Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 75 (2007) 1–13
Antiviral Research
www.elsevier.com/locate/antiviral

Review

The acyclic nucleoside phosphonates from inception to clinical use: Historical perspective

Erik De Clercq *

Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received 6 September 2006; accepted 13 October 2006

Tribute to Prof. Antonín Holý at his 70th birthday (1 September 2006).

Abstract

The collaboration between Antonín Holý [Institute of Organic Chemistry and Biochemistry (IOCB, Prague, Czech Republic)] and Erik De Clercq (Rega Institute for Medical Research, K.U. Leuven, Belgium) started exactly 30 years ago. It led to the discovery of a (rather small) series of acyclic nucleoside analogues (prototype: DHPA), followed by the discovery of a large number of nucleotide analogues [acyclic nucleoside phosphonates (ANPs)] (prototype: HPMPA). From HPMPA originated three compounds, which have been approved by regulatory agencies worldwide for clinical use: (i) HPMPC [cidofovir (Vistide®)] for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients, and "off label" for the treatment of polyoma-, papilloma-, adeno-, herpes- and poxvirus infections; (ii) PMEA [adefovir (in its oral prodrug form, adefovir dipivoxil (Hepsera®)] for the treatment of chronic HBV (hepatitis B virus) infections, and (iii) PMPA [tenofovir (in its oral prodrug form, tenofovir disoproxil fumarate (Viread®)] for the treatment of HIV infections (AIDS). The latter has also been approved, in combination with emtricitabine (Truvada®), and in combination with emtricitabine and efavirenz (Atripla®) for the treatment of HIV infections. Many other ANPs such as the DAP derivatives HPMPDAP, PMEDAP and PMPDAP, and the DAPy derivatives HPMPO-DAPy, PMEO-DAPy, and PMPO-DAPy, were found to exhibit an antiviral activity spectrum and potency comparable to that of the parent compounds HPMPA (and HPMPC), PMEA and PMPA, respectively.

Keywords: Acyclic nucleoside phosphonates (ANPs); HPMPA; Cidofovir (HPMPC); Adefovir (PMEA); Tenofovir (PMPA)

Contents

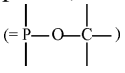
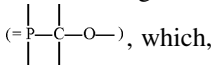
Abbreviations: ANPs, acyclic nucleoside phosphonates; DHPA, [(*S*)-9-(2,3-dihydroxypropyl)adenine]; HPMPA, [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine]; HPMPC, [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]; PMEA, [9-(2-phosphonylmethoxyethyl)adenine]; FPMPA, [(*S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine]; PMPA, [(*R*)-9-(2-phosphonylmethoxypropyl)adenine]; HPMPDAP, [(*S*)-9-(3-hydroxyl-2-phosphonylmethoxypropyl)-2,6-diaminopurine]; PMEDAP, [9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine]; PMPDAP, [(*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine]; HPMPO-DAPy, [(*R*)-6-(3-hydroxy-2-(phosphonylmethoxy)propoxy)-2,4-diaminopyrimidine]; PMEO-DAPy, [6-(2-(phosphonylmethoxy)ethoxy)-2,4-diaminopyrimidine]; PMPO-DAPy, [(*R*)-6-(2-(phosphonylmethoxy)propoxy)-2,4-diaminopyrimidine]

* Tel.: +32 16 337367; fax: +32 16 337340.
E-mail address: erik.declercq@rega.kuleuven.be.

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.10.006

1. Introduction

The acyclic nucleoside phosphonates (ANPs) represent a new dimension for the treatment of DNA virus and retrovirus infections (De Clercq and Holý, 2005). These ANPs encompass three compounds that have been formally licensed for the treatment of (i) HCMV (human cytomegalovirus) infections (i.e. HCMV retinitis) in AIDS patients (cidofovir, Vistide®), (ii) chronic HBV (hepatitis B virus) infections (adefovir dipivoxil, Hepsera®), and (iii) HIV (human immunodeficiency virus) infections (AIDS) [tenofovir disoproxil fumarate (TDF), Viread®]. The first (cidofovir) can also be used "off label" in the treatment of herpesvirus (other than HCMV) infections, as well as polyoma-, papilloma-, adeno- and poxvirus infections. The third (TDF) is also available, in a fixed-dose combination form with either emtricitabine (Truvada®), or emtricitabine and efavirenz (Atripla®), for the treatment of AIDS.

The ANPs have thus proved to be a cornerstone in antiviral therapy. What is the significance of an acyclic nucleoside "phosphonate" and from where stems the designation "phosphonate"? In regular nucleotides (or nucleoside phosphates), the phosphate group is attached through an ester bound  to the nucleoside, as, for example, when a phosphate group has been linked to acyclovir during the first phosphorylation step of acyclovir (to acyclovir monophosphate) by the herpesvirus [HSV (herpes simplex virus)-encoded] thymidine kinase (TK). In the ANPs, the phosphate group, in the form of a phosphonate group, has already been attached to the nucleoside analogue, thus resulting in the formation of a phosphonomethyl ether  , which, unlike the phosphate ester linkage, should resist any attack by esterases, or any catabolic enzymes at large.

The concept of the ANPs was born in 1986 with the identification of the ANP prototype, (*S*)-9-(3-hydroxyl-2-phosphonylmethoxypropyl)adenine (HPMPA), as a broad-spectrum anti-DNA virus agent (De Clercq et al., 1986). HPMPA itself could be envisaged as a kind of construct resulting from the replacement of the carboxylate group of phosphonoacetic acid [PAA, the predecessor of the antiviral agent phosphonoformic acid (foscarnet, Foscavir®] by the acyclic nucleoside analogue DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine], which was described in 1978 as an acyclic nucleoside analogue with broad-spectrum antiviral activity (De Clercq et al., 1978).

Here I will describe the genealogy (Fig. 1) of the acyclic nucleoside phosphonates (ANPs) starting from DHPA, via HPMPA as the prototype of the ANPs, leading to the three ANPs that are currently in clinical use (cidofovir, adefovir, tenofovir) and several others, i.e. 2,6-diaminopurine (DAP) and 2,4-diaminopyrimidine (DAPy) derivatives, which show high potential as therapeutic agents.

According to their activity spectrum, the ANPs can be classified in two categories: (i) the "HPMP" derivatives, represented by HPMPC (cidofovir), which are active against a broad variety of DNA viruses (polyoma-, papilloma-, adeno-, herpes- and poxviruses), and (ii) the "PME" and "PMP" derivatives, represented by, respectively, PMEA (adefovir) and PMPA (tenofovir), which are primarily active against hepadna- and retroviruses (Fig. 2). Their clinical indications, whether approved or potential ("off label"), are presented in Fig. 3. The (molecular) mechanism of action of the ANPs is not the subject of the present review. This aspect has been addressed in previous reports (i.e. De Clercq, 2003b; De Clercq and Holý, 2005).

2. DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine]

From the beginning, it was quite clear that the antiviral activity spectrum and mode of action of DHPA was clearly distinct from that of the acyclic guanosine analogue acyclovir, which had been reported (Elion et al., 1977; Schaeffer et al., 1978) a few months before our paper appeared in Science (De Clercq et al., 1978). While acyclovir was to become the "gold standard" for the treatment of HSV-1 and -2 infections, DHPA only had a limited appearance on the market (as Duvira® gel) in Czechoslovakia for the treatment of herpes labialis (cold sores). However, DHPA served as an experimental tool to identify and probe for inhibitors of *S*-adenosyl-L-homocysteine (SAH) hydrolase inhibitors (Votruba and Holý, 1980). It would later become apparent that SAH hydrolase, because of its key role in methylation reactions such as those involved in the maturation of viral mRNAs, is an attractive target for the design of antiviral agents.

Following DHPA, and several structurally related aliphatic nucleoside analogues (De Clercq and Holý, 1979), which act as reversible inhibitors of SAH hydrolase, D-eritadenine, the active component isolated from the edible Japanese mushroom *Lentinus edodes shiitake* (Holý et al., 1982), and alkyl esters of 3-adenin-9-yl-2-hydroxypropanoic acid (AHPA) were described as potent (irreversible) inhibitors of SAH hydrolase (De Clercq

and Holý, 1985), with an antiviral activity spectrum similar to that of DHPA. This antiviral activity spectrum extends, in contrast to that of acyclovir (which encompasses primarily HSV-1 and HSV-2), to various other DNA viruses (i.e. poxviruses) as well as RNA viruses, particularly (−)RNA viruses such as arena-, rhabdo- and paramyxoviruses, which heavily depend on methylations for virus replication (i.e. 5′-capping of their mRNA) (see, for example, De Clercq, 1987; Snoeck et al., 1993).

