Acyclic Nucleosides as Antiviral Compounds

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Abstract

Acyclovir is an effective drug for the treatment of HSV and VZV infections, which after phosphorylation to the triphosphate, inhibits viral DNA polymerase. Acyclovir has low oral bioavailability, therefore prodrugs have been developed, and the L-valyl ester, valaciclovir, recently has been licensed for the treatment of shingles. Ganciclovir is used against CMV, and famciclovir, a lipophilic prodrug of penciclovir, is marketed for shingles. The acyclic nucleoside phosphonates are active against thymidine kinase-resistant viral strains. Promising analogs are PMEA (in clinical trial for the treatment of AIDS) and (S)-HPMPC (good in vivo activity against HSV, VZV, CMV, and EBV). Oligonucleotides incorporating acyclic nucleosides at the 3'- and 5'-ends, or constituted of amino acyclic nucleosides, are resistant to cleavage by nucleases and may be useful in antisense and/or antigene therapy. HEPT is active against HIV-1: It binds in a hydrophobic pocket on reverse transcriptase, rather than in the polymerase active site. Some acyclic nucleosides are potent inhibitors of purine and pyrimidine nucleoside phosphorylase. These compounds may have a therapeutic niche in combination therapy with antiviral and anticancer nucleosides, and in the treatment of diseases involving the T-cell.

Index Entries: Acyclic nucleosides; acyclic nucleoside phosphonates; acyclic oligonucleotides; acyclovir; valaciclovir; ganciclovir; penciclovir; famciclovir; PMEA; *(S)*-HPMPC; HEPT; antiviral; purine nucleoside phosphorylase inhibitors.

1. Introduction

Clinical applications and potential therapeutic uses of acyclic nucleosides mainly have been directed toward the treatment of viral infections. Viral multiplication involves the host cell's biochemical machinery, and proceeds by a number of steps *(1,2)*. Infection commences with attachment of the virus to the host cell, and is followed by virus penetration through the host cell membrane. Once inside the cell, the virus sheds its coat. Viral replication can involve one or more viral enzymes, but also generally utilizes specific host enzymes. For both RNA and DNA viruses, key steps in the viral replicative process involve conversion of the viral genome into viral mRNA. For RNA viruses this can be catalyzed by RNA replicases or polymerases, or, for retroviruses, by reverse transcriptase, and for DNA viruses generally requires virus-specific DNA polymerases. The viral mRNA formed is translated by host enzymes into viral proteins that are used to coat the virus, before transport of the virus out of the host cell. Antiviral therapies have been targeted against each step of the life cycle *(1,2)*, but to date most clinical advance has been made by targeting viral nucleic acid synthesis and replication stages, as these are most distinct from host processes (though increasing understanding of the biochemical details of other processes, such as carbohydrate-lectin cell-surface recognition, will certainly lead to future applications). The focus here is to review antiviral acyclic nucleosides, which function mostly as inhibitors of viral reverse transcriptase or DNA polymerase *(3)*. This review will also discuss acyclic nucleosides that are inhibitors of nucleoside phosphorylase *(4)* (*see*

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Section 9.) and may find a therapeutic use in cancer chemotherapy and in the treatment of autoimmune diseases, and the possible use of certain acyclic nucleoside analogs in modified oligonucleotides as potential antisense or antigene agents, potentially targeted against viral RNA or DNA, respectively. Any recent and more specific reviews have been included in the references for particular sections. Research into acyclic nucleosides is an active and exciting area, however, it is beyond the scope of this review to consider all recent developments and only those showing the greatest therapeutic promise will be discussed.

2. Acyclovir

Acyclovir [Structure 1, aciclovir, Zovirax, 9-(2-hydroxyethoxymethyl)guanine] *(5–8)* marketed by The Wellcome Foundation Ltd. as topical, oral, and intravenous formulations, is a potent and selective inhibitor of herpes simplex virus (HSV-1 and -2). Since its discovery in 1974, and first product launch in 1981, it has achieved a secure market position and has earned an impressive reputation as a well tolerated and effective drug. In the UK, acyclovir became available as an over-the-counter medicine in 1993 for the treatment of *herpes labialis* (cold sores). Its minimal cytotoxicity can be attributed to its enhanced uptake into virus-infected cells, and to its selective monophosphorylation by the herpes-encoded thymidine kinase enzyme: In cells not infected by the virus, acyclovir remains unphosphorylated. In virus-infected cells, host enzymes convert the monophosphate to acyclovir triphosphate that is a substrate and potent inhibitor of viral (but not host) DNA polymerase. When acyclovir is incorporated into the growing viral DNA strand, further chain elongation is blocked, halting viral replication *(5)*.

