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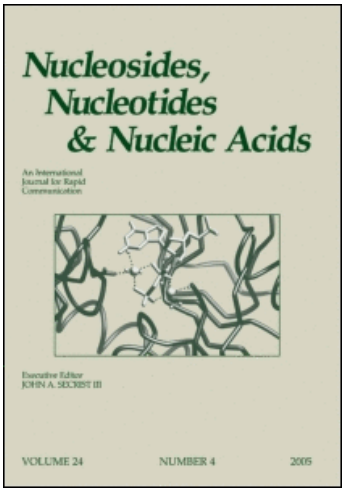

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ACYCLIC/CARBOCYCLIC GUANOSINE ANALOGUES AS ANTI-HERPESVIRUS AGENTS

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ACYCLIC/CARBOCYCLIC GUANOSINE ANALOGUES AS ANTI-HERPESVIRUS AGENTS

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ABSTRACT

Several guanosine analogues, i.e. acyclovir (and its oral prodrug valaciclovir), penciclovir (in its oral prodrug form, famciclovir) and ganciclovir, are widely used for the treatment of herpesvirus [i.e. herpes simplex virus type 1 (HSV-1), and type 2 (HSV-2), varicella-zoster virus (VZV) and/or human cytomegalovirus (HCMV)] infections. In recent years, several new guanosine analogues have been developed, including the 3-membered cyclopropylmethyl and -methenyl derivatives (A-5021 and synguanol) and the 6-membered D- and L-cyclohexenyl derivatives. The activity of the acyclic/carbocyclic guanosine analogues has been determined against a wide spectrum of viruses, including the HSV-1, HSV-2, VZV, HCMV, and also human herpesviruses type 6 (HHV-6), type 7 (HHV-7) and type 8 (HHV-8), and hepatitis B virus (HBV). The new guanosine analogues (i.e. A-5021 and D- and L-cyclohexenyl G) were found to be particularly active against those viruses (HSV-1, HSV-2, VZV) that encode for a specific thymidine kinase (TK), suggesting that their antiviral activity (at least partially) depends on phosphorylation by the virus-induced TK. Marked antiviral activity was also noted with A-5021 against HHV-6 and with D- and L-cyclohexenyl G against HCMV and HBV. The antiviral activity of the acyclic/carbocyclic nucleoside analogues could be markedly potentiated by mycophenolic acid, a potent inhibitor of inosine 5′-monophosphate (IMP) dehydrogenase. The new carbocyclic guanosine analogues (i.e. A-5021 and D- and L-cyclohexenyl G) hold great promise, not only as antiviral agents for the treatment of herpesvirus infections, but also an antitumor agents for the

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combined gene therapy/chemotherapy of cancer, provided that (part of) the tumor cells have been transduced by the viral (HSV-1, VZV) TK gene.

INTRODUCTION: ACYCLOVIR, PENCICLOVIR AND GANCICLOVIR

Foremost among the most frequently used antiherpetic drugs are the guanosine analogues acyclovir (and its oral prodrug form, valaciclovir), penciclovir (under its oral prodrug form, famciclovir) and ganciclovir (which can be administered either intravenously or orally). These guanosine analogues have been pursued primarily for the treatment of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV) and human cytomegalovirus (HCMV) infections. Acyclovir has been the gold standard for the treatment of mucosal, cutaneous and systemic HSV-1 and HSV-2 infections (including herpes encephalitis and genital herpes) and VZV infections (varicella and herpes zoster), and can also be used in the prophylaxis of genital and orofacial HSV infections, and VZV and CMV infections (1). The L-valyl ester, valaciclovir, the oral prodrug of acyclovir, has been found to achieve substantially higher plasma levels of acyclovir than oral acyclovir itself; oral valaciclovir has proven to be particularly useful in the treatment of herpes zoster (2) and in the prevention of HCMV disease after renal transplantation (3). The indications for the use of famciclovir, the oral prodrug form of penciclovir (4), are identical to those of valaciclovir, *viz*. the treatment of HSV and VZV infections. The total systemic availability of penciclovir, following oral famciclovir, can be considered similar to that of acyclovir following oral valaciclovir. Upon intravenous administration, penciclovir and acyclovir afforded equivalent efficacy in the treatment of HSV infections in immunocompromised patients (5). Although ganciclovir is at least as effective, if not more so, than penciclovir and acyclovir against HSV-1 and HSV-2, it has been developed mainly for the treatment of HCMV infections (i.e., HCMV retinitis in AIDS patient) (6), where it can be administered either intravenously or orally (as mentioned above), or locally, as an intravitreal implant. Acyclovir was the first guanosine analogue described as an antiviral agent: it has served as the prototype for the development of a whole series of new acyclic or carbocyclic guanosine analogues, including penciclovir and ganciclovir, as well as a number of both three- and six-membered sugar derivatives (Fig. 1). The comparative antiviral potency, based on the 50% effective concentration (EC_{50}), of these compounds against the herpesviruses HSV-1, HSV-2, VZV and HCMV is presented in Table 1.