In fact, a close correlation has been established between the inhibitory effects of the acyclic adenosine analogues (i.e. DHPA, AHPA) and carbocyclic adenosine analogues (i.e. neplanocin A,

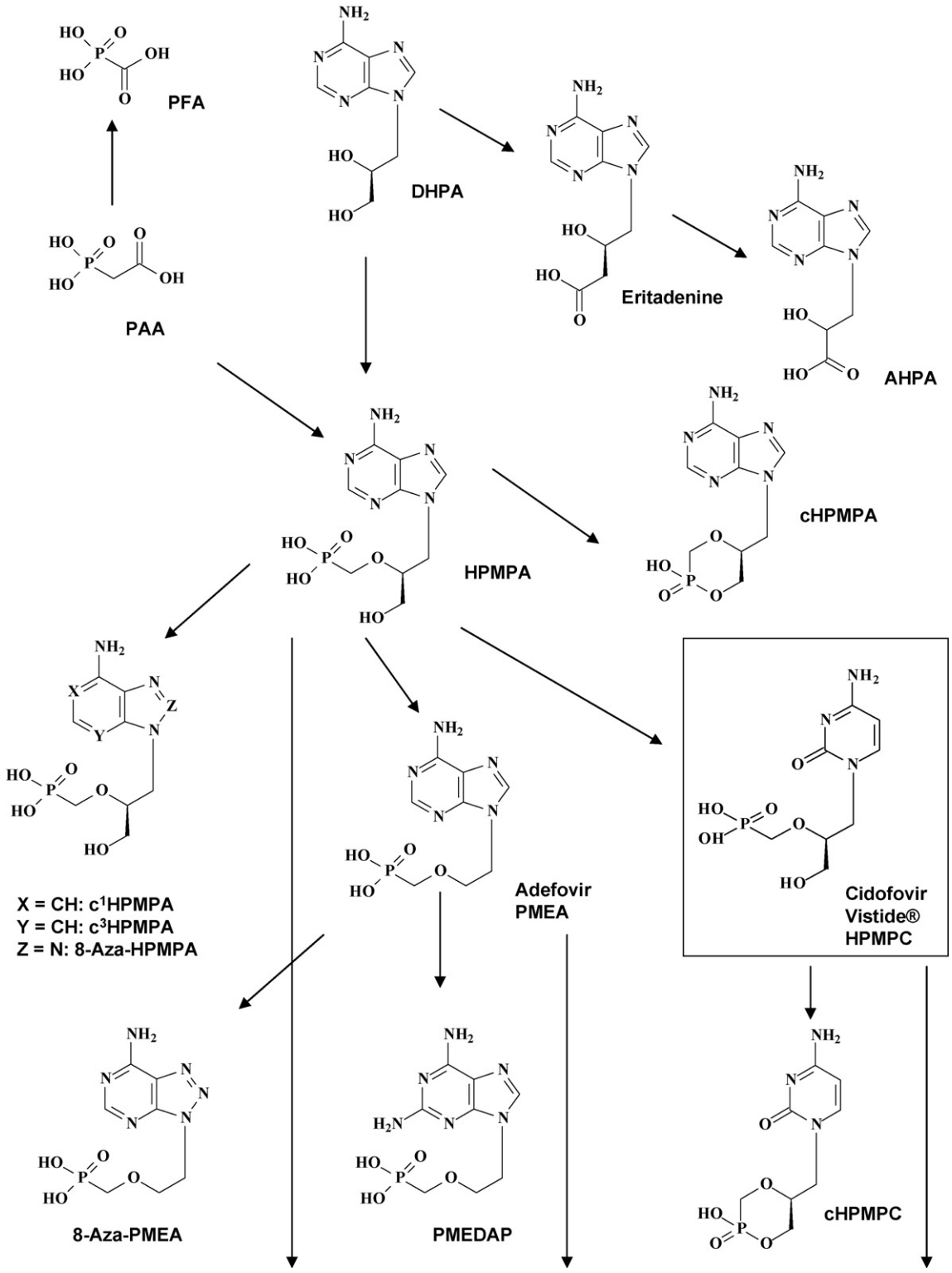


Fig. 1. Genealogy of acyclic nucleoside phosphonates (boxed in: marketed compounds).

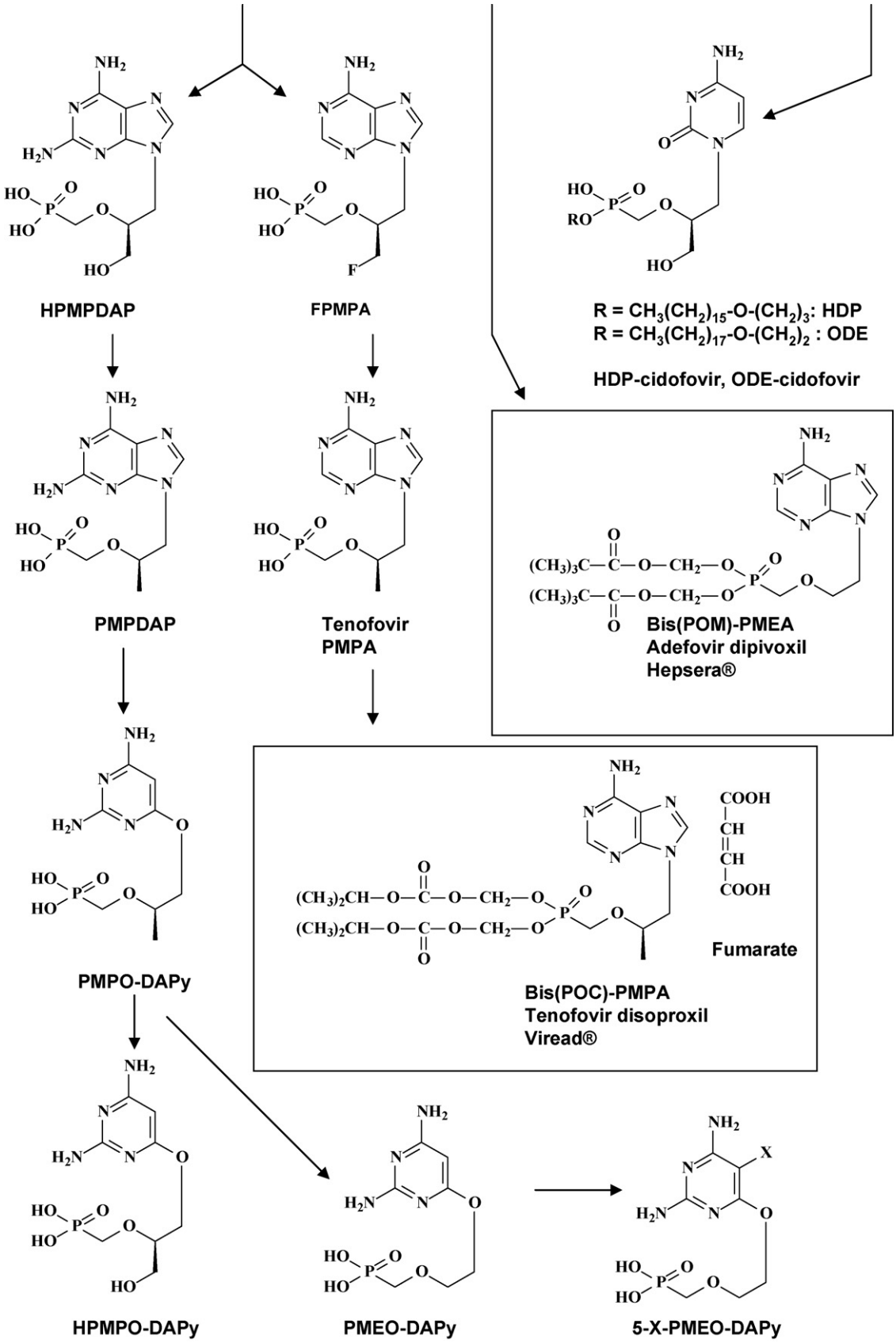


Fig. 1. (*Continued*).

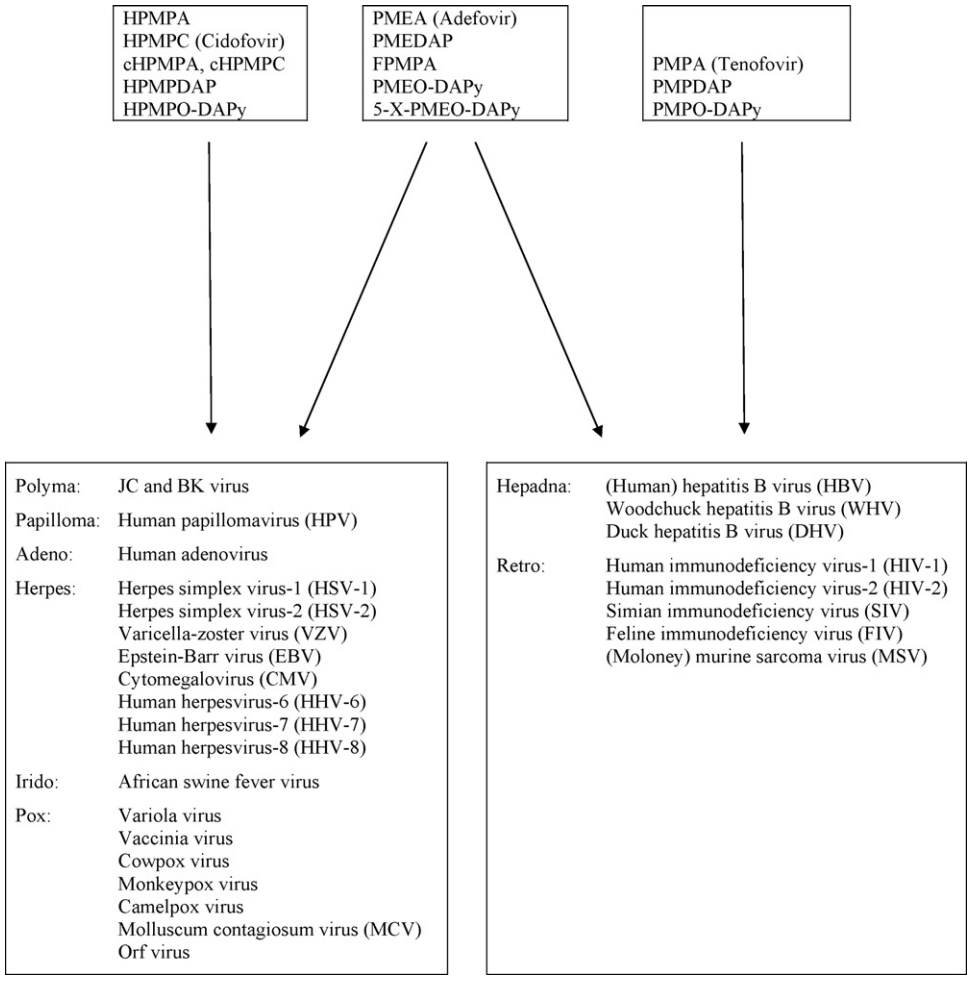


Fig. 2. Antiviral activity spectrum of acyclic nucleoside phosphonates.

