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REVIEW

Antiviral prodrugs – the development of successful prodrug strategies for antiviral chemotherapy

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Following the discovery of the first effective antiviral compound (idoxuridine) in 1959, nucleoside analogues, especially acyclovir (ACV) for the treatment of herpesvirus infections, have dominated antiviral therapy for several decades. However, ACV and similar acyclic nucleosides suffer from low aqueous solubility and low bioavailability following oral administration. Derivatives of acyclic nucleosides, typically esters, were developed to overcome this problem and valaciclovir, the valine ester of ACV, was among the first of a new series of compounds that were readily metabolized upon oral administration to produce the antiviral nucleoside *in vivo*, thus increasing the bioavailility by several fold. Concurrently, famciclovir was developed as an oral formulation of penciclovir. These antiviral 'prodrugs' thus established a principle that has led to many successful drugs including both nucleoside and nucleotide analogues for the control of several virus infections, notably those caused by herpes-, retro- and hepatitisviruses. This review will chart the origins and development of the most important of the antiviral prodrugs to date.
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Abbreviations: ACV, acyclovir; AZT, azidothymidine; BVDU, bromovinyldeoxyuridine; cCDV, cHPMPC, cyclic cidofovir; CMV, cytomegalovirus; ddC, dideoxycytidine; ddI, dideoxyinosine; d4T, didehydrodideoxythymidine; DP, diphosphate; FddGuo, FLG, 3′-fluoro-2′,3′-dideoxyguanosine; FddThd, FLT, 3′-fluoro-2′,3′-dideoxythymidine; (−)FTC, emtricitabine; GCV, ganciclovir; HBV, hepatitis B virus; 3′-Val-L-dC, 3′-valine ester of L-dC (valtorcitabine); HDP-CDV, hexadecyloxypropyl cidofovir; HDP-cCDV, hexadecyloxypropyl (cyclic) cidofovir; HPMPC, cidofovir, (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; H2G, (−)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine ((−)-2HM-HBG) L-dC, β-L-2′-deoxycytidine; L-dT, β-L-thymidine; MP, monophosphate; MIV-606, L-valine, (3*R*)-3[2-amino-1, 6-dihydro-6-oxo-purin-9-yl]methyl]-4[(1-oxooctadecyl)oxo]butylester; MIV-210, bis(5'-*O*-[(*S*)-2-(L-valoyloxy)propionyl)-FLG; NANA, *N*-acetylneuraminic acid; ODE-cCDV, octadecyloxyethyl (cyclic) cidofovir; ODE-CDV, octadecyloxyethyl cidofovir; PCV, penciclovir; PMEA, adefovir, 9-(2-phosphonylmethoxyethyl)adenine; PMPA, tenofovir, (*R*)-9-(2-phosphonylmethoxypropyl)adenine; TDF, tenofovir disoproxil fumarate; TK, thymidine kinase; TP, triphosphate; VZV, varicella-zoster virus

Introduction

Nucleoside analogue origins of modern antiviral chemotherapy

In the 21st century, antiviral chemotherapy is well established for the prevention and treatment of many important virus infections; there are now more than 40 licensed drugs for diseases caused by herpesviruses, retroviruses, orthomyxoviruses, hepatitis B and hepatitis C viruses. Without exception, all viruses are obligate, intracellular parasites and until the 1950s were widely believed not to be susceptible to 'antibiotic' therapy. This dogma was reversed with the discovery of 5-iodo-2′-deoxyuridine (idoxurine) (Prusoff, 1959), the first antiviral drug to be widely used having acknowledged clinical utility for the topical treatment of herpes keratitis. For the next two decades, the field of antiviral therapy was dominated by this and other nucleoside analogues especially trifluorothymidine, adenine arabinoside and subsequently acyclovir (ACV), bromovinyldeoxyuridine, ganciclovir and penciclovir (Field & De Clercq, 2004).

Antiviral nucleosides as prodrugs

All these nucleosides are essentially 'prodrugs' since their antiviral activity depends upon metabolism within herpes-infected cells to form sequentially the mono-, di- and triphosphates (MP, DP, and TP, respectively). It is these nucleotides (especially the nucleoside TP) that inhibit essential processes in virus replication. Most important, the nucleoside TP may inhibit herpesvirus-encoded DNA-polymerase. Furthermore, some nucleoside TPs (notably ACV-TP) are obligate chain terminators of herpesvirus DNA while others (notably penciclovir-TP) may be facultative chain terminators (Wutzler & Thust, 2001) but in most cases virus DNA replication is blocked (Darby, 1995). For the purpose of this review, however, 'antiviral prodrug' will be defined as a compound

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that requires metabolic conversion *in vivo* before entering the infected cell wherein further metabolism may or may not be required to yield the active inhibitor.

First- and second-generation nucleoside analogues

The first-generation antiviral nucleosides (including idoxuridine and trifluorothymidine) were poorly selective for virus-infected cells and proved to be too toxic for systemic administration; they are used only for topical application. A major advance occurred with the discovery that ACV – the first of the second-generation nucleoside analogues – is a highly selective inhibitor of herpes simplex virus (HSV-1) and varicella-zoster virus (VZV), and, moreover, safe for oral administration.

The development of antiviral prodrugs

ACV: 9-(2-hydroxyethoxymethyl)guanine (Figure 1)

The discovery of ACV as a selective antiherpetic agent (Elion *et al*., 1977; Schaeffer *et al*., 1978) heralded a new era in antiviral chemotherapy, that of a selective approach to attack virus infections. ACV would, later on, become the gold standard for the treatment of herpesvirus (HSV-1, HSV-2 and VZV) infections.