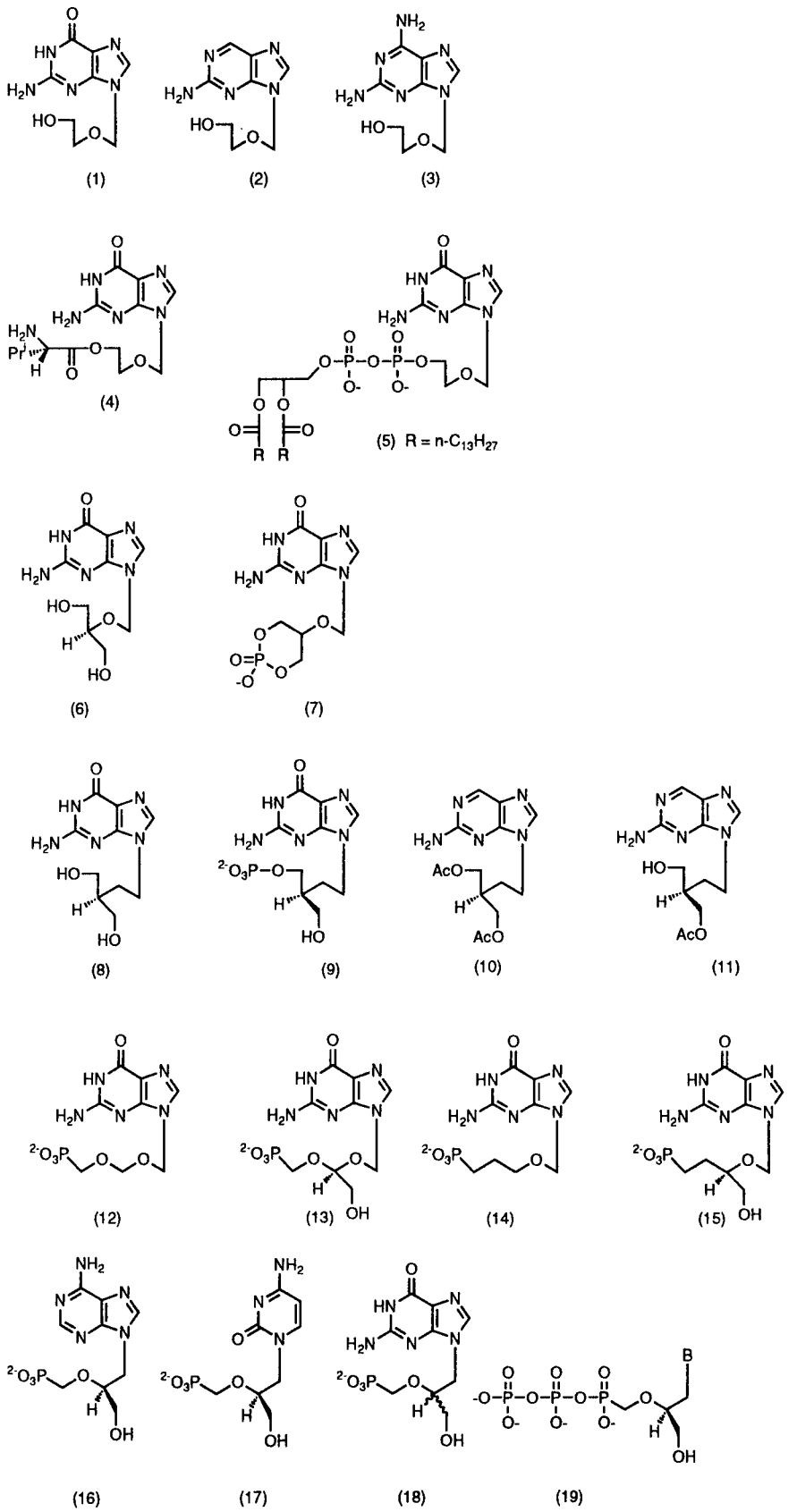
Acyclovir is also the first-line therapy for the treatment of varicella zoster virus (VZV), both in the primary infection (varicella or chicken pox) (although there is controversy over its benefits here), and in the recurrent indication (zoster or shingles) *(5,9–11)*. However, it is ineffective in treating cytomegalovirus (CMV), because although the triphosphate of acyclovir is an inhibitor of the viral DNA polymerase *(12)*, CMV does not possess a virally encoded thymidine kinase and only poor phosphorylation of acyclovir is achieved by the host enzyme.