3-deazaneplanocin A, carbocyclic 3-deazaadenosine) on SAH hydrolase and their antiviral activity, i.e. against the rhabdovirus vesicular stomatitis virus (VSV) and the poxvirus vaccinia virus, suggesting that inhibition of SAH hydrolase actually accounted for the antiviral activity of the acyclic and carbocyclic adenosine analogues (De Clercq and Cools, 1985; Cools and De Clercq, 1989). Further studies have ascertained that the antiviral activity of DHPA, AHPA and the carbocyclic adenosine analogues is duly mediated by inhibition of SAH hydrolase (Cools et al., 1990; Cools and De Clercq, 1990).

Compound (Commercially available)	Dosage and route of administration	Approved clinical use	Off-label (potential) clinical use
Cidofovir (Vistide®)	Intravenous (5 mg/kg weeky or two-weekly) Topical (gel/cream) 1%	• CMV retinitis in AIDS patients	• Severe polyoma-, papilloma-, adeno-, herpes- and poxvirus infections (in immunocompromised patients) • HPV-, HSV-1-, HSV-2-, MCV-, orf- and other poxvirus-associated infections
Adefovir dipivoxil (Hepsera®)	Oral (10 mg daily)	• Chronic hepatitis B (HBV infection)	
Tenofovir disoproxil fumarate (TDF) (Viread®)	Oral (300 mg TDF daily)	• AIDS (HIV infection)	• Chronic hepatitis B (HBV infection)
TDF in fixed-dose combination with emtricitabine (Truvada®)	Oral (300 mg TDF daily) (200 mg emtricitabine daily)	• AIDS (HIV infection)	• Chronic hepatitis B (HBV infection)
TDF in fixed-dose combination with emtricitabine and efavirenz (Atripla®)	Oral (300 mg TDF daily) (200 mg emtricitabine daily) (600 mg efavirenz daily)	• AIDS (HIV infection)	

Fig. 3. Clinical application of acyclic nucleoside phosphonates.

3. HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine]

Linking DHPA with PAA (phosphonoacetic acid), while removing the carboxylate group of the latter, resulted in a drastic change in the antiviral activity spectrum of DHPA: the resulting construct, HPMPA, exhibited the activity spectrum of PAA, rather than that of the larger component, DHPA, in that it proved mainly active against DNA viruses (De Clercq et al., 1986). HPMPA, the prototype of the acyclic nucleoside phosphonates, showed, from the very start, a truly broad-spectrum anti-DNA virus activity, encompassing herpes-, adeno-, pox-, irido- and papovaviruses, as well as those RNA viruses replicating through a proviral DNA intermediate (i.e. retroviruses). Numerous studies have confirmed the *in vitro* antiviral activity of HPMPA, i.e. against varicella-zoster virus (Baba et al., 1987a), adenovirus (Baba et al., 1987b), African swine fever virus (Gil-Fernandez et al., 1987; Arzuza et al., 1988), Epstein-Barr virus (EBV) (Lin et al., 1987), seal herpesvirus (phocid herpesvirus 1) (Osterhaus et al., 1987), and vaccinia virus (De Clercq et al., 1987). The inhibitory effects of HPMPA and its cyclic analogue (cHPMPA) on vaccinia virus replication has also been demonstrated in primary human keratinocytes and organotypic "raft" cultures of epithelial cells (Snoeck et al., 2002). *In vivo*, HPMPA was found highly efficacious in the treatment of various experimental herpes simplex virus (HSV-1, HSV-2) and vaccinia virus infections (De Clercq et al., 1989), including intracutaneous and intracerebral TK^- (thymidine kinase-deficient) HSV-1 infections, and TK^- HSV-1 keratitis (Maudgal et al., 1987). This points to the potential of HPMPA for the treatment of HSV infections that have become resistant to treatment with the conventional antiherpes drugs (i.e. acyclovir). Of a large series of acyclic nucleoside phosphonates evaluated for their inhibitory effects on the *in vitro* growth of *Plasmodium falciparum* HPMPA and its 3-deaza analogue (c^3HPMPA) proved to be the most potent (Smeijsters et al., 1999). The trypanocidal activity of HPMPA has also been demonstrated *in vivo* (Kaminsky et al., 1996).

4. HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]

HPMPC (cidofovir) and its cyclic analogue (cHPMPC), exhibit an antiviral activity spectrum that is quite similar to that of HPMPA (and cHPMPA). The antiviral properties of HPMPC were first reported by De Clercq et al. (1987). In 1996 it was formally licensed (Vistide®) for clinical use (by the intravenous route) in the treatment of CMV retinitis in patients with AIDS. The key clinical data the approval of cidofovir was based upon was the difference in progression of CMV retinitis following immediate treatment with cidofovir as compared to deferred treatment (Lalezari et al., 1997).

Which were the principal observations that led HPMPC (cidofovir) during its initial development? HPMPC was found to be a potent and selective inhibitor of CMV replication *in vitro* (Snoeck et al., 1988), with a prolonged antiviral response, lasting for several days after a short-pulse exposure (Neyts et al., 1991). Its selective anti-CMV activity is due to a preferential inhibition of CMV DNA synthesis (Neyts et al., 1990). The *in vivo* efficacy of HPMPC was originally demonstrated in various models of HSV infection in mice, whether due to HSV-1, HSV-2 or TK^- HSV (De Clercq and Holý, 1991). The most remarkable feature of HPMPC was that a single administration of the compound, even as late as 4 days after infection, conferred significant protection against HSV infection. This pronounced *in vivo* efficacy of HPMPC has further been demonstrated in severe combined immune deficiency (SCID) mice infected with murine cytomegalovirus (Neyts et al., 1992) or vaccinia virus (Neyts and De Clercq, 1993).

The great potential of cidofovir in the treatment of poxvirus infections, whether due to vaccinia, variola (smallpox), cowpox, monkeypox, camelpox, molluscum contagiosum or orf (ecthyma contagiosum), has been reviewed (De Clercq, 2002). Cidofovir should be efficacious in the therapy and short-term prophylaxis of smallpox and related poxvirus infections in humans. Based on the efficacy seen with cidofovir in the treatment of disseminated/progressive vaccinia in a murine model (Neyts et al., 2004), it may also be advocated in the treatment of the complications of vaccinia that may arise in immunocompromised patients inadvertently inoculated with the smallpox vaccine (vaccinia) (De Clercq, 2002). In fact, antiviral treatment with, for example, cidofovir, was found to be more effective than (post-exposure) smallpox vaccination after lethal (intratracheal) infection of cynomolgus monkeys with monkeypox (Stittelaar et al., 2006).

From a clinical viewpoint, the potential use of cidofovir should focus, in particular, on poxvirus infections and human papilloma virus (HPV) infections. This recommendation is based upon the compelling results obtained with systemic or topical administration of cidofovir in the treatment of, for example, hypopharyngeal papilloma (Van Cutsem et al., 1995), laryngeal papilloma (Snoeck et al., 1998), recurrent respiratory papillomatosis in children (Pransky et al., 1999), plantar warts (Davis et al., 2000), molluscum contagiosum in AIDS patients (Meadows et al., 1997) and orf (ecthyma contagiosum) in immunocompromised patients (Geerinck et al., 2001).

5. PMEA [9-(2-phosphonylmethoxyethyl)adenine]

The antiviral potential of PMEA (adefovir) was first mentioned by De Clercq et al. (1986). After it had been initially pursued for clinical use in the treatment of HIV infections (AIDS), adefovir, in its oral prodrug form, adefovir dipivoxil (Hepsera®) was formally licensed in 2002 for the treatment of chronic hepatitis B. The key clinical data that led to this approval were generated by the studies of Hadziyannis et al. (2003), demonstrating the significant decrease in HBV DNA levels in HBeAg-negative HBV patients, as well as those of Marcellin et al. (2003), demonstrating an equally impressive of HBV DNA levels in HBeAg-positive HBV infections.

Which were the pivotal observations that led to the early development of PMEA (adefovir)? In 1988, PMEA and its congeners (including HPMPA) were announced as a new class of antiretroviral agents with potent and selective (*in vitro*) activity against HIV (Pauwels et al., 1988). Soon thereafter, PMEA was shown to inhibit retrovirus infections *in vivo*, i.e. (Moloney)

murine sarcoma virus (MSV)-induced tumor formation in mice (Balzarini et al., 1989) and simian immunodeficiency virus (SIV) infection in rhesus monkeys (Balzarini et al., 1991b). PMEA has also been shown to suppress feline immunodeficiency virus (FIV) infection *in vivo*, in cats (Egberink et al., 1990). Of note, the anti-retrovirus activity of PMEA *in vivo* increased when it was less frequently administered (Balzarini et al., 1990). The first indication that PMEA may be effective against human hepatitis B stemmed from the observations of Yokota et al. (1991) reporting a suppressive effect of PMEA on HBV DNA synthesis *in vitro*. Since PMEA has, as such, limited oral bioavailability, the bis(pivaloylmethyl) ester of PMEA, bis(POM)-PMEA (adefovir dipivoxil) was designed to ensure the compound could be administered by the oral route (Naesens et al., 1996).

Adefovir dipivoxil has evolved to an established therapy for chronic HBV infections. A recent study conducted by Hadziyannis et al. (2005) showed that in HBeAg-negative chronic hepatitis B, adefovir dipivoxil continued to achieve suppression of HBV DNA levels if treatment was extended from 48 to 96 weeks, whereas the benefits from the first 48 weeks of adefovir dipivoxil treatment were lost if treatment was stopped after 48 weeks.

6. FPMPA [(*S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine]

In 1991 we reported the *in vitro* and *in vivo* antiretroviral activity of (*RS*)-9-(3-fluoro-2-phosphonylmethoxypropyl) purine derivatives (Balzarini et al., 1991a). Description of their chemical synthesis followed in 1993 (Jindrich et al., 1993). The (*S*)-isomer of FPMPA has been further evaluated for its efficacy against FIV in cats (Hartmann et al., 1998). Both FPMPA and PMEA had a pronounced beneficial effect on the clinical symptoms of FIV-infected field cats. FPMPA proved less antivirally effective, but also less toxic, than PMEA in cats. Although these findings argued for further investigations on the efficacy and usefulness of acyclic nucleoside phosphonates in the treatment of FIV infections in cats and related lentivirus infections in other animal species (Hartmann et al., 1998), with the advent of PMPA (tenofovir) (see next section), FPMPA was not further pursued for its potential in the treatment of retrovirus infections in either humans or animals.