The compound (first known as acycloguanosine) was synthesized in the U.S.A. as part of the Burroughs Wellcome programme for the development of guanosine nucleosides resistant to phosphorylase degradation with the first observation of antiviral activity being made by Collins & Bauer in the U.K. at the Beckenham laboratories of the former Wellcome Foundation. The key to selectivity was subsequently shown to be selective phosphorylation of the acyclic guanosine nucleoside, ACV to ACV-MP by the herpesvirus-encoded pyrimidine deoxynucleoside kinase, thymidine kinase (TK). Further highly selective nucleosides with similar mechanisms of selectivity followed with the reports of bromovinyldeoxyuridine (BVDU), ganciclovir (GCV) and penciclovir (PCV). While these and similar compounds were very effective inhibitors of HSV in cell culture with effective inhibitory concentrations of 1 μM or lower and ACV proved safe for systemic administration, there remained an important disadvantage; that of relatively low oral bioavailability and short plasma half-life. Comparatively large doses and frequent administration were thus required to maintain trough values for plasma concentrations of ACV above the threshold required for virus inhibition. Thus, ACV has limited oral bioavailability (15–20%); possibly as low as 10% following an 800 mg dose (Weller *et al*., 1993) and also limited solubility in water ($\sim$0.2%, 25°C). This means that the compound cannot be given as intramuscular injections (and therefore must be administered intravenously as a bolus infusion of 5 mg kg^{-1} every 8 h), or, for the treatment of herpetic keratitis, cannot be given as eyedrops (and therefore must be applied as a 3% eye ointment). In attempts to make ACV more water soluble, several ester derivatives of ACV were prepared, taht is, 2′-*O*-glycyl and 2′-*O*-alanyl ACV esters (Figure 1) (Colla *et al*., 1983). The 2′-*O*-glycyl ester proved to be efficacious in the topical treatment of herpetic keratitis (in rabbits) when administered as a 1% eyedrop formulation (Maudgal *et al*., 1984) and, as the patent of ACV drew towards its end and generic ACV was to become widely available, much research effort was directed to improve methods of drug delivery including methods of slow release by mechanical or chemical methods leading ultimately to the first successful antiviral prodrugs.

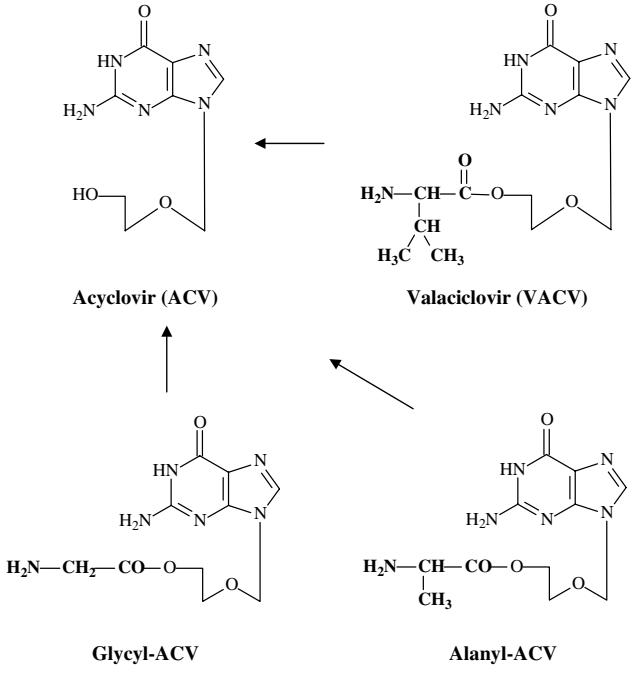


Figure 1 Structures of ACV and amino-acid ester derivatives thereof.

Penciclovir: 9-(4-hydroxy-3-hydroxymethyl-but-1-yl) guanine (Figure 2)

Penciclovir (Boyd *et al*., 1987) is an acyclic guanosine nucleoside analogue with a structure that is comparable to that of ACV; there is, however, no oxygen atom in the acyclic 'sugar' moiety although an OH group exists in the position equivalent to that of the 3′-OH group in the normal deoxynucleoside, 2′-deoxyguanosine. Like ACV, penciclovir is converted to penciclovir-MP by the HSV or VZV TK

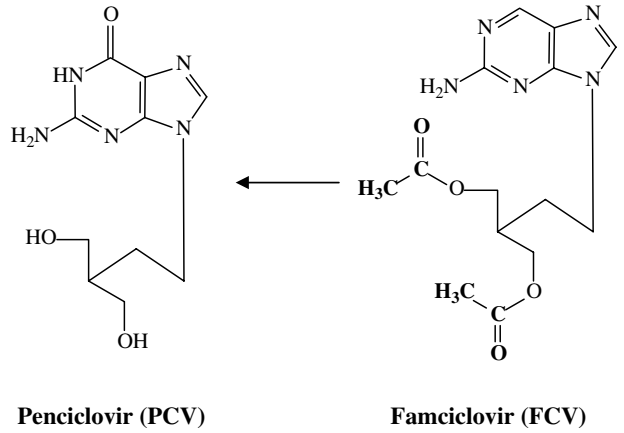


Figure 2 Structures of penciclovir and famciclovir.

(Vere Hodge & Cheng 1993a, b), and penciclovir-TP inhibits herpesvirus DNA polymerase but with the possibility of internal incorporation of penciclovir residues into viral DNA and further chain extension (Vere Hodge & Perkins, 1989). The initial conversion of penciclovir to penciclovir-MP is more efficient than the phosphorylation of ACV, but the penciclovir-TP formed in infected cells is less active than ACV-TP as an inhibitor of HSV DNA polymerase (Earnshaw *et al*., 1992). When penciclovir was developed in the laboratories of the former Beecham Laboratories (Harnden & Jarvest, 1985), it was realized at an early stage that, although penciclovir showed a similar spectrum of antiviral activity and selectivity compared with ACV, the oral bioavailability was even lower than that of ACV. These were the drivers that resulted in an intense programme leading to the compounds valaciclovir and famciclovir that were to become the first antiviral prodrugs with clinical utility.