Although resistance to acyclovir is not serious in immunocompetent patients, immunocompromised individuals, resulting from HIV infection or organ transplant, are very prone to disease recurrence, and acyclovir-resistant HSV and VZV strains have been identified. Resistance can arise from nucleotide mutations or deletions in either the thymidine kinase gene, resulting in the production of inactive protein *(13)*, or in the gene encoding for viral DNA polymerase, resulting in altered inhibition of this enzyme by acyclovir triphosphate *(5)*.

The resistance and recurrence issues, the limitation that only ~20% of orally administered acyclovir is absorbed by passive diffusion in the gastrointestinal tract *(5,14)*, coupled with the expiry of the acyclovir patent in 1995, ensures that there is continued interest in the search for novel anti-HSV compounds and in the design of prodrugs of acyclovir.

3. Prodrugs of Acyclovir

Early attempts to find an orally active prodrug of acyclovir focused on two derivatives with modifications in the guanine ring. The 6-deoxy analog, desciclovir (Structure 2), rapidly is absorbed principally by passive diffusion *(15)* and then metabolized by cellular xanthine oxidase to give a 70% oral bioavailability of acyclovir. The substantial increase in the oral absorption of desciclovir, when compared with acyclovir, despite similar lipophilicity and structures, has been proposed to arise through the lower desolvation energy of desciclovir, a property important to passive diffusion *(15)*. The 6-amino analog (Structure 3) is converted to acyclovir by cellular adenosine deaminase. However, both (Structure 2) and (Structure 3) showed greater toxicity than acyclovir in animal studies, which was attributed to some phosphorylation of (Structure 2) and (Structure 3) prior to their metabolism to acyclovir. Attention was then focused on the design of prodrugs that could only be phosphorylated after their conversion to acyclovir.



Structures 1–19.

The Wellcome Foundation Ltd. have recently launched valaciclovir (Structure 4; Valtrex), the oral L-valyl ester prodrug of acyclovir *(16–18)* for the treatment of shingles. From toxicology studies and clinical trials, valaciclovir shows the same safety profile as acyclovir. In agreement with the results in monkeys *(19)* and rats *(20)*, in humans the orally administered prodrug is absorbed rapidly and then completely metabolized to the parent drug and valine, giving rise to a 3–5-fold greater oral bioavailability of acyclovir than that achieved using the parent drug *(21)*. The greater absorption of valaciclovir, when compared with acyclovir, appears to be attributable to the uptake of valaciclovir by a dipeptide transporter in the intestinal brush border *(22)*. The metabolism of valaciclovir to acyclovir is catalyzed by the hepatic mitochondrial enzyme, valaciclovir hydrolase, which is related to rat liver methionine aminopeptidase *(22)*. This prodrug is especially useful where high concentrations of acyclovir are required to achieve a therapeutic effect, for example, in the treatment of the less sensitive VZV *(10,17)*.

Recently, Hostetler and coworkers *(23)* evaluated acyclovir diphosphate dimyristoylglycerol (Structure 5) as a phospholipid prodrug designed to have activity against acyclovir-resistant HSV. This analog was cleaved by a mitochondrial pyrophosphatase enzyme to give phosphatidic acid and acyclovir monophosphate that was active against thymidine kinase-deficient acyclovir-resistant strains of HSV.

4. Ganciclovir

Ganciclovir [6, 9'-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG], the 2-hydroxymethyl analog of acyclovir, is significantly more potent than acyclovir for the treatment of CMV and Epstein-Barr viral infections *(24,25)* prevalent in immunocompromised (AIDS or organ transplant) patients. For example, 20–30% of adult AIDS patients have CMV retinitis, which would result in loss of sight if left untreated. Marketed by Syntex as Cymevene, ganciclovir shares treatment and prophylactic prevention of this indication with foscarnet, and these drugs have been shown to act synergistically.