7. PMPA [(*R*)-9-(2-phosphonylmethoxypropyl)adenine]

The original publication on the anti-retrovirus activity of PMPA (tenofovir) dates from 1993 (Balzarini et al., 1993): the compound was shown to inhibit HIV replication *in vitro* and MSV-induced tumor formation *in vivo* (mice). In 2001 PMPA would be approved, in its oral prodrug form, tenofovir disoproxil fumarate (TDF) (Viread®), for clinical use in the treatment of HIV infection (AIDS). Historical comparisons pointed out that the combination of TDF with lamivudine (3TC) and efavirenz was superior to any previously used drug combination regime, based on the percentage of patients with undetectable HIV load at 48 weeks of treatment (reviewed by De Clercq and Holý, 2005). A crucial 3-year randomized trial comparing the efficacy and safety of TDF and stavudine (D4T), both in combination with lamivudine and efavirenz, in HIV-infected individuals not previously exposed to antiretroviral drug treatment, clearly revealed the superiority of the TDF over the D4T arm, especially from a safety viewpoint (Gallant et al., 2004). In a further comparative study, TDF, in combination with emtricitabine ((−)FTC) and efavirenz, proved superior through 48 weeks of treatment to zidovudine (AZT), in combination with lamivudine (3TC) and efavirenz, in terms of viral load suppression, CD4 response and adverse events resulting in discontinuation of the study drugs (Gallant et al., 2006). This study was of crucial importance for the formulation of the fixed-dose drug combination of TDF with emtricitabine (Truvada®, and of TDF with emtricitabine and efavirenz (Atripla®).

Which were the milestone observations that guided PMPA (tenofovir) during its swift development to the clinical stage? Of paramount importance was the observation of Tsai et al. (1995) that parenteral SIV infection in macaques could be completely prevented by PMPA, even if administered from 24 h after virus inoculation. Otten et al. (2002) showed that PMPA treatment when started either 12 or 36 h after virus inoculation prevented intravaginal HIV in macaques. Van Rompay et al. (2001) found that PMPA, administered as a single dose of 30 mg/kg at 1 h after virus inoculation prevented newborn macaques against perinatal (oral) SIV infection. Since PMPA (tenofovir), like PMEA (adefovir), is poorly absorbed by the oral route, an orally bioavailable prodrug has been designated, namely bis(isopropyloxycarbonyloxymethyl)PMPA, or bis(POC)-PMPA, or tenofovir disoproxil, which is then formulated with fumarate as TDF. Tenofovir disoproxil, when given by the oral route, was found to achieve effective levels of the active metabolite, allowing infrequent administration of the parent drug (Naesens et al., 1998; Robbins et al., 1998).

Given the experimental observations that tenofovir can prevent SIV/HIV infection by either the parenteral, intravaginal or perinatal route, combined with the safety/efficacy profile that has been established for tenofovir disoproxil fumarate (TDF, Viread®) in the treatment of AIDS over the past 5-year period (2001–2006) since TDF was approved for clinical use, TDF, whether or not in combination with emtricitabine (Truvada®) could be strongly endorsed (as a single daily pill) for the pre- and post-exposure prophylaxis of HIV infections in humans. In the abeyance of any effective vaccine to prevent HIV infection, Viread® and Truvada®, given once daily by the oral route, may confer the desired protection, and if applied, at a sufficiently wide scale, may prevent further spread of the disease.

TDF (Viread®) may also be considered for the treatment of chronic hepatitis B, especially for HBV infections which have developed resistance to lamivudine, since tenofovir retains sensitivity to HBV mutants that have developed resistance to lamivudine and other anti-HBV agents such as entecavir, emtricitabine, clevudine and telbivudine (Yang et al., 2005). Tenofovir would even retain sensitivity towards some rare HBV variants which have developed resistance towards adefovir (Schildgen et

al., 2006). Furthermore, much along the considerations given for their prophylactic use against HIV infections, Viread® and/or Truvada® might also be envisaged for use in the prophylaxis of HBV infections, i.e. in patients who have not been vaccinated (or did not respond to the hepatitis B vaccine). HBV is transmitted by the same routes (parenteral, sexual and perinatal) as HIV, and may, therefore, be amenable to similar preventive measures.

8. The 2,6-diaminopurine (DAP) derivatives: HPMPDAP [(*S*)-9-(3-hydroxyl-2-phosphonylmethoxypropyl)-2,6-diaminopurine], PMEDAP [9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine] and PMPDAP [(*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine]

The DAP (2,6-diaminopurine) derivatives show an antiviral potency and activity spectrum comparable to that of their adenine counterparts. Thus, HPMPDAP is equivalent to HPMPA, i.e. with regard to their activity against poxvirus infections, such as vaccinia virus (De Clercq and Neyts, 2004) and orf virus (Dal Pozzo et al., 2005).

PMEDAP is more active as an anti-retrovirus agent than PMEA, but also more toxic, so that its therapeutic index, based on its *in vivo* activity against MSV is equivalent to that of PMEA (Naesens et al., 1989). Single doses of either PMEA or PMEDAP afforded a marked antiviral protection when administered one day before MSV infection in newborn mice (Naesens et al., 1991). Oral PMEDAP has proven highly efficacious in the treatment of retrovirus (i.e. Moloney murine sarcoma virus and Friend leukemia virus) as well as murine CMV infections in mice (Naesens et al., 1993). PMEDAP retains its antiviral efficacy when dosed infrequently (i.e., once or twice weekly), and achieves a higher efficacy (and thus therapeutic ratio) when given orally. These remarkable properties make PMEDAP an attractive candidate for the treatment of both retrovirus (i.e. HIV) and herpesvirus (i.e. CMV) infections. I recommend it should be further followed up from this viewpoint.

In the original publication pertaining to the anti-retrovirus activity of PMPA (tenofovir), PMPDAP was accredited with even higher anti-retrovirus potency than PMPA (Balzarini et al., 1993). PMPDAP has also proved active against (both wild-type and lamivudine-resistant) HBV at a potency comparable to that of PMPA (tenofovir) (Ying et al., 2000a,b). The therapeutic potential of PMPDAP in the treatment of (*in vivo*) HIV and HBV infections remains to be further explored.

9. 1-Deaza-, 3-deaza and 8-aza HPMPA [(*S*)-9-(3-hydroxyl-2-phosphonylmethoxypropyl)adenine derivatives

Whereas the 1-deaza and 3-deaza derivatives of PMEA did not shown much antiviral activity, the corresponding 1-deaza-HPMPA and, particularly, 3-deaza-HPMPA showed pronounced activity against VZV and CMV (Dvoráková et al., 1990). Of a series of ANPs that were evaluated for activity against HHV-6 in HSB-2 cells, 3-deaza-HPMPA showed the highest selectivity index (Reymen et al., 1995). Also, more recent studies have confirmed that 3-deaza-HPMPA is remarkably active against HHV-6 (Naesens and De Clercq, 2006). There is, at present, no animal model for HHV-6. Nor has any compound been formally approved for the treatment of HHV-6 infections (De Bolle et al., 2005). It would seem mandatory, therefore, to further explore 3-deaza-HPMPA for its potential in the treatment of HHV-6 and other herpesvirus infections.

The synthesis of acyclic nucleotide analogues derived from 8-azapurine was first reported in 1993 by Dvoráková et al. (1993). Of these analogues, the 8-aza-HPMPA exhibited a marked activity against HSV-1, TK$^-$ HSV, HSV-2, VZV, TK$^-$ VZV and vaccinia virus (Holý et al., 1996). HPMPA and 8-aza-HPMPA proved more potent as inhibitors of poxvirus (i.e. vaccinia virus replication *in vitro*) than HPMPC (cidofovir) (De Clercq, 2001). The antiviral potency of 8-aza-HPMPA was comparable to that of HPMPA, which justifies it should be further examined for its antiviral potential.

10. Prodrugs of HPMPC [(*S*)-1-(3-hydroxyl-2-phosphonylmethoxypropyl)cytosine] and HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine]

The cyclic analogue of HPMPC (cidofovir), cHPMPC, is a chemically stable prodrug of HPMPC, and is converted to HPMPC by an intracellular cyclic CMP phosphodiesterase (Mendel et al., 1997). However, both HPMPC and cHPMPC are only slowly taken up (by pinocytosis) by the cells, and also their oral bioavailability is limited. To increase the cellular uptake and oral bioavailability of HPMPC (and cHPMPC), alkoxyalkyl esters have been prepared, that is hexadecyloxypropyl (HDP)-HPMPC and -cHPMPC and octadecyloxyethyl (ODE)-HPMPC and -cHMPC (Painter and Hostetler, 2004). These alkoxyalkyl esters of HPMPC (cidofovir) exhibited a 2.5- to 4.0-log increase in antiviral activity against herpesvirus (i.e. CMV) replication *in vitro* (Beadle et al., 2002). The HDP and ODE derivatives of HPMPC and cHPMPC were taken up more rapidly by human lung fibroblasts than the parent compounds (Aldern et al., 2003). Conversion of HPMPC to HDP-HPMPC also increased oral bioavailability and diminished drug accumulation in the kidneys (Ciesla et al., 2003). *In vivo*, oral HDP-HPMPC and/or ODE-HPMPC proved as effective as parenteral cidofovir in the treatment of human CMV, and pox (i.e. vaccinia) virus infections in a variety of murine models (Bidanset et al., 2004; Buller et al., 2004; Kern et al., 2004; Smee et al., 2004) (as reviewed by De Clercq and Field, 2006). The HDP and ODE prodrugs of HPMPC and HPMPA should also be further evaluated for their potential to treat adenovirus infections, as they have been found to inhibit adenovirus replication *in vitro* at significantly lower concentrations and with significantly higher selectivity indices than those noted for the parent compounds HPMPC and HPMPA (Hartline et al., 2005). The HDP and ODE prodrugs of HPMPA have also been reported to be potent inhibitors of HIV replication *in vitro* under conditions where

the parent compound (HPMPA) was inactive (Hostetler et al., 2006).