LOBUCAVIR

Lobucavir {1R(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine}, which has also been referred to as (R)-BHCG, SQ-34,514, BMS-180194 and cygalovir, corresponds to the active enantiomer of (±)BHCG (SQ-33,054, cyclobut-G)

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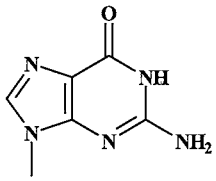

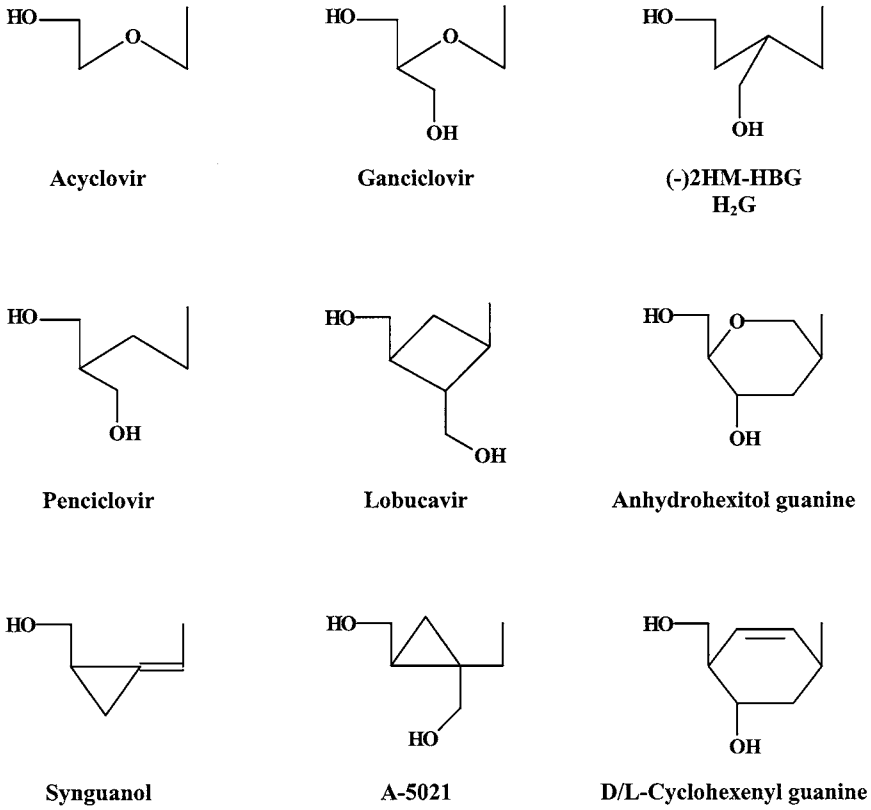

Figure 1. Structural formulae of carbocyclic and acyclic guanosine analogues.

(8,9), which was originally shown to possess broad-spectrum antiviral activity against several herpesviruses (i.e. HSV-1, HSV-2, VZV, HCMV,...) as well as HIV (21). The phosphorylation of lobucavir is initiated by the HSV- or VZV-encoded thymdine kinase (TK) (8), and the eventual action of lobucavir against HSV-1, HSV-2 and VZV is based upon inhibition of the viral DNA polymerase by the triphosphate of lobucavir (22). The triphosphate of lobucavir is also a potent inhibitor of the HCMV DNA polymerase (Ki: 5 nM) (23), although lobucavir inhibits HCMV replication only at a 100-fold higher concentration (Table 1) than that required to inhibit HSV-1, HSV-2 or VZV. The reason is that lobucavir is only

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Table 1. Comparative Potency of Different Antiherpetic Compounds Against Different Herpesviruses