Famciclovir: diacetyl-6-deoxy-9-(4-hydroxy-3-hydroxymethyl-but-1-yl)guanine (Figure 2)

Famiclovir is the diacetyl derivative of penciclovir (Harnden *et al* 1989; Vere Hodge *et al*., 1989), an oral prodrug that yields penciclovir *in vivo* (Figure 2). Following an oral dose, famciclovir is efficiently converted to penciclovir requiring the action of two enzymes that catalyse (i) removal of the two acetyl groups and (ii) oxidation of the purine nucleoside (Vere Hodge, 1993). The oxidative step was initially attributed to the action of xanthine oxidase (a molybdenum hydroxylase) but further studies have revealed that aldehyde oxidase, another molybdenum hydroxylase, would account for the conversion of 6-deoxypenciclovir to penciclovir (Rashidi *et al*., 1997). From studies with rat and human tissue, it was apparent that the major product detected was penciclovir. In extracts of intestinal wall, hydrolysis of the first ester group was much faster than that of the second; while the liver was considered to be the most important site for oxidation (Vere Hodge *et al*., 1989). Both steps are rapid and efficient in man producing an absolute bioavailability of pencicloivr from famciclovir of 77% following a single oral dose; the final products being exclusively penciclovir and the harmless by-product, acetic acid (Vere Hodge, 1993). Famciclovir is inactive in standard tissue cultures for antiviral activity (since the oxidation step in the conversion to penciclovir does not occur), but many studies have shown that, if administered orally, it is highly effective against HSV in a variety of different animal infection models (as reviewed by Field, 1996). In an experimental HSV-1 infection model in immunosuppressed mice, famciclovir was found more efficacious than valaciclovir in clearing the virus from its target sites (ear and brain) (Field *et al*., 1995). In a murine model for HSV-2 infection, famciclovir again compared favorably with valaciclovir in preventing viral recurrences (Thackray & Field, 1996). Also, in a quantitative study on the prevention of HSV-1 latency in mice, significantly less latent virus could be detected in the ganglia from mice that had been treated with famciclovir, as compared to valaciclovir (Thackray & Field, 1998). The reason(s) why famciclovir is able to prevent recurrences of infectious virus in neural tissues (in mice) under conditions where valaciclovir is unable to do so (Thackray & Field, 2000) remain(s) to be elucidated.

After more than 10 years clinical experience, famciclovir has been established to be effective against HSV-1-and -2 for both therapy and long-term suppression of recurrences (Sacks *et al*., 1996; Mertz *et al*., 1997) and is also claimed to be efficacious in treatment of herpes zoster (Degreef & Famciclovir Herpes Zoster Clinical Study Group, 1994; Tyring *et al*., 1995) with beneficial effects on acute disease and postherpetic neuralgia (Tyring *et al*., 1995).

Valaciclovir: L-valyl ester of ACV (Figure 1)

As mentioned above, Burroughs Wellcome and others had investigated a number of water-soluble esters of ACV. The first of these to be reported was found to be toxic (Purifoy *et al*., 1993) but the valine ester of ACV, valaciclovir (Crooks, 1995), proved to be a safe and efficacious drug. The increased oral bioavailability of valaciclovir can be attributed to a carrier-mediated intestinal absorption, *via* the human intestinal peptide transporter hPEPT1 (Guo *et al*., 1999), followed by the rapid conversion to ACV by ester hydrolysis in the small intestine. One of the enzymes that may act as a 'valACVase' is the biphenyl hydrolase-like protein, previously cloned from human breast carcinoma and recently purified from Caco-2 cells derived from the human intestine (Kim *et al*., 2003). Upon oral administration, valaciclovir undergoes rapid first-pass metabolism to yield only ACV and the essential amino acid valine (Perry & Faulds, 1996). As with famciclovir, this results in a significant increase in the bioavailability of ACV following oral administration. Thus, multiple oral doses of valaciclovir (1 g three times daily) resulted in plasma ACV concentrations similar to those achieved with intravenous ACV (5 or 10 mg kg^{-1} three times daily) without the sharp peak concentrations (Carrington, 1994; 1997; Crooks, 1995). In this case, the enzymatic conversion occurs in cell culture and the antiviral activity can be demonstrated in standard tissue culture assays for the inhibition of HSV (H.J. Field, unpublished observations). Both valaciclovir and famciclovir have been widely used in the clinic and, although there are few data comparing one with the other (Field, 1996), they are now both accepted to be safe and effective antiviral compounds.

Valaciclovir (administered orally at 1 g three times daily for 7 days) has proved as efficacious and safe as ACV (800 mg five times daily), while offering a simpler dosing regime, in the therapy of herpes zoster in immunocompetent adults (Beutner *et al*., 1995). Valaciclovir (administered orally at 2 g twice daily for 1 day) has shown efficacy (and has recently been approved in the United States) for the episodic treatment of herpes labialis (cold sores) (Spruance *et al*., 2003). Valaciclovir (500 mg twice daily) has also proved effective for the suppression of recurrent genital herpes in HIV-infected subjects (DeJesus *et al*., 2003), and valaciclovir (500 mg once daily) was found to reduce the risk of transmission of genital herpes (monitored for a period of 8 months) (Corey *et al*., 2004).