11. The 2,4-diaminopyrimidine (DAPy) derivatives: HPMPO-DAPy [(*R*)-6-(3-hydroxy-2-(phosphonylmethoxy)propoxy)-2,4-diaminopyrimidine], PMEO-DAPy [6-(2-(phosphonylmethoxy)ethoxy)-2,4-diaminopyrimidine], and 5-X-substituted derivatives thereof, and PMPO-DAPy [(*R*)-6-(2-(phosphonylmethoxy)propoxy)-2,4-diaminopyrimidine]

"Second generation" ANPs have been recently described, the "open ring" or "O-linked" ANP analogues or 6-[2-phosphonylmethoxyalkoxy]-2,4-diaminopyrimidines (DAPys) (Holý et al., 2002; Balzarini et al., 2002; Hocková et al., 2003, 2004), which show substantial potential for the treatment of a broad range of DNA virus and retrovirus infections (De Clercq et al., 2005). Like HPMPA and HPMPC (cidofovir), HPMPO-DAPy holds potential for the treatment of polyoma-, papilloma-, adeno-, herpes- and poxvirus infections, whereas PMEO-DAPy (and 5-X-substituted derivatives thereof) and PMPO-DAPy hold potential for the treatment of retrovirus (HIV) and hepadnavirus (HBV) infections.

Specifically, the 5-methyl, 5-chloro-, 5-bromo-, 5-iodo-, 5-cyano- and 5-formyl derivatives of PMEO-DAPy showed pronounced antiretroviral activity, comparable to that of PMEA (adefovir) and PMPA (tenofovir) (Hocková et al., 2003, 2004). The 5-cyano derivative of PMEO-DAPy also exhibited high potency *in vitro* against the replication of both wild type and lamivudine-resistant HBV (Ying et al., 2005). 5-Methyl PMEO-DAPy has proved markedly more inhibitory to MSV-induced tumor cell formation and associated animal death than either PMEO-DAPy or PMEA, at the dosage of 5 mg/kg day (Balzarini et al., 2006).

Akin to HPMPA and HPMPC (cidofovir), HPMPO-DAPy proved to be a potent and selective inhibitor of adenovirus replication *in vitro* [as monitored by a number of parameters (Naesens et al., 2005)], and again akin to HPMPC and HPMPC (cidofovir), HPMPO-DAPy was found to exhibit selective and potent activity against orf virus in both human and ovine cell monolayers and organotypic ovine raft cultures (Dal Pozzo et al., 2005). *In vivo*, HPMPO-DAPy, similarly to HPMPC (cidofovir), was shown to cause healing of cutaneous vaccinia lesions in athymic-nude mice (which corresponds to an experimental model infection for disseminated vaccinia in immunosuppressed patients inadvertently vaccinated with live smallpox vaccine (De Clercq and Neyts, 2004). Like cidofovir, HPMPO-DAPy also proved more effective than smallpox vaccination when used in cynomolgus monkeys 24 h after they had been infected with lethal monkey pox virus challenge (Stittelaar et al., 2006).

It is at present unpredictable whether the DAPy derivatives may surpass the established nucleotide analogues cidofovir, adefovir or tenofovir in terms of potency and/or safety, or any other properties. Therefore, their activity spectrum, *in vivo* efficacy, tolerability, safety, bioavailability, pharmacokinetics, tissue distribution and resistance development should be further examined.

12. Antitumor potential of the acyclic nucleoside phosphonates

Although cidofovir, adefovir and tenofovir have been clinically pursued primarily for their antiviral activity, their antitumor activity should be further explored so as to assess their potential in anticancer therapy. PMEA (adefovir) has been found to induce tumor cell differentiation of several tumor (i.e. erythroleukemia, myeloid neuroblastoma, and choriocarcinoma) cell lines (Balzarini et al., 1995; Hatse et al., 1998a). *In vivo*, adefovir was found to inhibit choriocarcinoma growth in the rat (Hatse et al., 1998b). Likewise, HPMPC (cidofovir) has demonstrated remarkable activity as an antitumor agent in several animal models such as human nasopharyngeal carcinoma xenografts in athymic-nude mice (Neyts et al., 1998; Murono et al., 2001), human cervical carcinoma xenografts in athymic-nude mice (Andrei et al., 1998), polyomavirus-induced hemangiomas in rats (Liekens et al., 1998), hemangiosarcoma development in athymic-nude mice (Liekens et al., 2001b), fibroblast growth factor-2-induced vascular tumor formation in athymic-nude mice and severe combined immunodeficient (SCID) mice (Liekens et al., 2001a), and murine melanoma B16 in mice (Redondo et al., 2000). The inhibitory effects of cidofovir on the growth of these tumors (i.e. nasopharyngeal carcinoma, hemangiosarcoma and endothelium-derived tumors) could be attributed to the induction of apoptosis.

Similarly, cidofovir was shown to cause cell death of HPV-infected, but not uninfected, human keratinocytes, due to the induction of apoptosis (Andrei et al., 2001). Induction of apoptosis in HPV-positive cells by cidofovir was associated with the accumulation of the tumor suppressor protein p53 (Andrei et al., 2001). Cidofovir was shown to restore the function of p53 and another tumor-suppressor protein pRb which in HPV-infected cells are neutralized by the oncoproteins E6 and E7, respectively (Abdulkarim et al., 2002). Numerous clinical observations point to the efficacy of cidofovir in achieving complete and permanent remissions of HPV-associated papillomatous lesions following either topical gel application or direct intralesional injections of the drug (for a review, see De Clercq, 2003a). It would now seem mandatory to examine whether this antitumor potential of cidofovir extends to tumors other than those induced by viruses (HPV, EBV, ...) and acyclic nucleoside phosphonates other than cidofovir. In this sense PMEG [9-(2-phosphonylmethoxyethyl)guanine] may hold promise as an antitumor agent (Rose et al., 1990). It could be generated *in vivo* from PMEDAP (see above: Section 8) and prodrugs thereof (such as the N^6-cyclopropyl derivative of PMEDAP).

13. Conclusion

The era of the acyclic nucleoside phosphonates (ANPs) as effective antiviral drugs (and potentially effective anticancer drugs) started now 20 years ago with the discovery of HPMPA as a broad-spectrum anti-DNA virus agent (De Clercq et al., 1986). The predecessor of HPMPA was the acyclic nucleoside analogue DHPA, which we had discovered as a broad-spectrum antiviral agent with albeit relatively weak activ-

ity against both (−)RNA and DNA viruses in 1976 [personal communication from E. De Clercq to A. Holý), later published by De Clercq et al. (1978)]. From HPMPA then originated a large progeny of new ANPs, three of which, HPMPC (cidofovir), PMEA (adefovir) and PMPA (tenofovir) were eventually approved and marketed for the treatment of, respectively, CMV retinitis (in AIDS patients), chronic HBV infections and HIV infections (AIDS). Additional indications include various polyoma-, papilloma-, adeno-, herpes-, and poxvirus infections for cidofovir, and HBV infections for tenofovir. Also from HPMPA originated the 3-deaza-HPMPA and 8-aza-HPMPA, the DAP (2,6-diaminopurine) derivatives HPMPDAP, PMEDAP and PMPDAP, and the DAPy (2,4-diaminopyrimidine) derivatives HPMPO-DAPy, PMEO-DAPy (and 5-substituted derivatives thereof) and PMPO-DAPy. To the extent that they have been explored, these "newer" ANPs display an antiviral activity spectrum and potency comparable to that of the "established" ANPs, but, in some cases they proved more potent and/or showed an extended activity spectrum. The newer ANPs, and, in particular, the DAPy derivatives should also be further explored for their *in vivo* efficacy, pharmacokinetics, safety and other properties, and in addition to their antiviral efficacy, the antitumor potential of the ANPs, whether old or new, should be further evaluated in the appropriate experimental (and, eventually,) clinical settings.

Acknowledgments

This paper is dedicated to Prof. Antonin Holý at his 70th birthday in remembrance of the Prague-Leuven connection which started 30 years ago. From the Leuven side, I want to acknowledge the help of my many collaborators (see list of references), and, in particular, my collaborators at the Rega Institute, Graciela Andrei, Masanori Baba [now in Japan (Kagoshima)], Jan Balzarini, Ria Bernaerts, Marina Cools, Sigrid Hatse, Piet Herdewijn, Sandra Liekens, Prabhat C. Maudgal, Lieve Naesens, Johan Neyts, Rudi Pauwels [now in Switzerland (Lausanne)], Dominique Schols, Takashi Sakuma and Robert Snoeck. For their excellent technical assistance, I gratefully acknowledge Ann Absillis, Anita Camps, Fabiana Dal Pozzo (Steven Carmans), Frieda De Meyer, Leentje Persoons, Lizette van Berckelaer, Lies Van den Heurck, Anita Van Lierde and Willy Zeegers. Both A. Holý and I appreciate the enormous efforts of John C. Martin, Norbert Bischofberger and W.A. ("Bill") Lee and their colleagues at Gilead Sciences (Foster City, California, USA) to turn our compounds [as Antonín Holý phrased it in Collect. Czech. Chem. Commun. 76 (6) (2006) v–xi] "from bench chemicals to shelf drugs". Very special thanks go to Christiane Callebaut for her invaluable editorial assistance.

References

Abdulkarim, B., Sabri, S., Deutsch, E., Chagraoui, H., Maggiorella, L., Thierry, J., Eschwege, F., Vainchenker, W., Chouaib, S., Bourhis, J., 2002. Antiviral agent cidofovir restores p53 function and enhances the radiosensitivity in HPV-associated cancers. Oncogene 21, 2334–2346.

Aldern, K.A., Ciesla, S.L., Winegarden, K.L., Hostetler, K.Y., 2003. Increased antiviral activity of 1-*O*-hexedecyloxypropyl-[2-^{14}C]cidofovir in MRC-5 human lung fibroblasts is explained by unique cellular uptake and metabolism. Mol. Pharmacol. 63, 678–681.