	50% Effective Concentration EC_{50} (μM)				
Compound	HSV-1	HSV-2	VZV	HCMV	References
Acyclovir	+++	+++	++	+	1,7
Penciclovir	+++	++	++	(+)	4
Ganciclovir	+++++	+++++	++	++	6,7
Lobucavir	++++	++++	+++	++	8,9
H2G	++++	++++	++++	(+)	§
A-5021	++++	+++	+++	+	10, this report
Synguanol	+	−	+	++	11,12
Anhydrohexitol G	++(+)	+++(+)	++	++	13,14
D-Cyclohexenyl G	++++	+++	++	++	15
L-Cyclohexenyl G	+++(+)	+++	++	++	15
References	16,17	16,18	19	20	

§E. De Clercq, unpublished data.
*−, if $EC_{50} > 100\,\mu$M.
+, if $EC_{50} = 10$–$100\,\mu$M.
++, if $EC_{50} = 1$–$10\,\mu$M.
+++, if $EC_{50} = 0.1$–$1\,\mu$M.
++++, if $EC_{50} = 0.01$–$0.1\,\mu$M.
+++++, if $EC_{50} = 0.001$–$0.01\,\mu$M.
( ) if only borderline, or if not accurately determined.

weakly phosphorylated in HCMV-infected cells, this phosphorylation being independent of the UL97 protein kinase (the HCMV-encoded phosphotransferase that is responsible for the phosphorylation of ganciclovir) (23).

H2G

H2G corresponds to (-)2HM-HBG or the (-)enantiomer of 9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine. Like acyclovir, penciclovir and lobucavir, H2G is primarily active against HSV-1, HSV-2 and VZV (24). H2G has been found to suppress simian varicella virus infection in African green monkeys at a dose as low as 1 mg/kg/day (25) and has been pursued for the treatment of VZV infections in humans.

A-5021

A new class of compounds, based on the cycloprophylmethyl entity as the sugar substitute, namely A-5021 or (1′*S*,2′*R*)-9-{[1′,2′-bis(hydroxymethyl)cyclopropr-1′-yl]methyl}guanine, has been recently identified as potent and selective

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inhibitors of HSV-1, HSV-2 and VZV (10). A-5021 proved even more active against HSV-1, HSV-2 and VZV than either acyclovir or penciclovir (Table 1), and, as for acyclovir and penciclovir, little activity was noted against HCMV. Again, as for acyclovir and penciclovir, the anti-HSV and anti-VZV activity of A-5021 was found to depend on the phosphorylation by the HSV- and VZV-encoded TK. The intracellular half-life of A-5021 triphosphate was longer than that of acyclovir triphosphate but shorter than that of penciclovir triphosphate. Inhibition of the viral DNA polymerase was strongest with acyclovir triphosphate, followed by A-5021 triphosphate and then penciclovir triphosphate (26). Thus, in comparison with the triphosphates of acyclovir and penciclovir, A-5021 triphosphate accumulates for a longer time in the infected cells than acyclovir triphosphate and effects a stronger inhibition of the viral DNA polymerase than penciclovir triphosphate. In several murine models of HSV infection, A-5021 has proven more effective than acyclovir and penciclovir, i.e. with regard to reduction in the severity of herpetic skin lesions and protection against herpetic encephalitis (27).

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SYNGUANOL

Synguanol corresponds to (*Z*)-2-(hydroxymethyl)cyclopropylidene)methylguanine. Synguanol is particularly active against HCMV (EC_{50}: 0.04–2.0 μM, depending on the assay system) (11,12); it has also marked activity against Epstein-Barr virus (EBV) but only limited potency against HSV-1, HSV-2 and VZV (11). The antiviral potency of synguanol is enantioselective. This enantioselectivity likely reflects differences in the rates of intracellular phosphorylation and/or affinities of the corresponding triphosphates for the target viral DNA polymerases (28). In addition to synguanol, various other (*Z*)-2-(hydroxymethyl)cyclopropylidene) methylpurines and–pyrimidines have been described as antiviral agents (i.e. synadenol, syncytol, synthymol, so named in analogy with synguanol) (29). Synguanol, as well as other methylenecyclopropane analogues of nucleosides, have proven effective in the treatment of murine cytomegalovirus (MCMV) infections in mice (30).

ANHYDROHEXITOL G

Various 1,5-anhydrohexitol nucleoside analogues have been found to exhibit marked activity against HSV-1, HSV-2, VZV and HCMV, the most active congeners being those with guanine (13), 5-iodouracil (13), 5-ethyluracil (14) or 5-trifluoromethyluracil (31) as the base moiety. Their antiviral activity must depend, at least partially, on a specific phosphorylation by the virus-encoded TK, since these compounds are less active against TK-deficient mutants of HSV-1. As HCMV does not encode for a virus-specific thymidine kinase, the activity noted with 1,5-anhydrohexitol guanine against HCMV would suggest that the compound must be phosphorylated by the UL97 protein kinase, or another phosphotransferase, in the HCMV-infected cells.