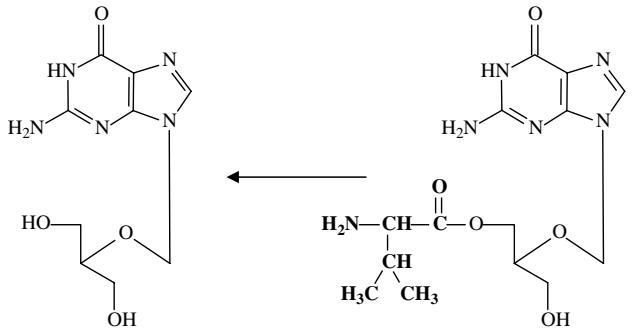
The pioneering success of these two compounds in the clinic thus paved the way for further similar synthetic modification to many other antiviral compounds; the antiviral prodrug concept proved to be an important land mark and many other prodrugs were soon to follow.

In humans, valaciclovir (or ACV) and famciclovir have only occasionally been the subject of a comparative study. In one such study (Shafran *et al*., 2004), famciclovir 750 mg once daily, 500 mg twice daily or 250 mg three times daily, and ACV 800 mg five times daily, all for 7 days, achieved a similar

beneficial effect on an acute herpes zoster episode (post-herpetic neuralgia was not assessed). In another double-blind, placebo-controlled trial, famciclovir (500 mg twice daily) was found to confer clinically and statistically significant reductions in the symptoms associated with HSV infection and the symptomatic and asymptomatic shedding of HSV in HIV-infected persons (Schacker *et al*., 1998).

Ganciclovir: 9-(1,3-dihydroxy-2-propoxymethyl)guanine (Figure 3)

Ganciclovir is an acyclic guanosine analogue that is structurally related to ACV. Ganciclovir, under certain conditions, is even more effective than ACV against HSV, although it has greater potential toxicity (Crumpacker, 1996). It is, however, much more effective than ACV against cytomegalovirus (CMV) and has been primarily used for the treatment of CMV infections in immunosuppressed patients (e.g., CMV retinitis in AIDS patients). As for ACV, the oral bioavailability of ganciclovir is limited, and, to overcome this problem the valine ester of ganciclovir (valganciclovir (Figure 3)) has been synthesized. Valganciclovir can be conveniently administered at 950 mg twice daily (De Clercq, 2004). Valganciclovir may be assumed to be taken up in the intestine by the same intestinal peptide transporter (hPEPT1) as valaciclovir, and then to be converted (by valACVase; Kim *et al*., 2003) to release the parent compound, ganciclovir.

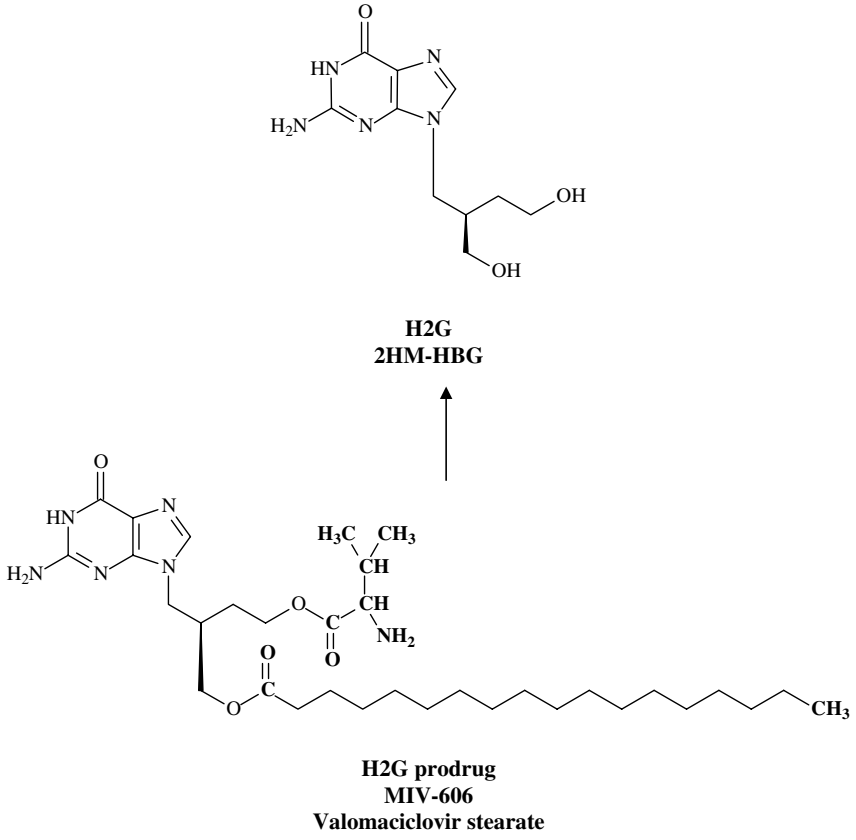


Ganciclovir (GCV) **Valganciclovir (VGCV)**

Figure 3 Structures of ganciclovir and valganciclovir.

H2G: (−)-9-[4-hydroxy-2-(hydroxymethyl)butyl] guanine ((−)-2HM-HBG) (Figure 4)

H2G corresponds to the (*R*) or (−)-enantiomer of the racemate, (*RS*)- or (±)-2HM-HBG (9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine) (Figure 4), which, like its structural analogue, ACV, was found to inhibit VZV replication in cell culture (Abele *et al*., 1987). The TP of 2HM-HBG is a potent inhibitor of VZV DNA polymerase, less active than ACV TP (Abele *et al*., 1988), but with a longer intracellular half-life (Lowe *et al*., 1995). As for ACV, the anti-VZV activity of H2G depends on phosphorylation by the VZV-encoded TK. H2G-resistant VZV mutants, generated *in vitro* contained deletions in the TK gene, and proved cross-resistant to ACV, and *vice versa* (Ng *et al*., 2001).