However, ganciclovir has only 6% oral bioavailability, and therefore the drug is administered by intravenous infusion. The adverse effects of ganciclovir treatment include neutropenia and thrombocytopenia, which are usually reversible on withdrawal of the drug. For the treatment of retinitis, controlled release intravitreal implants and weekly intraocular injections of encapsulated liposomal ganciclovir are promising formulations that avoid systemic side effects.

The mode of action of ganciclovir, which requires phosphorylation to the trisphosphate before inhibition of the viral DNA polymerase, was at first unclear, because CMV does not encode for a viral thymidine kinase. However, the rate-limiting monophosphorylation of ganciclovir is catalyzed by the UL97 CMV gene product *(26)*, with the monophosphate of ganciclovir being converted to the triphosphate by cellular kinases. Some resistance to ganciclovir has been reported: This has been attributed to point mutations in either the CMV UL97 or DNA polymerase genes. Although ganciclovir has been shown to have activity against some acyclovir-resistant strains, cross-resistance was also observed.

Interestingly, the cyclic phosphate of ganciclovir (Structure 7) is a broad spectrum antiviral compound *(27)*. In contrast to the mechanism of action to ganciclovir, the antiviral activity of (Structure 7) has been attributed to its structural similarity to the second messenger cGMP *(28)*.

5. Penciclovir and Famciclovir

The SmithKline Beecham group has replaced the ether oxygen in ganciclovir with a methylene substituent to give the carboanalog penciclovir (8, 9-[4-hydroxy-3-hydroxymethylbut-1-yl]guanine) *(29)*. Penciclovir shows an activity profile qualitatively similar to that observed for acyclovir, rather than ganciclovir, with potent activity against HSV and VZV *(18,30)*, but only low activity against CMV. Faster onset, improved potency, and longer duration of action were observed for penciclovir when compared with acyclovir, which was rationalized in terms of an increase in the half-life of penciclovir triphosphate *(30–33)*. Penciclovir also showed good activity against

the duck hepatitis B virus in vitro, with an IC_{50} value of 0.7 μ*M*, suggesting that it (or its oral form discussed in the following) may be useful in the treatment of hepatitis B *(34)*. When injected intravenously, penciclovir was well tolerated by healthy subjects and 70% of the drug was excreted unchanged in the urine *(35)*. Acyclovir-resistant HSV strains are also resistant to penciclovir *(36)*, supporting a similar mechanism of action. Using ^{13}C-labeled penciclovir it has been shown that the monophosphorylation catalyzed by HSV-1-encoded thymidine kinase shows high stereospecificity giving 75% of the *(S)*-enantiomer (Structure 9), which is stereochemically analogous to the natural nucleotide *(37)*. The enantiomeric excess is enriched on stereoselective further phosphorylation by cellular kinases, giving rise to 95% of the *(S)*-enantiomer of penciclovir triphosphate.

Like acyclovir and ganciclovir, penciclovir is poorly absorbed by oral administration *(22)*, which led to the design of the prodrug famciclovir (Structure 10; diacetyl 6-deoxypenciclovir) *(22)*. The principle route of metabolism of the prodrug is a fast stereospecific esterase catalyzed hydrolysis of the pro-*(S)*-acetoxymethyl group to give alcohol (Structure 11), with subsequent slower hydrolysis of the remaining acetyl group. Final oxidation at the 6-position is catalyzed in the liver by the cytosolic enzyme aldehyde oxidase *(22)* (and not by cytochrome P-450; *38*) to give penciclovir *(39)*. Famciclovir has good oral bioavailability, with 60% of the dose being excreted via the kidneys as penciclovir *(40)*. In clinical studies, famciclovir has been shown to be effective in the treatment of shingles (herpes zoster) *(10, 18,41)* with minimal adverse effects *(42)*. It has recently been launched as Famvir by SmithKline Beecham for the treatment of shingles.