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D- AND L-CYCLOHEXENYL G

The cyclohexene nucleoside analogues can be considered as analogues of the natural furanose nucleosides where the ring oxygen has been replaced by a double bond. D-cyclohexenyl G and L-cyclohexenyl G were found to possess similar potency (15) against HSV-1, HSV-2, VZV and CMV (Table 1). This is not the case for the D- and L-anhydrohexitol nucleoside analogues where only the D-analogues demonstrated antiviral activity (32). As the activity of D- and L-cyclohexenyl G was lower against TK-deficient (TK^-) HSV-1 strains that against TK^+ strains, the virus-induced phosphorylation may be an important determinant in their anti-HSV activity. In fact, both enantiomers of cyclohexenyl G could be readily accommodated in the active site of the viral TK (33). D- and L-cyclohexenyl guanine represent the first example of a broad-spectrum anti-herpesvirus compound exhibiting similar activity in their L- and D-form for which an explanation can be offered at the molecular (i.e. viral TK) level (33).

ANTIVIRAL ACTIVITY SPECTRUM OF ACYCLIC/CARBOCYCLIC GUANOSINE ANALOGUES

In comparison with acyclovir, the "gold standard" for the chemotherapy of HSV and VZV infections, the D- and L-cyclohexenyl guanines (15) were slightly more potent against HSV-1 and slightly less potent against HSV-2. Also A-5021 has proven to be more potent than acyclovir against both HSV-1 and HSV-2 (10). In our investigations we found D- and L-cyclohexenyl G to be equipotent with acyclovir, and A-5021 to be 20-fold more potent than acyclovir, in inhibiting VZV replication (Table 2). The virus-encoded thymidine kinase clearly contributed to the activity

Table 2. Activity of Guanosine Analogues Against Various Strains of Varicella-Zoster Virus (VZV) in Human Embryonic Lung (HEL) Cells

	EC_{50} (μg/ml)[a]			
	TK^+ VZV		TK^- VZV	
Compound	YS	OKA	07/1	YS/R
Acyclovir	1.1	0.8	13	28
Penciclovir	0.6	0.6	8	29
A-5021	0.04	0.05	3	16
D-Cyclohexenyl G	0.5	0.6	2.1	2.8
L-Cyclohexenyl G	1.2	1.9	5.8	6.8

[a]50% Effective concentration, or concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). The compounds did not show a microscopically detectable alteration of normal cell morphology or inhibition of host cell growth at the highest concentration tested (50 μg/ml), except for D- and L-cyclohexenyl G that inhibited HEL cell growth by 50% at a concentration of 11 and 38 μg/ml, respectively.

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Table 3. Activity of Guanosine Analogues Against Various Strains of Human Cytomegalovirus (HCMV) in Human Embryonic Lung (HEL) Cells

	EC_{50} (μg/ml)[a]			
Compound	AD-169	Davis	Ly 9990	U 9070
Acyclovir	16	16	...	...
Penciclovir	>50	>50	...	...
Ganciclovir	0.77	0.62	7.5	10.8
A-5021	8	12	...	...
D-Cyclohexenyl G	0.47	0.83	0.38	0.13
L-Cyclohexenyl G	1.05	3.97	0.79	0.4

[a]50% Effective concentration, or concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). The compounds did not show a microscopically detectable alteration of normal cell morphology or inhibition of host cell growth at the highest concentration tested (50 μg/ml), except for D- and L-cyclohexenyl G that inhibited HEL cell growth by 50% at a concentration of 11 and 38 μg/mL, respectively.

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of the compounds against VZV, since they were less active against TK^- than TK^+ VZV strains (Table 2).

Like acyclovir, A-5021 did not shown much activity against HCMV (Table 3). However, both D- and L-cyclohexenyl G proved active against HCMV at roughly the same concentration as ganciclovir, and, in addition, D- and L-cyclohexenyl retained marked activity against HCMV strains that were resistant to ganciclovir (Table 3).