H2G
2HM-HBG

H2G prodrug
MIV-606
Valomaciclovir stearate

Figure 4 Structures of H2G and its prodrug valomaciclovir stearate.

Both (±)-2HM-HBG and H2G have proved efficacious in African green monkeys infected with simian varicella virus, for H2G even at a dosage down to 1 mg kg^{-1} day^{-1} (Lake-Bakaar *et al*., 1988; Soike *et al*., 1993). However, because of their low oral bioavailability, (±)-2HM-HBG and H2G have to be injected parenterally (intramuscularly) to achieve therapeutic efficacy at such low dose. Interestingly, H2G was quoted to have an oral bioavailability of 17% in cynomolgus monkeys, which is similar to that of ACV (Soike *et al*., 1993). MIV-606, the prodrug of H2G [L-valine, (3*R*)-3[2-amino-1,6-dihydro-6-oxo-purin-9-yl]methyl]-4[(1-oxooctadecyl)oxo]butylester] has an oral bioavailability of >70% in rats and monkeys, and >60% in humans (B. Öberg, unpublished observations).

3′-Fluoro-2′,3′-dideoxynucleosides FLT and FLG (Figure 5)

In the 1980s various 2′,3′-dideoxynucleosides, 3′-substituted 2′,3′-dideoxynucleosides and 2′,3′-didehydro-2′,3′-dideoxynucleosides were described as antiretroviral agents, among which azidothymidine (zidovudine, AZT), dideoxyinosine (didanosine, ddI), dideoxycytidine (zalcitabine, ddC) and didehydrodideoxythymidine (stavudine, d4T) were later marketed for the treatment of HIV infections (AIDS). Also described in that period was the anti-HIV activity of a number of 3′-fluoro-substituted 2′,3′-dideoxynucleoside analogues such as 3′-fluoro-2′,3′-dideoxythymidine (FddThd, FLT) and 3′-fluoro-2′,3′-dideoxyguanosine (FddGuo, FLG) (Figure 5) (Herdewijn *et al*., 1987; Balzarini *et al*., 1988), and these (and other) compounds are still being pursued for their clinical potential in the treatment of HIV infections. All 2′,3′-dideoxynucleoside (ddN) analogues act in the same fashion, in that they have to undergo three consecutive (intracellular) phosphorylations to be converted to their 5′-TP form (ddNTP), which then interacts as a competitive inhibitor/alternate substrate for the reverse transcriptase (RNA-dependent DNA polymerase). If incorporated into the DNA chain, the ddNMP will inevitably terminate chain elongation.

FLG has an oral bioavailability of 10–20% in various animal species and this can be increased by 4- to 5-fold when using the bis(5′-*O*-[(*S*)-2-(L-valoyloxy)propionyl]) prodrug (MIV-210) (Figure 5): the oral bioavailability of MIV-210 in man is greater than 80% (B. Öberg, unpublished observations).

L-Nucleosides: β-L-thymidine and β-L-2′-deoxycytidine (Figure 6)

The 'unnatural' L-nucleosides*β*-L-thymidine (L-dT) and *β*-L-2′-deoxycytidine (L-dC) have been identified as specific inhibitors of hepatitis B virus (HBV) infections (Bryant *et al*., 2001). These compounds were reported to achieve an unprecedented (up to 10^8 genome equivalents per ml serum) reduction in viral load in the woodchuck model of chronic HBV infection. Intracellularly, L-dT and L-dC are rapidly and extensively phosphorylated to their active intracellular metabolites which have long intracellular half-lives (>15 h) (Hernandez-Santiago *et al*., 2002). Neither L-dT nor L-dC interfered with the level of phosphorylation of the other compound, suggesting that they could be combined with one another in the treatment of HBV infection. In fact, L-dT (telbivudine) is currently in phase III and the 3′-valine ester of L-dC (valtorcitabine) is currently in phase II, and a combination (telbivudine + valtorcitabine) has been planned, for the treatment of hepatitis B (J.P. Sommadossi, presented at the XVI International Round Table on

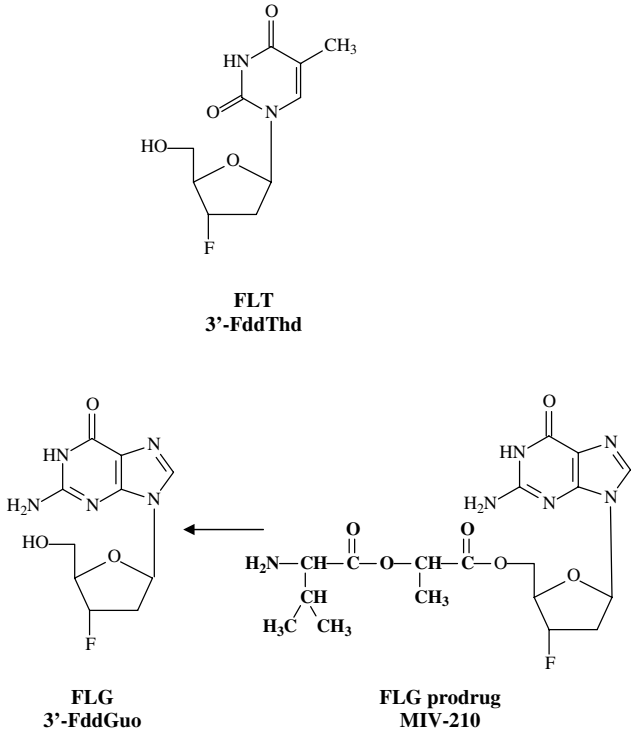


Figure 5 Structures of FLT, FLG and the FLG prodrug MIV-210.