6. Acyclic Nucleoside Phosphonates

The phosphonate group is an isosteric and isoelectronic replacement for the phosphate substituent. Therefore, acyclic nucleoside phosphonates—possessing the biologically stable carbon–phosphorus bond—are analogs of acyclic nucleotide monophosphates, and do not require initial phosphorylation by thymidine kinase. Several acyclic nucleoside phosphonates show excellent therapeutic potential as inhibitors of viral infections that lack a viral thymidine kinase, for example CMV *(43)*. They are also promising for the treatment of thymidine kinase deficient HSV-acyclovir resistant strains. For example, the phosphonate analogs (Structure 12) and (Structure 13) of acyclovir and ganciclovir monophosphates, respectively *(44,45)* are good inhibitors of both HSV-1 and CMV, and their carboanalogs (Structure 14) and (Structure 15) have also been prepared *(46)*.

The acyclic nucleoside phosphonate, *(S)*-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [*(S)*-HPMPA, Structure 16] shows potent, long-acting, and selective activity against a broad range of DNA viruses *(47)*. In vivo studies on mice infected with HSV-1, HSV-2, or thymidine kinase deficient HSV-1 supported the efficacy of this compound *(48)*. Activity was also observed against intracerebral HSV, suggesting that these compounds cross the blood-brain barrier and therefore should be useful for the treatment of HSV encephalitis *(48)*. Both the cytosine [*(S)*-HPMPC (Structure 17)] *(49)* and the guanosine [*(R,S)*-HPMPG (Structure 18)] *(50)* analogs shows similar in vitro and in vivo activity to that of *(S)*-HPMPA. The cytosine analog *(S)*-HPMPC was the least toxic and is a promising drug candidate for the treatment of the DNA viruses HSV, VZV, CMV, and Epstein-Barr (EBV, IC_{50} 0.03 μ*M*) *(51)*, as well as for acyclovir- and foscarnet-resistant thymidine-kinase deficient strains of HSV, ganciclovir-resistant CMV *(52,53)*, and acyclovir-resistant VZV in immunocompromised patients *(54)*.

(S)-HPMPA and *(S)*-HPMPC are believed to share the same mechanism of action. The phosphonate is taken up into cells, most probably via an endocytosis mechanism, and is then phosphorylated by cellular enzymes to the bisphosphate (Structure 19). As this process is independent of viral kinases, this accounts for their potency against thymidine kinase-deficient viral strains. The bisphosphorylation of *(S)*-HPMPA and analogs by ATP may occur in two steps, each phosphorylation being catalyzed by adenylate kinase *(55)*. Adenylate kinase only phosphorylates the *(S)*-