Marked inhibitory activity was noted with A-5021 against both human herpesvirus type 6 strains HHV-6A and HHV-6B (Table 4): it was about 10-fold more

Table 4. Activity of Guanosine Analogues Against Human Herpesvirus (HHV-6) in Human Lymphoblast Cell Lines

	EC_{50} (μg/ml)[a]			
	HHV-6A (GS) in HSB-2 Cells		HHV-6B (Z-29) in MOLT-3 Cells	
Compound	CPE	DNA Synthesis	CPE	DNA Synthesis
Acyclovir	27	41	36	41
Penciclovir	71	85	>100	>100
Ganciclovir	10	8	16	15
Labucavir	2.17	1.96	2.36	4.85
A-5021	2.64	3.45	3.39	3.48

[a]50% Effective concentration, or concentration required to reduce virus-induced CPE (cytopathic effect, determined microscopically) or viral DNA synthesis (determined by hybridisation with a digoxigenin-labelled probe) by 50%. The minimum cytotoxic concentrations, causing a microscopically detectable alteration of normal cell morphology, were 100 μg/ml (acyclovir), $\geq$100 μg/ml (penciclovir), 20 μg/ml (ganciclovir), 10 μg/ml (lobucavir) and $\geq$50 μg/ml (A-5021).

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potent than acyclovir. A-5021 was about equipotent as lobucavir, but, as it was less toxic to the host cells (Table 4, footnote), it achieved a greater selectivity index against HHV-6 than lobucavir.

A-5021 did not inhibit the replication of human herpesvirus type 7 (HHV-7) in human T-lymphocyte (sup T1) cells at the highest (subtoxic) concentration tested (60 μg/ml). Nor did D/L-cyclohexenyl G (20 μg/ml), acyclovir (250 μg/ml), penciclovir (170 μg/ml), ganciclovir (50 μg/ml) or H2G (100 μg/ml). Lobucavir proved active against HHV-7 at an EC_{50} of 5 μg/ml, that is a concentration that was 3-fold lower than its CC_{50} (50% cytotoxic concentration).

A-5021 was also evaluated for its activity against Epstein-Barr virus (EBV) and human herpesvirus type 8 (HHV-8), in P3HR-1 and BCBL-1 cells, respectively; anti-EBV and anti-HHV-8 activity was assessed by monitoring viral DNA synthesis with digoxigenin-labelled probes specific for EBV and HHV-8. A-5021 inhibited EBV DNA synthesis at an EC_{50} of 1.0 μg/ml (as compared to 1.4 μg/ml for acyclovir). Neither A-5021 nor acyclovir inhibited HHV-8 at a concentration of 20 μg/ml.

The guanosine analogues acyclovir, penciclovir, ganciclovir, lobucavir, synguanol and A-5021 were also evaluated against a member of the Flaviviridae, namely Yellow fever virus [as a surrogate substitute for hepatitis C virus (HCV)], but as expected, no activity was found with any of the compounds at concentrations up to 200 μg/ml.

When the guanosine analogues were evaluated against hepatitis B virus (HBV), marked activity was noted for lobucavir and D/L-cyclohexenyl G (Table 5). The latter was about 10-fold more potent against HBV than penciclovir, which, in its prodrug form (famciclovir), has been the subject of clinical studies in HBV-infected patients. A-5021 and the other guanosine analogues that were tested did not show appreciable activity against HBV. Those that did, inhibited the replication of wild-type HBV (Hep AD38 cells) and lamivudine-resistant HBV, containing the methionine $\rightarrow$ valine substitution (M550V) in the DNA polymerase (34), at similar EC_{50} values (Table 5).

ENHANCEMENT OF THE ANTIVIRAL ACTIVITY OF ACYCLIC/CARBOCYCLIC GUANOSINE ANALOGUES BY MYCOPHENOLATE MOFETIL

Mycophenolate mofetil (which is currently used as an immunosuppressant in kidney transplant recipients) is the morpholinoethyl ester of mycophenolic acid (Fig. 2). The latter is known to be a potent inhibitor of IMP dehydrogenase (35). Like other IMP dehydrogenase inhibitors (Fig. 2), such as ribavirin, tiazofurin, EICAR (36) and VX-497 (37), mycophenolic acid is expected to reduce the intracellular pool levels of GTP and dGTP through its inhibitory effect on the conversion of IMP to XMP (Fig. 3). Through depletion of the intracellular dGTP pools, mycophenolic acid may then facilitate the effectiveness of the triphosphates of the acyclic guanosine analogues (acyclovir, penciclovir, ganciclovir, etc.) in their competitive

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Table 5. Activity of Guanosine Analogues Against Hepatitis B Virus (HBV) in Hep AD38 and Hep AD79 Cells

	EC_{50} (μg/ml)[a]	
Compound	Hep AD38	Hep AD79
Acyclovir	>50	>50
Penciclovir	46	45
Ganciclovir	>50	>50
Lobucavir	0.5	1.1
Synguanol	>100	>100
A-5021	>20	>20
D/L-Cyclohexenyl G	6	3
Adefovir (PMEA)	0.05	0.14
Tenofovir (PMPA)	0.05	0.05
Lamivudine (3TC)	0.0037	0.82