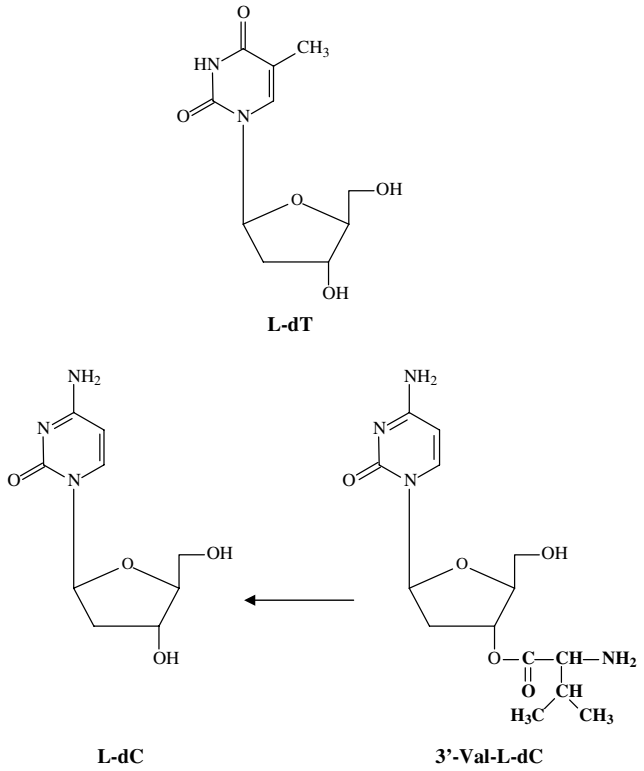


Figure 6 Structures of L-nucleosides.

Nucleosides, Nucleotides & Nucleic Acids, Minneapolis, MN, U.S.A., 12–16 September 2004).

Neuraminidase inhibitors: zanamivir and oseltamivir (Figure 7)

Based on structural information generated from X-ray crystallographic studies of the neuraminidase of influenza virus, transition-state analogues of sialic acid (*N*-acetylneuraminic acid, NANA) were designed as inhibitors of the influenza neuraminidase, zanamivir (von Itzstein *et al*., 1993) and oseltamivir (Kim *et al*., 1997) being the prototypes of this class of compounds (Figure 7). Oseltamivir (Figure 7) actually corresponds to the ethyl ester of the carbocyclic sialic acid analogue that was originally designed as a transition state-based neuraminidase inhibitor, the reason for this esterification being that it affords sufficient oral bioavailability to the neuraminidase inhibitor. Oseltamivir has obtained wide acceptance for the oral therapy and prophylaxis of influenza virus infections, irrespective of the type concerned (influenza A (H3N2, H1N1, H5N1, H7N7) or B).

Cidofovir: (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) (Figure 8)

The acyclic nucleoside phosphonate HPMPC (cidofovir, CDV) was first described in 1987 by De Clercq *et al* (1987) as a broad-spectrum anti-DNA virus agent, active against herpesviruses (HSV-1, HSV-2, VZV, CMV), adeno- and poxviruses; 9 years later it became the first acyclic nucleoside phosphonate to be licensed (for the intravenous treatment of CMV retinitis in AIDS patients). It is also being used 'off label' for the intravenous or topical treatment of various herpes- and adenovirus infections, poxvirus infections (i.e. molluscum contagiosum) and papillomavirus-associated diseases such as genital warts and laryngeal papillomas (De Clercq, 2003). The nephrotoxicity of cidofovir necessitates limiting the dosage to $5\,mg\,kg^{-1}$ once weekly (or every other week) and the concomitant administration of fluid (hydration) and probenecid.

As demonstrated in rats, the cyclic analogue (cCDV, Figure 8) is a chemically stable prodrug of CDV. Within the cells, it is converted to CDV by an intracellular cyclic CMP phosphodiesterase (Mendel *et al*., 1997). It has reduced nephrotoxicity as compared to CDV in rats (Bischofberger *et al*., 1994), and also in humans (Cundy *et al*., 1999). In (immunocompromised) guinea-pigs, cCDV was shown to be safe and effective against systemic guinea-pig CMV infection (Bourne *et al*., 2000).

However, CDV and cCDV are only slowly taken up (by pinocytosis) by the cells and also their oral bioavailability is limited. To increase cellular uptake of CDV (and cCDV), alkoxyalkyl esters have been prepared, that is, hexadecyloxypropyl (cyclic) cidofovir (HDP-CDV, HDP-cCDV) and octadecyloxyethyl (cyclic) cidofovir (ODE-CDV, ODE-cCDV) (Figure 8) (Painter & Hostetler, 2004). These alkoxyalkyl esters of CDV and cCDV exhibited a 2.5- to 4-log increase in antiviral activity against herpesvirus (i.e. CMV) replication *in vitro* (Beadle *et al*., 2002). HDP-CDV and HDP-cCDV were taken up more rapidly by human lung fibroblasts than CDV and cCDV (Aldern *et al*., 2003). Esterification of CDV, as in HDP-CDV, also increased oral bioavailability and diminished drug accumulation in the kidney (Ciesla *et al*., 2003). *In vivo*, oral HDP-CDV and/or ODE-CDV proved as effective as parenteral cidofovir in the treatment of human CMV, pox and vaccinia infection in a range of mouse models (Bidanset *et al*., 2004; Buller *et al*., 2004; Kern *et al*., 2004; Smee *et al*., 2004).

These data point to the potential of the alkoxyalkyl esters of cidofovir in the oral treatment (and prophylaxis) of poxvirus infections such as smallpox (Painter & Hostetler, 2004) but also various other DNA virus (i.e. herpes-, adeno-, and papillomavirus) infections that have so far proved amenable to parenteral cidofovir therapy.