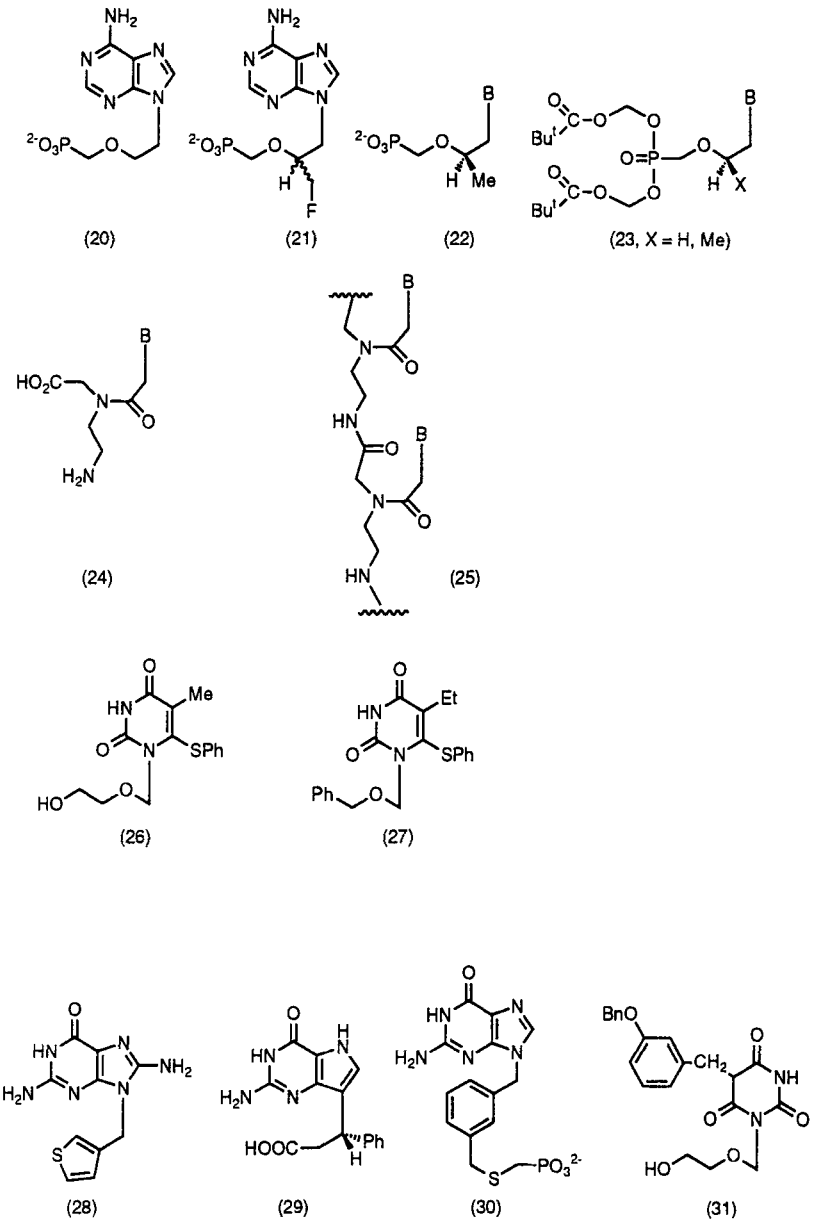
enantiomers of HPMPA and analogs, which may be one reason why the *(R)*-enantiomers show significantly lower antiviral activity *(55–57)*. Alternatively, the bisphosphates (Structure 19) can be formed by the direct transfer of the pyrophosphate group from 5-phosphoribosyl 1-pyrophosphate (PRPP) to the phosphonates. This reaction is catalyzed by PRPP synthetase *(58)*, however, it has been suggested that this transformation is only important for *E. coli (55)*. The bisphosphates (Structure 19) interact selectively with viral DNA polymerase to inhibit replication by three possible mechanisms *(52,59)*. First, bisphosphate (Structure 19) could be a competitive inhibitor, thus preventing the inclusion of the natural substrate into DNA. Second, bisphosphate (Structure 19) could be attached at the 3'-end of DNA leading to chain termination. Third, bisphosphate (Structure 19) could be incorporated into DNA utilizing also its 3-hydroxy group. Although further research is required, one study *(60)* supports the third option with HPMPA. The bisphosphate of *(S)*-HPMPA has also been shown to inhibit HSV-1 encoded ribonucleotide reductase and this may also be linked to its antiviral activity *(61)*.

The N-(2-phosphonylmethoxyethyl) (PME) purine and pyrimidine derivatives *(49)* are less active than the HPMP analogs against HSV, CMV, and VZV. However, they are more active against hepatitis-B and thymidine kinase-deficient, acyclovir resistant HSV strains *(62)*. Moreover, the PME derivatives are potent and selective inhibitors of RNA retroviruses, for example, HIV *(63,64)*. The adenine analog (PMEA, Structure 20) *(65)* shows low toxicity while being a potent in vitro inhibitor of retroviruses with IC_{50} values of 0.006–2 μM. PMEA shows some cross resistance with highly AZT-resistant (50–200-fold) strains of HIV, however, with less resistant (8–25-fold) strains, PMEA was active *(66)*. For HIV-infected T-cells, synergistic antiviral activity was observed between PMEA and AZT, therefore PMEA may be important in combination therapy. Whereas long-term AZT therapy leads to immunosuppression, PMEA has immunostimulatory effects that may contribute to its antiviral response and increase host resistance against viral infections *(67)*. Activity of PMEA was demonstrated in vivo in mice in the AIDS model Molony murine sarcoma retrovirus (MSV) and HSV *(64,68)*. Against MSV, PMEA was 25-fold more active than AZT with a better therapeutic index. Activity was also demonstrated in mice with intracerebral MSV or HSV infections suggesting that PMEA shows some ability to cross the blood-brain barrier.