[a]50% Effective concentration, or concentration required to reduce viral DNA synthesis (as monitored by hybridisation with a digoxigenin-labelled probe) by 50%. Hep AD38 and Hep AD79 cells represent hepatoma cells transfected with a cDNA copy of the pregenomic RNA of wild-type virus or mutant virus (containing the M550V mutation in the DNA polymerase, which leads to resistance to 3TC), respectively. Data taken from reference 34; and unpublished data (C. Ying, J. Neyts and E. De Clercq).

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inhibitory effect (with respect to dGTP) at the viral DNA polymerase level. Mycophenolic acid was found to markedly (up to 100-fold) potentiate the inhibitory effects of acyclovir, penciclovir and ganciclovir on HSV-1, HSV-2, VZV and CMV *in vitro* (Table 6) and *in vivo* (38); and a similar marked enhancement was noted for the activity of H2G against HSV-1, HSV-2 and both TK^+ and TK^- VZV (39). In hairless mice infected intracutaneously with HSV-1, combined use of systemic acyclovir (20 mg/kg/day) and topical mycophenolate mofetil (5%) resulted in an almost complete protection, whereas single use of either compound had virtually no protective effect (40). Mycophenolic acid has also been found to markedly potentiate the activity of lobucavir against HSV-1, HSV-2, TK^- HSV-1 and HCMV: for TK^-HSV-1 the 50% effective concentration (EC_{50}) of lobucavir was decreased up to 1400-fold upon combination with mycophenolic acid (41). Similarly, mycophenolic acid brought about a significant enhancement (up to 200-fold) of the activity of A-5021 against HSV-1, HSV-2 and TK^- HSV-1 [J. Neyts and E. De Clercq (unpublished data, 1999)]. These observations may have important implications in transplant recipients, that under therapy with mycophenolate mofetil, develop opportunistic herpesvirus infections that are amenable to treatment with any of the acyclic guanosine analogues mentioned above (acyclovir, penciclovir, ganciclovir, etc.). It would now seem imperative to examine whether mycophenolate mofetil also potentiates the antiviral effects of the other guanosine analogues, i.e., synguanol, anhydrohexitol guanine and L- and D-cyclohexenyl guanine.

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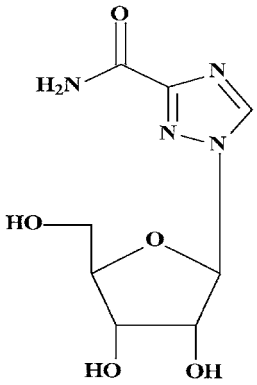

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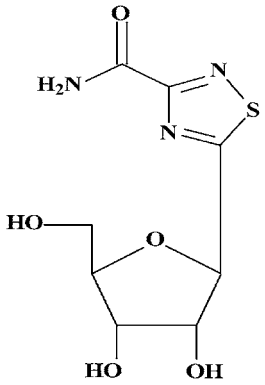

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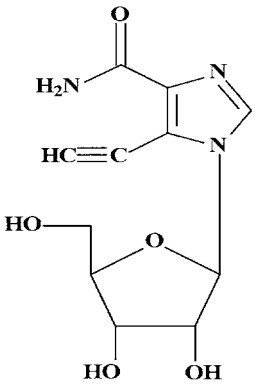

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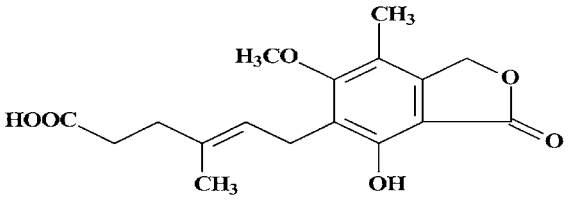
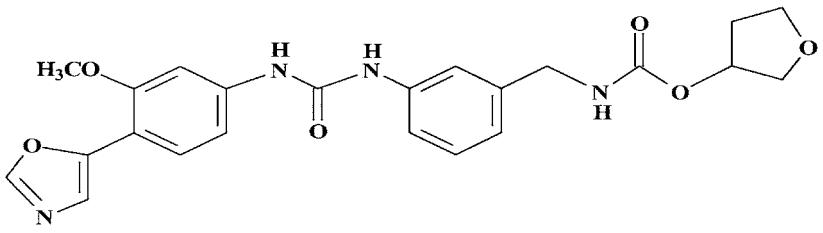


Mycophenolic acid



VX-497

Figure 2. Mycophenolic acid and other IMP dehydrogenase inhibitors (ribavirin, tiazofurin, EICAR and VX-497).