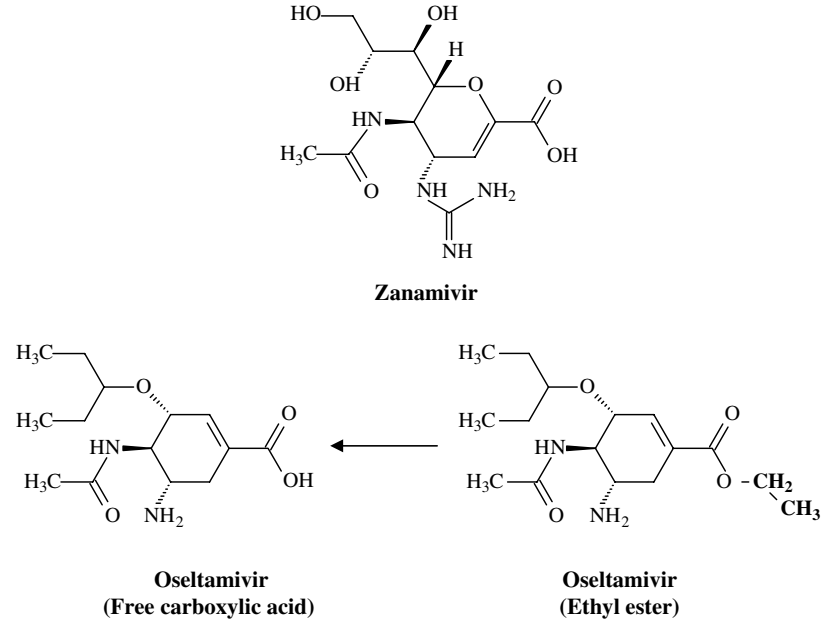


Figure 7 Structures of neuraminidase inhibitors.

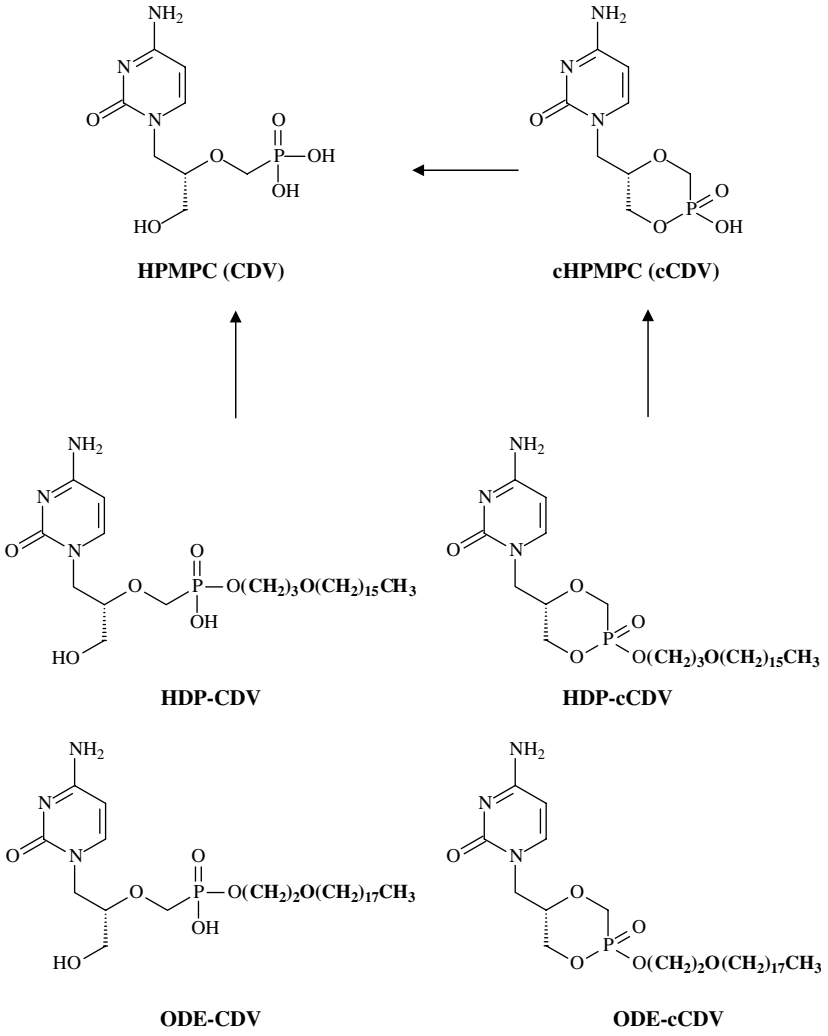


Figure 8 Structures of cidofovir and its prodrugs.