The cellular uptake of PMEA is controversial: Naesens has reported that PMEA is transported by an endocytosis process *(65)*, whereas the transport of [^{14}C]-PMEA across the plasma membrane of HeLa S3 cells has been shown to be protein-mediated, and a 50-kDa protein that may mediate transport has been identified *(69)*. The bisphosphorylation of PMEA to the active bisphosphate may be catalyzed either by AMP kinase or by PRPP synthetase *(65)*. The bisphosphate of PMEA, which has a long intracellular half-life (~17 h), is a potent competitive inhibitor of both HSV-1 DNA polymerase (K_i 0.1 μM with respect to the natural substrate dATP) *(60)* and HIV reverse transcriptase (K_i 0.18 μM), which is thought to be responsible for its antiviral activity. Because of the absence of a 3'-hydroxy group in PMEA, the bisphosphate cannot be incorporated fully into DNA and recent studies have shown that the bisphosphate of PMEA gives rise to DNA chain termination *(70)*. PMEA shows great promise as a drug for the treatment of retroviral infections and it is being evaluated in phase I/II clinical trials in patients with AIDS.

In contrast to the broad spectrum of antiviral activity reported for *(S)*-HPMPA, the nontoxic fluoroanalog, 9-[*(2RS)*-3-fluoro-2-phosphonylmethoxypropyl]adenine [Structure 21, *(RS)*-FPMPA] shows selective activity toward HIV-1 and -2, with much lower activity against other viruses *(71,72)*. The fluoroanalog (Structure 21) shows a similar mechanism of antiretroviral activity to PMEA, with the bisphosphate of FPMPA acting as a DNA chain terminator competing with dATP for HIV-1 reverse transcriptase.

The *(R)*-enantiomers of 9-[2-phosphonylmethoxypropyl]adenine (PMPA) and 2,6-diaminopurine (PMPDAP) (Structure 22) were particularly active in vitro against visna virus *(56)*, human and duck



Structures 20–31.

hepatitis-B virus *(62,73),* HIV and murine MSV, with activity demonstrated in MSV-infected mice following oral administration *(57).*

The acyclic nucleoside phosphonates are dianionic that typically show poor oral bioavailability and poor cellular uptake. The lipophilicity of the acyclic nucleoside phosphonates, PMEA, PMPA, and PMPDAP, recently have been enhanced with the design of the bis(pivaloyloxymethyl) prodrugs (Structure 23) *(74–76),* which cleave to the parent drug in the presence of esterases. In each case, intracellular uptake was improved and the prodrug showed enhanced antiviral activity when compared with the drug. However, the prodrugs were more cytotoxic giving rise either to a comparable (PMPA) or a slightly decreased (PMEA, PMPDAP) therapeutic index. These prodrugs increased the bioavailability of PMEA to 30% in monkeys, from 6% observed with orally administered PMEA.

7. Acyclic Oligonucleotides

Inhibition of gene expression using the antisense or antigene approach requires that the oligonucleotide shows stability towards nucleases, penetrates through the cell membrane and binds with high specificity to the target gene *(77)*. Oligonucleotides incorporating one or more acyclic nucleotides bearing the *(S)*-3,4-dihydroxybutyl, *(S)*-3,5-dihydroxypentyl and *(3S,4R)*-4-methoxy-3,5-dihydroxypentyl side chains have been evaluated for their base pairing properties and their stability toward nucleases *(78,79)*. Substitution in the middle of an oligonucleotide decreases duplex stability, whereas incorporation of an acyclic nucleoside at the 3'- and 5'-ends of DNA makes the oligonucleotide resistant to cleavage by nucleases, while retaining good base pairing properties. The end-modified oligonucleotides therefore have potential in antisense or antigene therapy.

A promising further development *(80–83)* involves the use of amino acyclic nucleoside analogs (Structure 24). Construction of oligomers of these affords peptide nucleic acids (Structure 25, PNA). These show strong duplex association with ssDNA, forming PNA-DNA duplexes that are stronger than the native dsDNA. They can also form PNA-DNA-PNA triplexes with ssDNA, and will displace a DNA oligonucleotide from dsDNA. They also show interesting RNA selectivity properties. These agents are nuclease-resistant, are uncharged, and hold considerable promise as antisense agents.