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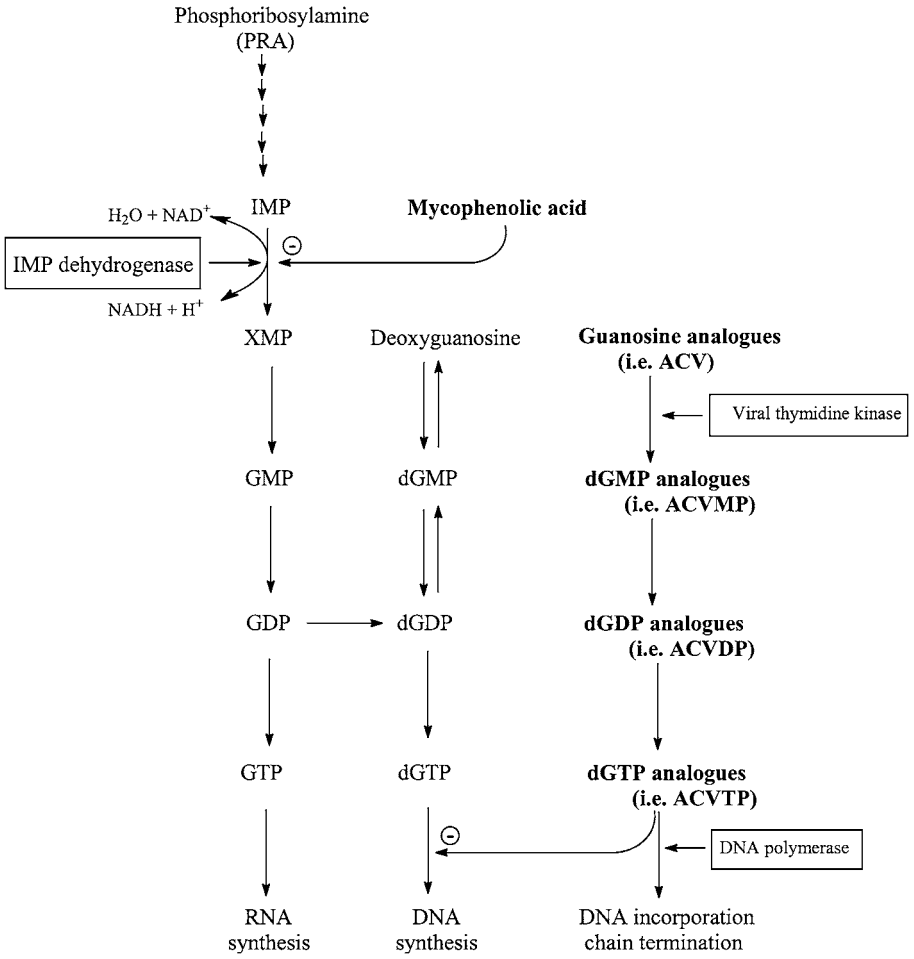

Figure 3. Interaction of mycophenolic acid (*via* inhibition of IMP dehydrogenase) with inhibitory effects of the guanosine analogues (triphosphates) at the DNA polymerase level.

ACYCLIC/CARBOCYCLIC GUANOSINE ANALOGUES IN THE COMBINED GENE THERAPY/CHEMOTHERAPY OF CANCER

As originally demonstrated 15 years ago (42), transformation of tumor cells, i.e. murine mammary FM3A carcinoma cells, with the HSV-1 thymidine kinase (TK) gene makes them highly sensitive to the cytostatic action of all those anti-herpesvirus agents (including ganciclovir) that for their antiviral activity rely upon phosphorylation by the viral TK. For ganciclovir, this cytostatic activity results from the incorporation of the compound (following its conversion to the triphosphate) into the DNA of the HSV-1 (or HSV-2) TK gene-transfected cells (43). Like ganciclovir, various other guanosine analogues also become significantly more cytostatic to tumor cells, i.e. murine mammary carcinoma (FM3A) cells, after these

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Table 6. Potentiating Effect of Mycophenolic Acid (MPA) on the Antiherpesvirus Activity of Guanosine Analogues