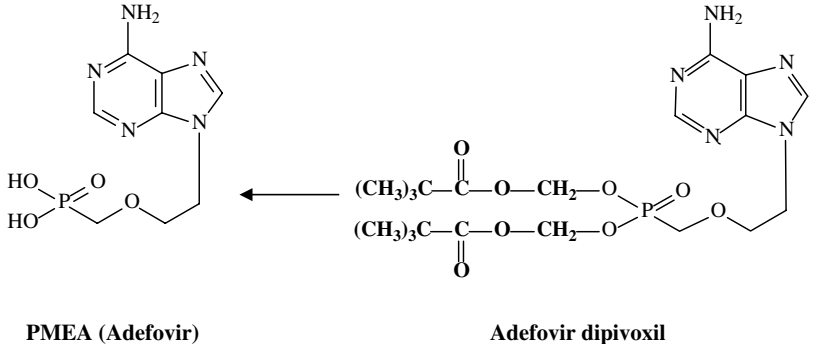
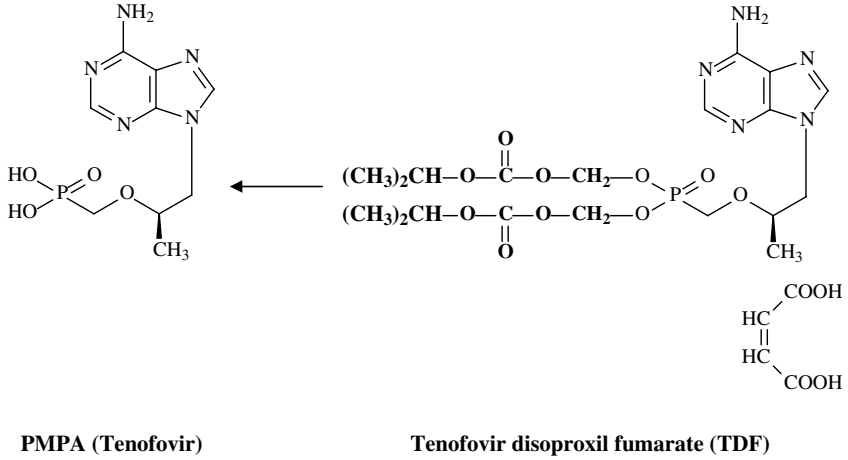


Figure 9 Structures of adefovir and its prodrug adefovir dipivoxil.

Adefovir: 9-(2-phosphonylmethoxyethyl)adenine (PMEA) (Figure 9)

The acyclic nucleoside phosphonate PMEA was first mentioned by De Clercq *et al.* (1986). After it was shown to be markedly active against HIV and other retroviruses, both *in vitro* (Pauwels *et al.*, 1988) and *in vivo* (Balzarini *et al.*, 1989), adefovir (as its oral prodrug form, adefovir dipivoxil, the bis(pivaloyloxymethyl) ester of adefovir, (Figure 9)) was initially tested in HIV infections (Naesens *et al.*, 1996; De Clercq, 2003), but eventually abandoned on account of toxicity. Instead, adefovir dipivoxil was further pursued, with success, for the treatment of HBV infections, where it was able to cause significant reductions in viral load at a (non-toxic) oral dose as low as 10 mg per day (Hadziyannis *et al.*, 2003; Marcellin *et al.*, 2003). Adefovir dipivoxil has now become



PMPA (Tenofovir) **Tenofovir disoproxil fumarate (TDF)**

Figure 10 Structures of tenofovir and its prodrug tenofovir disoproxil fumarate.

a standard treatment for chronic hepatitis B, particularly in patients with a lamivudine-resistant HBV infection.

Tenofovir: (R)-9-(2-phosphonylmethoxypropyl)adenine (PMPA) (Figure 10)

The inhibitory effects of the acyclic nucleoside phosphonate PMPA (Figure 10) on the replication of HIV and other retroviruses were first reported by Balzarini *et al*. (1993). After 8 years, in 2001, tenofovir, as its oral prodrug form (tenofovir disoproxil fumarate (TDF)), was licensed for the treatment of HIV infections (AIDS). Tenofovir disoproxil corresponds to the bis(isopropyloxycarbonyloxymethyl) ester of tenofovir (Figure 10). The *in vivo* efficacy of oral tenofovir disoproxil was first demonstrated in a murine (Moloney sarcoma) retrovirus model (Naesens *et al*., 1998). The intestinal absorption of TDF depends on the P-glycoprotein transporter and can be enhanced by defined ester mixtures (Van Gelder *et al*., 2002).

Oral TDF (300 mg once daily) has now become a widely accepted component of antiretroviral drug regimens, used in both treatment-experienced and treatment-naïve patients. One such antiretroviral regimen is based on the combination of TDF with lamivudine and efavirenz: this drug combination regimen is as efficacious as the combination of stavudine with lamivudine and efavirenz, but is associated with a better lipid profile and less lipodystrophy (Gallant *et al*., 2004). An even more efficacious drug regimen may be based on the combination of TDF with emtricitabine (instead of lamivudine) and efavirenz. TDF and emtricitabine ((−)FTC) have already been formulated as a single pill once daily (at a fixed dose of 300 mg TDF and 200 mg emtricitabine), and a once daily formulation with three anti-HIV compounds (TDF, (−)FTC and efavirenz) is forthcoming.

In addition to its use in the treatment of HIV infections, TDF may also become an effective alternative for the treatment of patients with wild-type HBV or lamivudine-resistant HBV infection (van Bömmel *et al*., 2004).

Concluding remarks

Antiviral chemotherapy was slow to be accepted. That reluctance was due, at least in part, to the fact that a generation of clinicians had been brought up on the notion that a virus inhibitor must, inevitably, be a toxic substance for the host. As explained in this review, ACV was the drug that changed this concept. Used correctly and at moderate doses, ACV is essentially devoid of toxic side effects. For over a decade this fact obscured the major limitations of this compound; its relatively poor solubility and low oral bioavailability were sometimes overlooked. As confidence in the safety of ACV and its antiviral successors has grown so thoughts have turned to problems other than safety. In response to the antiviral management of HIV patients, a new approach, unthinkable in the 1950s was the use of combinations of two or more compounds. Drug combinations for HIV including nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors and protease inhibitors are now fully accepted as a vital strategy to combat resistance development in the HIV field, and combinations are also coming to be accepted for hepatitis therapy. This approach has continued to develop apace with drug combinations for the first time becoming commercially available in a single pill (tenofovir disoproxil fumarate with emtricitabine, see above).

Similarly, this review has shown how the prodrug strategy for antiviral compounds is now readily accepted and has already been applied to many successful drugs. The prodrug concept is now an integral part of the drug discovery process and will continue to be widely exploited by the medicinal chemist in relation to the many novel compounds currently under investigation and development.

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