Andrei, G., Snoeck, R., Piette, J., Delvenne, P., De Clercq, E., 1998. Inhibitory effects of cidofovir (HPMPC) on the growth of the human cervical carcinoma (SiHa) xenografts in athymic-nude mice. Oncol. Res. 10, 533–539.

Andrei, G., Snoeck, R., Schols, D., De Clercq, E., 2001. Induction of apoptosis by cidofovir in human papillomavirus (HPV)-positive cells. Oncol. Res. 12, 397–408.

Arzuza, O., García-Villalón, D., Tabarés, E., Gil-Fernández, C., De Clercq, E., 1988. Inhibition of African swine fever virus DNA synthesis by (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine. Biochem. Biophys. Res. Commun. 154, 27–32.

Baba, M., Konno, K., Shigeta, S., De Clercq, E., 1987a. *In vitro* activity of (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine against newly isolated clinical varicella-zoster virus strains. Eur. J. Clin. Microbiol. 6, 158–160.

Baba, M., Mori, S., Shigeta, S., De Clercq, E., 1987b. Selective inhibitory effect of (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine and 2'-nor-cyclic GMP on adenovirus replication *in vitro*. Antimicrob. Agents Chemother. 31, 337–339.

Balzarini, J., Naesens, L., Herdewijn, P., Rosenberg, I., Holý, A., Pauwels, R., Baba, M., Johns, D.G., De Clercq, E., 1989. Marked *in vivo* antiretrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine, a selective anti-human immunodeficiency virus agent. Proc. Natl. Acad. Sci. U.S.A. 86, 32–336.

Balzarini, J., Naesens, L., De Clercq, E., 1990. Anti-retrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) *in vivo* increases when it is less frequently administered. Int. J. Cancer 46, 337–340.

Balzarini, J., Holý, A., Jindrich, J., Dvorakova, H., Hao, Z., Snoeck, R., Herdewijn, P., Johns, D.G., De Clercq, E., 1991a. 9-[(2*RS*)-3-fluoro-2-phosphonylmethoxypropyl] derivatives of purines: a class of highly selective antiretroviral agents *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 88, 4961–4965.

Balzarini, J., Naesens, L., Slachmuylders, J., Niphuis, H., Rosenberg, I., Holý, A., Schellekens, H., De Clercq, E., 1991b. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits retrovirus replication in vitro and simian immunodeficiency virus infection in rhesus monkeys. AIDS 5, 21–28.

Balzarini, J., Holý, A., Jindrich, J., Naesens, L., Snoeck, R., Schols, D., De Clercq, E., 1993. Differential antiherpesvirus and antiretrovirus effects of the (*S*) and (*R*) enantiomers of acyclic nucleoside phosphonates: potent and selective *in vitro* and *in vivo* antiretrovirus activities of (*R*)-9-(2-phosphonomethoxypropyl)-2, 6-diaminopurine. Antimicrob. Agents Chemother. 37, 332–338.

Balzarini, J., Verstuyf, A., Hatse, S., Goebels, J., Sobis, H., Vandeputte, M., De Clercq, E., 1995. The human immunodeficiency virus (HIV) inhibitor 9-(2-phosphonylmethoxyethyl)ade-nine (PMEA) is a strong inducer of differentiation of several tumor cell lines. Int. J. Cancer 61, 130–137.

Balzarini, J., Pannecouque, C., De Clercq, E., Aquaro, S., Perno, C.-F., Egberink, H., Holý, A., 2002. Antiretrovirus activity of a novel class of acyclic pyrimidine nucleoside phosphonates. Antimicrob. Agents Chemother. 46, 2185–2193.

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, J.R., Hartline, C., Aldern, K.A., Rodriguez, N., Harden, E., Kern, E.R., Hostetler, K.Y., 2002. Alkoxyalkyl esters of cidofovir and cyclic cidofovir exhibit multiple-log enhancement of antiviral activity against cytomegalovirus and herpesvirus replication *in vitro*. Antimicrob. Agents Chemother. 46, 2381–2386.

Bidanset, D.J., Beadle, J.R., Wan, W.B., Hostetler, K.Y., Kern, E.R., 2004. Oral activity of ether lipid ester prodrugs of cidofovir against experimental human cytomegalovirus infection. J. Infect. Dis. 190, 499–503.

Buller, R.M., Owens, G., Schriewer, J., Melman, L., Beadle, J.R., Hostetler, K.Y., 2004. Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. Virology 318, 474–481.

Ciesla, S.L., Trahan, J., Wan, W.B., Beadle, J.R., Aldern, K.A., Painter, G.R., Hostetler, K.Y., 2003. Esterification of cidofovir with alkoxyalkanols increases oral bioavailability and diminishes drug accumulation in kidney. Antiviral Res. 59, 163–171.

Cools, M., De Clercq, E., 1989. Correlation between the antiviral activity of acyclic and carbocyclic adenosine analogues in murine L929 cells and their inhibitory effect on L929 cell *S*-adenosylhomocysteine hydrolase. Biochem. Pharmacol. 38, 1061–1067.

Cools, M., De Clercq, E., 1990. Influence of *S*-adenosylhomocysteine hydrolase inhibitors on *S*-adenosylhomocysteine and *S*-adenosylmethionine pool levels in L929 cells. Biochem. Pharmacol. 40, 2259–2264.

Cools, M., Hasobe, M., De Clercq, E., Borchardt, R.T., 1990. Mechanism of the synergistic antiviral and cytostatic activity of (*RS*)-3-(adenin-9-yl)-2-hydroxypropanoic acid isobutyl ester and D,L-homocysteine. Biochem. Pharmacol. 39, 195–202.

Dal Pozzo, F., Andrei, G., Holý, A., Van Den Oord, J., Scagliarini, A., De Clercq, E., Snoeck, R., 2005. Activity of acyclic nucleoside phosphonates against orf virus in human and ovine cell monolayers and organotypic ovine raft cultures. Antimicrob. Agents Chemother. 49, 4843–4852.

Davis, M.D., Gostout, B.S., McGovern, R.M., Persing, D.H., Schut, R.L., Pittelkow, M.R., 2000. Large plantar wart caused by human papillomavirus-66 and resolution by topical cidofovir therapy. J. Am. Acad. Dermatol. 43, 340–343.

De Bolle, L., Naesens, L., De Clercq, E., 2005. Update on human herpesvirus 6 biology, clinical features, and therapy. Clin. Microbiol. Rev. 18, 217–245.

De Clercq, E., 1987. S-Adenosylhomocysteine hydrolase inhibitors as broad-spectrum antiviral agents. Biochem. Pharmacol. 36, 2567–2575.

De Clercq, E., 2001. Vaccinia virus inhibitors as a paradigm for the chemotherapy of poxvirus infections. Clin. Microbiol. Rev. 14, 382–397.

De Clercq, E., 2002. Cidofovir in the treatment of poxvirus infections. Antiviral Res. 55, 1–13.

De Clercq, E., 2003a. Clinical potential of acyclic nucleoside phosphonates cidofovir, adefovir, and tenofovir in treatment of DNA virus and retrovirus infections. Clin. Microbiol. Rev. 16, 569–596.

De Clercq, E., 2003b. Potential of acyclic nucleoside phosphonates in the treatment of DNA virus and retrovirus infections. Expert Rev. Anti-infect. Ther. 1, 21–43.

De Clercq, E., Cools, M., 1985. Antiviral potency of adenosine analogues: correlation with inhibition of *S*-adenosylhomocysteine hydrolase. Biochem. Biophys. Res. Commun. 129, 306–311.

De Clercq, E., Field, H.J., 2006. Antiviral prodrugs—the development of successful prodrug strategies for antiviral chemotherapy. Br. J. Pharmacol. 147, 1–11.

De Clercq, E., Holý, A., 1979. Antiviral activity of aliphatic nucleoside analogues: structure–function relationship. J. Med. Chem. 22, 510–513.

De Clercq, E., Holý, A., 1985. Alkyl esters of 3-adenin-9-yl-2-hydroxypropanoic acid: a new class of broad-spectrum antiviral agents. J. Med. Chem. 28, 282–287.

De Clercq, E., Holý, A., 1991. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine in various models of herpes simplex virus infection in mice. Antimicrob. Agents Chemother. 35, 701–706.

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

De Clercq, E., Neyts, J., 2004. Therapeutic potential of nucleoside/nucleotide analogues against poxvirus infections. Rev. Med. Virol. 14, 289–300.

De Clercq, E., Descamps, J., De Somer, P., Holý, A., 1978. (*S*)-9-(2, 3-Dihydroxypropyl)-adenine: an aliphatic nucleoside analog with broad spectrum antiviral activity. Science 200, 563–565.

De Clercq, E., Holý, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C., 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., Holý, A., Rosenberg, I., 1989. Efficacy of phosphonylmethoxyalkyl derivatives of adenine in experimental herpes simplex virus and vaccinia virus infections *in vivo*. Antimicrob. Agents Chemother. 33, 185–191.

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.

Dvoráková, H., Holý, A., Snoeck, R., Balzarini, J., De Clercq, E., 1990. Acyclic nucleoside and nucleotide analogues derived from 1-deaza and 3-deazaadenine. Collect. Czech. Chem. Commun. 55, 113–116.

Dvoráková, H., Holý, A., Masojídková, M., Votruba, I., Balzarini, J., Snoeck, R., De Clercq, E., 1993. Synthesis and antiviral activity of acyclic nucleoside and nucleotide derivatives of 8-azaadenine. Collect. Czech. Chem. Commun. 58, 253–255.

Egberink, H., Borst, M., Niphuis, H., Balzarini, J., Neu, H., Schellekens, H., De Clercq, E., Horzinek, M., Koolen, M., 1990. Suppression of feline immunodeficiency virus infection *in vivo* by 9-(2-phosphonomethoxyethyl)adenine. Proc. Natl. Acad. Sci. U.S.A. 87, 3087–3091.

Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L., Schaeffer, H.J., 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A. 74, 5716–5720.