	EC_{50} (μg/ml)[a]					
	HSV-1		HSV-2		TK^- HSV-1	
Compound	−MPA	+MPA	−MPA	+MPA	−MPA	+MPA
Acyclovir	5.3	0.1	2.6	0.04	56	0.3
Penciclovir	6.6	0.5	5.3	0.7	>100	2.6
Ganciclovir	1.0	0.01	1.4	0.05	18	0.4
Lobucavir	1.8	0.03	0.7	0.004	11	0.06
H2G	5.3	0.2	11	0.2	>100	0.08
A-5021	1.3	0.006	1.5	0.08	≥70	1.3

[a]50% Effective concentration, or concentration required to reduce virus-induced cytopathicity in Vero cells by 50%.
MPA was used at a concentration of 2.5 μg/ml, at which it had by itself no antiviral effect.
Data taken from references 38, 39 and 41; and unpublished data (J. Neyts and E. De Clercq).

cells have been transfected by the HSV-1 TK gene (44). These observations have been extended to human osteosarcoma cells transfected by the HSV-1 TK/GFP fusion gene (45) (Table 7). A-5021 and D/L-cyclohexenyl guanine, but not synguanol, exhibited potent cytostatic activity against OST/TK^- HSV-1 TK $(GFP)^+$ cells, as

Table 7. Inhibitory Effects of Different Guanosine Analogues on the Proliferation of HSV Thymidine Kinase (TK) Gene-Transfected Tumor Cells

	EC_{50} (μM)[a]		
Compound	OST/TK^-	OST/TK^- HSV-1 TK $(GFP)^+$	S.I.[b]
Acyclovir	73 ± 29	0.059 ± 0.015	1,237
Penciclovir	231 ± 13	0.013 ± 0.002	17,769
Ganciclovir	44 ± 22	0.001 ± 0.0005	44,000
Lobucavir	18 ± 0.4	0.008 ± 0.0001	2,250
H2G	>250	0.009 ± 0.003	>27,778
A-5021	137 ± 10	0.0006 ± 0.0002	228,333
Synguanol	13 ± 4	14 ± 3.3	0.9
D/L-Cyclohexenyl G	3.2 ± 0.5	0.005 ± 0.004	640

[a]50% Effective concentration, or concentration required to inhibit cell proliferation by 50%. OST/TK: human osteosarcoma cells deficient in cytosol TK; OST/TK^- HSV-1 TK$(GFP)^+$: OST/TK^- cells transduced by the HSV-1 TK GFP (green fluorescence protein) fusion gene.
[b]Selectivity index, or ratio of EC_{50} (OST/TK^-) to EC_{50} [OST/TK^- HSV-1 TK $(GFP)^+$].
Data taken from reference 45; and unpublished data (B. Degrève, E. De Clercq and J. Balzarini).

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did H2G, lobucavir and ganciclovir. Of all the compounds tested, A-5021 showed the highest ratio (about 200,000) in the EC_{50} for non-transfected *versus* the EC_{50} for transfected osteosarcoma cells. The acyclic/carbocyclic guanosine analogues could thus be envisaged as potent anticancer agents, provided the tumor cells are engineered in such a way that they express the viral (HSV-1) thymidine kinase. It is not even necessary to transform all the tumor cells, since the guanosine analogues are endowed with a marked bystander killing effect, i.e. they are able to kill up to 90% of the cells of a population that contain only 10% viral TK gene-transfected tumor cells (45).

CONCLUSION

The most novel congeners among the carbocyclic guanosine analogues contain either a 3- or 6-membered sugar substitute: *viz*. the cyclopropylmethyl derivative A-5021 and D- and L-cyclohexenyl guanine. These new guanosine analogues were found to possess an extended activity spectrum as compared to that of acyclovir. While equally active (D- and L-cyclohexenyl guanine) or more active (A-5021) than acyclovir against HSV-1, HSV-2 and VZV, D- and L-cyclohexenyl guanine showed marked activity against HCMV, including HCMV strains that were resistant to ganciclovir. A-5021 was quite effective against HHV-6, as so was D/L-cyclohexenyl guanine against HBV. Combination with mycophenolic acid (MPA) markedly potentiated the antiviral activity of the new guanosine analogues (i.e., A-5021), as it did with the older congeners (acyclovir, ganciclovir and penciclovir). Given their dependence on phosphorylation by the virus (i.e. HSV-1)-encoded thymidine kinase (TK), all these guanosine analogues, and particularly A-5021 and D- and L-cyclohexenyl guanine, should also be pursued from combined gene therapy/chemotherapy viewpoint, based on the observation that transduction of the tumor cells by the viral TK make them exqusitely sensitive to the cytostatic action of these antiviral compounds.

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