2004) Synthesis and antiviral activity of (Z)- and (E)-2,2-[bis(hydroxymethyl) cyclopropylidene]methylpurines and -pyrimidines: second-generation methylenecyclopropane analogues of nucleosides. J Med Chem 47:566–575

Zoulim F (2006) Entecavir: a new treatment option for chronic hepatitis B. J Clin Virol 36:8–12