8. HEPT and Analogs

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio) thymine (Structure 26; HEPT) is a highly specific, nontoxic, anti-HIV-1 agent with no activity against HIV-2 *(84,85)*. Although it interacts with reverse transcriptase, unlike other antiviral nucleoside analogs, HEPT, or its triphosphate does not show inhibition of HIV-1 reverse transcriptase by acting as a competitive inhibitor or chain terminator *(85)*. In addition, the phosphorylation of HEPT is not catalyzed by thymidine kinase, and the benzyl analog, 1-benzyloxymethyl-5-ethyl-6-phenylthiouracil (Structure 27) (EC_{50} 0.006 μ*M*), which lacks the 2-hydroxy group, is a more potent inhibitor of HIV-1 than HEPT (EC_{50} 6.5 μ*M*) *(86,87)*. Unlike other nucleosides, HEPT and analogs do not need to be phosphorylated to show activity. Consistent with other non-nucleoside drugs (e.g., nevirapine and TIBO), HEPT, and the benzyl analog (Structure 27) are noncompetitive inhibitors that are thought to bind in a hydrophobic pocket beneath the polymerase active site by interaction with tyrosines 181 and 188 *(88,89)*. The lack of inhibition of HIV-2 by these compounds may be attributed to the absence of a hydrophobic pocket on this viral reverse transcriptase *(87)*. Of importance, both (Structure 26) and (Structure 27) are active against AZT-resistant strains of HIV-1.

9. Nucleoside Phosphorylase Inhibitors

As well as being useful antiviral agents, acyclic nucleosides are also among the most potent inhibitors of the salvage enzymes, purine, and pyrimidine nucleoside phosphorylase *(4)*. These enzymes catalyze a reaction in which inorganic phosphate displaces the nucleoside base, with inversion of stereochemistry at C-1', to give the base and the sugar 1-phosphate. The phosphorylase enzymes deactivate a number of nucleosides that are used in chemotherapy, for example, the anticancer drug, 5-fluoro-2'-deoxyuridine, and the anti-AIDS nucleoside, 2',3'-dideoxyinosine. It is probable that these drugs will show improved efficacy if administered in combination with inhibitors of nucleoside phosphorylase.

T-cells are important in various autoimmune diseases, for example, rheumatoid arthritis and psoriasis, as well as organ transplant rejection and T-cell leukemia. Low levels of purine nucleoside phosphorylase (PNP) results in severe T-cell immunodeficiency and T-cell death, therefore PNP inhibitors may be useful in these disease states *(4)*. PNP inhibitors may also be important for the treatment of gout as they should block the formation of uric acid.

The first potent inhibitor of PNP was the enzyme bisubstrate analog, acyclovir diphosphate (K_i of 8.7 n*M*) *(90)*. However, acyclovir is only phosphorylated in virally infected cells, and the diphosphate cannot penetrate cell membranes and is chemically and enzymatically unstable. Exten-

sive studies with 60 purine acyclic nucleoside analogs only gave inhibitors with K_i values of >2 μ*M (91)*. Rational design, using protein X-ray crystallography and molecular modelling, gave the potent PNP inhibitors (Structure 28) *(92)* and (Structure 29) *(4)*, with EC_{50}s of ~6 n*M*. Further studies by the Wellcome group showed that the phosphonate bisubstrate analog (Structure 30) was a potent inhibitor of PNP (K_i 1.1 n*M*) *(93)*. Similarly, 5-(*m*-benzyloxy)benzyl-1-[(2-hydroxyethoxy)methyl]barbituric acid (Structure 31) was a potent inhibitor (IC_{50} 1.1 n*M*) of uridine nucleoside phosphorylase *(94)*. The progress of PNP inhibitors in clinical studies is awaited with interest.

10. Conclusion

Acyclovir, ganciclovir, famciclovir, and valaciclovir are useful antiviral drugs. Several other acyclic nucleosides, especially, PMEA and *(S)*-HPMPC show promise for the therapy of HSV, VZV, CMV, and AIDS, including thymidine kinase-deficient strains. As well as an anticipated continued contribution to antiviral therapy, acyclic nucleosides may be important in cancer chemotherapy and diseases of the T-cell, and in the design and therapeutic applications of modified antisense and antigene agents.

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