Gallant, J.E., Staszewski, S., Pozniak, A.L., DeJesus, E., Suleiman, J.M., Miller, M.D., Coakley, D.F., Lu, B., Toole, J.J., Cheng, A.K., 2004. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients: a 3-year randomized trial. JAMA 292, 191–201.

Gallant, J.E., DeJesus, E., Arribas, J.R., Pozniak, A.L., Gazzard, B., Campo, R.E., Lu, B., McColl, D., Chuck, S., Enejosa, J., Toole, J.J., Cheng, A.K., 2006. Tenofovir DF, emtricitabine, and efavirenz vs zidovudine, lamivudine, and efavirenz for HIV. N. Engl. J. Med. 354, 251–260.

Geerinck, K., Lukito, G., Snoeck, R., De Vos, R., De Clercq, E., Vanrenterghem, Y., Degreef, H., Maes, B., 2001. A case of human orf in an immunocompromised patient treated successfully with cidofovir cream. J. Med. Virol. 64, 543–549.

Gil-Fernandez, C., Garcia-Villalon, D., De Clercq, E., Rosenberg, I., Holý, A, 1987. Phosphonylmethoxyalkylpurines and pyrimidines as inhibitors of African swine fever virus replication *in vitro*. Antiviral Res. 8, 273–282.

Hadziyannis, S.J., Tassopoulos, N.C., Heathcote, E.J., Chang, T.T., Kitis, G., Rizzetto, M., Marcellin, P., Lim, S.G., Goodman, Z., Wulfsohn, M.S., Xiong, S., Fry, J., Brosgart, C.L., 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N. Engl. J. Med. 348, 800–807.

Hadziyannis, S.J., Tassopoulos, N.C., Heathcote, E.J., Chang, T.T., Kitis, G., Rizzetto, M., Marcellin, P., Lim, S.G., Goodman, Z., Ma, J., Arterburn, S., Xiong, S., Currie, G., Brosgart, C.L., 2005. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N. Engl. J. Med. 352, 2673–2681.

Hartline, C.B., Gustin, K.M., Wan, W.B., Ciesla, S.L., Beadle, J.R., Hostetler, K.Y., Kern, E.R., 2005. Ether lipid-ester prodrugs of acyclic nucleoside phosphonates: activity against adenovirus replication *in vitro*. J. Infect. Dis. 191, 396–399.

Hartmann, K., Kuffer, M., Balzarini, J., Naesens, L., Goldberg, M., Erfle, V., Goebel, F.-D., De Clercq, E., Jindrich, J., Holý, A., Bischofberger, N., Kraft, W., 1998. Efficacy of the acyclic nucleoside phosphonates (*S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine (FPMPA) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) against feline immunodeficiency virus. J. Acquir. Immune Defic. Synd. Hum. Retrovirol. 17, 120–128.

Hatse, S., Naesens, L., De Clercq, E., Balzarini, J., 1998a. Potent differentiation-inducing properties of the antiretroviral agent 9-(2-phosphonyl-methoxyethyl)adenine (PMEA) in the rat choriocarcinoma (RCHO) tumor cell model. Biochem. Pharmacol. 56, 851–859.

Hatse, S., Naesens, L., Degrève, B., Segers, C., Vandeputte, M., Waer, M., De Clercq, E., Balzarini, J., 1998b. Potent antitumor activity of the acyclic nucleoside phosphonate 9-(2-phosphonyl-methoxyethyl)adenine in choriocarcinoma-bearing rats. Int. J. Cancer 76, 595–600.

Hocková, D., Holý, A., Masojídková, M., Andrei, G., Snoeck, R., De Clercq, E., Balzarini, J., 2003. 5-Substituted-2, 4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidines-acyclic nucleotide phosphonate analogues with antiviral activity. J. Med. Chem. 46, 5064–5073.

Hocková, D., Holý, A., Masojídková, M., Andrei, G., Snoeck, R., De Clercq, E., Balzarini, J., 2004. Synthesis and antiviral activity of 2, 4-diamino-5-cyano-6-[2-(phosphonomethoxy)ethoxy]pyrimidine and related compounds. Bioorg. Med. Chem. 12, 3197–3202.

Holý, A., Votruba, I., De Clercq, E., 1982. Synthesis and antiviral activity of stereoisomeric eritadenines. Collect. Czech. Chem. Commun. 47, 1392–1407.

Holý, A., Dvoráková, H., Jindrich, J., Masojídková, M., Budesínský, M., Balzarini, J., Andrei, G., De Clercq, E., 1996. Acyclic nucleotide analogs derived from 8-azapurines: synthesis and antiviral activity. J. Med. Chem. 39, 4073–4088.

Holý, A., Votruba, I., Masojídková, Andrei, G., Snoeck, R., Naesens, L., De Clercq, E., Balzarini, J., 2002. 6-[2-(Phosphonomethoxy)-alkoxy]pyrimidines with antiviral activity. J. Med. Chem. 45, 1918–1929.

Hostetler, K.Y., Aldern, K.A., Wan, W.B., Ciesla, S.L., Beadle, J.R., 2006. Alkoxyalkyl esters of (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine are potent inhibitors of the replication of wild-type and drug-resistant human immunodeficiency virus type 1 *in vitro*. Antimicrob. Agents Chemother. 50, 2857–2859.

Jindrich, J., Holý, A., Dvoráková, H., 1993. Synthesis of *N*-(3-fluoro-2-phosphonomethoxypropyl) (FPMP) derivatives of heterocyclic bases. Collect. Czech. Chem. Commun. 58, 1645–1667.

Kaminsky, R., Schmid, C., Grether, Y., Holý, A., De Clercq, E., Naesens, L., Brun, R., 1996. (*S*)-9-(3-Hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA]: a purine analogue with trypanocidal activity *in vitro* and *in vivo*. Trop. Med. Int. Health 1, 255–263.

Kern, E.R., Collins, D.J., Wan, W.B., Beadle, J.R., Hostetler, K.Y., Quenelle, D.C., 2004. Oral treatment of murine cytomegalovirus infections with ether lipid esters of cidofovir. Antimicrob. Agents Chemother. 48, 3516–3522.

Lalezari, J.P., Stagg, R.J., Kuppermann, B.D., Holland, G.N., Kramer, F., Ives, D.V., Youle, M., Robinson, M.R., Drew, W.L., Jaffe, H.S., 1997. Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS. A randomized, controlled trial. Ann. Intern. Med. 126, 257–263.

Liekens, S., Andrei, G., Vandeputte, M., De Clercq, E., Neyts, J., 1998. Potent inhibition of hemangioma formation in rats by the acyclic nucleoside phosphonate analogue cidofovir. Cancer Res. 58, 2562–2567.

Liekens, S., Neyts, J., De Clercq, E., Verbeken, E., Ribatti, D., Presta, M., 2001a. Inhibition of fibroblast growth factor-2-induced vascular tumor formation by the acyclic nucleoside phosphonate cidofovir. Cancer Res. 61, 5057–5064.

Liekens, S., Verbeken, E., De Clercq, E., Neyts, J., 2001b. Potent inhibition of hemangiosarcoma development in mice by cidofovir. Int. J. Cancer 92, 161–167.

Lin, J.-C., De Clercq, E., Pagano, J.S., 1987. Novel acyclic adenosine analogs inhibit Epstein-Barr virus replication. Antimicrob. Agents Chemother. 31, 1431–1433.

Marcellin, P., Chang, T.T., Lim, S.G., Tong, M.J., Sievert, W., Shiffman, M.L., Jeffers, L., Goodman, Z., Wulfsohn, M.S., Xiong, S., Fry, J., Brosgart, C.L., 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N. Engl. J. Med. 348, 808–816.

Maudgal, P.C., De Clercq, E., Huyghe, P., 1987. Efficacy of (*S*)-HPMPA against thymidine kinase-deficient herpes simplex virus-keratitis. Invest. Ophthalmol. Visual Sci. 28, 243–248.

Meadows, K.P., Tyring, S.K., Pavia, A.T., Rallis, T.M., 1997. Resolution of recalcitrant molluscum contagiosum virus lesions in human immunodeficiency virus-infected patiens treated with cidofovir. Arch. Dermatol. 133, 987–990.

Mendel, D.B., Cihlar, T., Moon, K., Chen, M.S., 1997. Conversion of 1-[((*S*)-2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine to cidofovir by an intracellular cyclic CMP phosphodiesterase. Antimicrob. Agents Chemother. 41, 641–646.

Murono, S., Raab-Traub, N., Pagano, J.S., 2001. Prevention and inhibition of nasopharyngeal carcinoma growth by antiviral phosphonated nucleoside analogs. Cancer Res. 61, 7875–7877.

Naesens, L., De Clercq, E., 2006. Antiviral activity of diverse classes of broad-acting agents and natural compounds in HHV-6-infected lymphoblasts. J. Clin. Virol. (Suppl.), S69–S75.

Naesens, L., Balzarini, J., Rosenberg, I., Holý, A., De Clercq, E., 1989. 9-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP): a novel agent with anti-human immunodeficiency virus activity in vitro and potent anti-Moloney murine sarcoma virus activity in vivo. Eur. J. Clin. Microbiol. Infect. Dis. 8, 1043–1047.

Naesens, L., Balzarini, J., De Clercq, E., 1991. Single-dose administration of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and 9-(2-phosphonylmethoxyethyl)-2, 6-diaminopurine (PMEDAP) in the prophylaxis of retrovirus infection in vivo. Antiviral Res. 16, 53–64.

Naesens, L., Neyts, J., Balzarini, J., Holý, A., Rosenberg, I., De Clercq, E., 1993. Efficacy of oral 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) in the treatment of retrovirus and cytomegalovirus infections in mice. J. Med. Virol. 39, 167–172.

Naesens, L., Balzarini, J., Bischofberger, N., De Clercq, E., 1996. Antiretroviral activity and pharmacokinetics in mice of oral bis(pivaloyloxymethyl)-9-(2-phosphony-lmethoxyethyl)adenine, the bis(pivaloyloxymethyl) ester prodrug of 9-(2-phosphonylmethoxyethyl)adenine. Antimicrob. Agents Chemother. 40, 22–28.

Naesens, L., Bischofberger, N., Augustijns, P., Annaert, P., Van den Mooter, G., Arimilli, M.N., Kim, C.U., De Clercq, E., 1998. Antiretroviral efficacy and pharmacokinetics of oral bis-(isopropyloxycarbonyloxymethyl)-9-(2-phosphonylmethoxypropyl)adenine in mice. Antimicrob. Agents Chemother. 42, 1568–1573.

Naesens, L., Lenaerts, L., Andrei, G., Snoeck, R., Van Beers, D., Holý, A., Balzarini, J., De Clercq, E., 2005. Antiadenovirus activities of several classes of nucleoside and nucleotide analogues. Antimicrob. Agents Chemother. 49, 1010–1016.

Neyts, J., De Clercq, E., 1993. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine for the treatment of lethal vaccinia virus infections in severe combined immune deficiency (SCID) mice. J. Med. Virol. 41, 242–246.

Neyts, J., Snoeck, R., Schols, D., Balzarini, J., De Clercq, E., 1990. Selective inhibition of human cytomegalovirus DNA synthesis by (*S*)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine [(*S*)-HPMPC] and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG). Virology 179, 41–50.

Neyts, J., Snoeck, R., Balzarini, J., De Clercq, E., 1991. Particular characteristics of the anti-human cytomegalovirus activity of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine (HPMPC) *in vitro*. Antiviral Res. 16, 41–52.

Neyts, J., Balzarini, J., Naesens, L., De Clercq, E., 1992. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1, 3-dihydroxy-2-propoxymethyl)guanine for the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice. J. Med. Virol. 37, 67–71.

Neyts, J., Sadler, R., De Clercq, E., Raab-Traub, N., Pagano, J.S., 1998. The antiviral agent cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] has pronounced activity against nasopharyngeal carcinoma grown in nude mice. Cancer Res. 58, 384–388.

Neyts, J., Leyssen, P., Verbeken, E., De Clercq, E., 2004. Efficacy of cidofovir in a murine model for disseminated/progressive vaccinia. Antimicrob. Agents Chemother. 48, 2267–2273.

Osterhaus, A.D.M.E., Groen, J., De Clercq, E., 1987. Selective inhibitory effects of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine and 1-(2′-deoxy-2′-fluoro-β-D-arabino-furanosyl)-5-iodouracil on seal herpesvirus (phocid herpesvirus 1) infection *in vitro*. Antiviral Res. 7, 221–226.

Otten, R.A., Smith, D.K., Adams, D.R., Pullium, J.K., Jackson, E., Kim, C.N., Jaffe, H., Janssen, R., Butera, S., Folks, T.M., 2002. Efficacy of postexposure prophylaxis after intravaginal exposure of pig-tailed macaques to a human-derived retrovirus (human immunodeficiency virus type 2). J. Virol. 74, 9771–9775.

Painter, G.R., Hostetler, K.Y., 2004. Design and development of oral drugs for the prophylaxis and treatment of smallpox infection. Trends Biotechnol. 22, 423–427.

Pauwels, R., Balzarini, J., Schols, D., Baba, M., Desmyter, J., Rosenberg, I., Holý, A., De Clercq, E., 1988. Phosphonylmethoxyethyl purine derivatives: a new class of anti-human immunodeficiency virus agents. Antimicrob. Agents Chemother. 32, 1025–1030.

Pransky, S.M., Magit, A.E., Kearns, D.B., Kang, D.R., Duncan, N.O., 1999. Intralesional cidofovir for recurrent respiratory papillomatosis in children. Arch. Otolaryngol. Head Neck Surg. 125, 1143–1148.

Redondo, P., Idoate, M., Galofre, J.C., Solano, T., 2000. Cidofovir inhibits growth of B16 melanoma cells *in vivo*. Br. J. Dermatol. 143, 741–748.

Reymen, D., Naesens, L., Balzarini, J., Holý, A., Dvoraková, H., De Clercq, E., 1995. Antiviral activity of selected acyclic nucleoside analogues against human herpesvirus type 6. Antiviral Res. 28, 343–357.

Robbins, B.L., Srinivas, R.V., Kim, C., Bischofberger, N., Fridland, A., 1998. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-*R*-(2-phosphonomethoxypropyl)adenine (PMPA), bis(isopropyloxymethylcarbonyl)PMPA. Antimicrob. Agents Chemother. 42, 612–617.

Rose, W.C., Crosswell, A.R., Bronson, J.J., Martin, J.C., 1990. *In vivo* antitumor activity of 9-[(2-phosphonylmethoxy)ethyl]-guanine and related phosphonate nucleotide analogues. J. Natl. Cancer Inst. 82, 510–512.

Schaeffer, H.J., Beauchamp, L., de Miranda, P., Elion, G.B., Bauer, D.J., Collins, P., 1978. 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature 272, 583–585.

Schildgen, O., Sirma, H., Funk, A., Olotu, C., Wend, U.C., Hartmann, H., Heim, M., Rockstroh, J.K., Willems, W.R., Will, H., Gerlich, W.H., 2006. Variant of hepatitis B virus with primary resistance to adefovir. N. Engl. J. Med. 354, 1807–1812.

Smee, D.F., Wong, M.H., Bailey, K.W., Beadle, J.R., Hostetler, K.Y., Sidwell, R.W., 2004. Effects of four antiviral substances on lethal vaccinia virus (HD strain) respiratory infections in mice. Int. J. Antimicrob. Agents 23, 430–437.

Smeijsters, L.J.J.W., Franssen, F.F.J., Naesens, L., de Vries, E., Holý, A., Balzarini, J., De Clercq, E., Overdulve, J.P., 1999. Inhibition of the *in vitro* growth of *Plasmodium falciparum* by acyclic nucleoside phosphonates. Int. J. Antimicrob. Agents 12, 53–61.

Snoeck, R.T, De Clercq, E., Rosenberg, I., Holý, A., 1988. (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine [(*S*)-HPMPC]: a potent and selective inhibitor of human cytomegalovirus replication. Antimicrob. Agents Chemother. 32, 1839–1844.

Snoeck, R., Andrei, G., Neyts, J., Schols, D., Cools, M., Balzarini, J., De Clercq, E., 1993. Inhibitory activity of *S*-adenosylhomocysteine hydrolase inhibitors against human cytomegalovirus replication. Antiviral Res. 21, 197–216.

Snoeck, R., Wellens, W., Desloovere, C., Van Ranst, M., Naesens, L., De Clercq, E., Feenstra, A., 1998. Treatment of severe laryngeal papillomatosis with intralesional injections of cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]. J. Med. Virol. 54, 219–225.

Snoeck, R., Holý, A., Dewolf-Peeters, C., Van Den Oord, J., De Clercq, E., Andrei, G., 2002. Antivaccinia activities of acyclic nucleoside phosphonate derivatives in epithelial cells and organotypic cultures. Antimicrob. Agents Chemother. 46, 3356–3361.

Stittelaar, K.J., Neyts, J., Naesens, L., van Amerongen, G., van Lavieren, R.F., Holý, A., De Clercq, E., Niesters, H.G.M., Fries, E., Maas, C., Mulder, P.G.H., van der Zeijst, B.A.M., Osterhaus, A.D.M.E., 2006. Antiviral treatment is more effective than smallpox vaccination upon lethal monkeypox virus infection. Nature 439, 745–748.

Tsai, C.C., Follis, K.E., Sabo, A., Beck, T.W., Grant, R.F., Bischofberger, N., Benveniste, R.E., Black, R., 1995. Prevention of SIV infection in macaques by (*R*)-9-(2-phosphonylmethoxypropyl)adenine. Science 270, 1197–1199.

Van Cutsem, E., Snoeck, R., Van Ranst, M., Fiten, P., Opdenakker, G., Geboes, K., Janssens, J., Rutgeerts, P., Vantrappen, G., De Clercq, E., 1995. Successful treatment of a squamous papilloma of the hypopharynx-esophagus by local injections of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC). J. Med. Virol. 45, 230–235.

Van Rompay, K.K., McChesney, M.B., Aguirre, N.L., Schmidt, K.A., Bischofberger, N., Marthas, M.L., 2001. Two low doses of tenofovir protect newborn macaques against oral simian immunodeficiency virus infection. J. Infect. Dis. 184, 429–438.

Votruba, I., Holý, A., 1980. Inhibition of *S*-adenosyl-L-homocysteine hydrolase by the aliphatic nucleoside analogue 9-(*S*)-(2, 3-dihydroxypropyl)adenine. Collect. Czech. Chem. Commun. 45, 3039–3044.

Yang, H., Qi, X., Sabogal, A., Miller, M., Xiong, S., Delaney IV., W.E., 2005. Cross-resistance testing of next-generation nucleoside and nucleotide analogues against lamivudine-resistant HBV. Antiviral Ther. 10, 625–633.

Ying, C., De Clercq, E., Neyts, J., 2000a. Lamivudine, adefovir and tenofovir exhibit long-lasting anti-hepatitis B virus activity in cell culture. J. Viral Hepatitis 7, 79–83.

Ying, C., De Clercq, E., Nicholson, W., Furman, P., Neyts, J., 2000b. Inhibition of the replication of the DNA polymerase M550V mutation variant of human hepatitis B virus by adefovir, tenofovir, L-FMAU, DAPD, penciclovir and lobucavir. J. Viral Hepatitis 7, 161–165.

Ying, C., Holý, A., Hocková, D., Havlas, Z., De Clercq, E., Neyts, J., 2005. Novel acyclic nucleoside phosphonate analogues with potent anti-hepatitis B virus activities. Antimicrob. Agents Chemother. 49, 1177–1180.

Yokota, T., Mochizuki, S., Konno, K., Mori, S., Shigeta, S., De Clercq, E., 1991. Inhibitory effects of selected antiviral compounds on human hepatitis B virus DNA synthesis. Antimicrob. Agents Chemother. 35, 394–397.