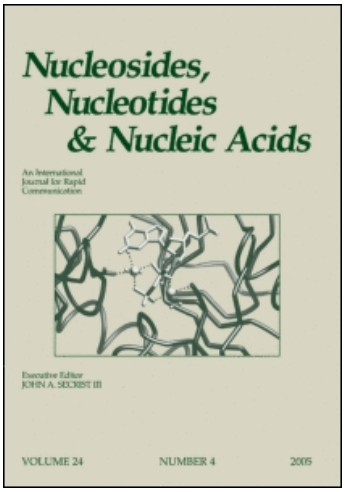

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Guanosine Analogues as Anti-Herpesvirus Agents

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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. HSV-1, HSV-2, VZV and HCMV) infections. In recent years, several new guanosine analogues have been developed, including the 3-membered (cyclopropyl) sugar derivative A-5021 and the 6-membered D- and L-cyclohexenyl derivatives. Prominent features shared by all guanosine analogues are the following. They depend for their phosphorylation on the virus-encoded thymidine kinase (TK), which makes them particularly effective against those viruses (HSV-1, HSV-2 and VZV) that encoded for such TK. They are also active against HCMV, whether or not they are subject of phosphorylation by the HCMV-induced UL97 protein kinase. Their antiviral activity can be markedly potentiated by mycophenolic acid, an IMP dehydrogenase inhibitor, and they hold great promise, not only as antiviral agents for the treatment of herpesvirus infections, but also as antitumor agents for the combined gene therapy/chemotherapy of cancer, provided that (part of) the tumor cells have been transfected by the viral TK gene.

INTRODUCTION

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, is the

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This paper is dedicated to the memory of Professor A. Krayevsky.

oral prodrug of acyclovir, which has been found to achieve substantially higher plasma levels of acyclovir than oral acyclovir itself; 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 patients,[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 {1*R*(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), 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.[17] 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.[18] The triphosphate of lobucavir is also a potent inhibitor of the HCMV DNA polymerase (Ki: 5 nM),[19] 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 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).[19]

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

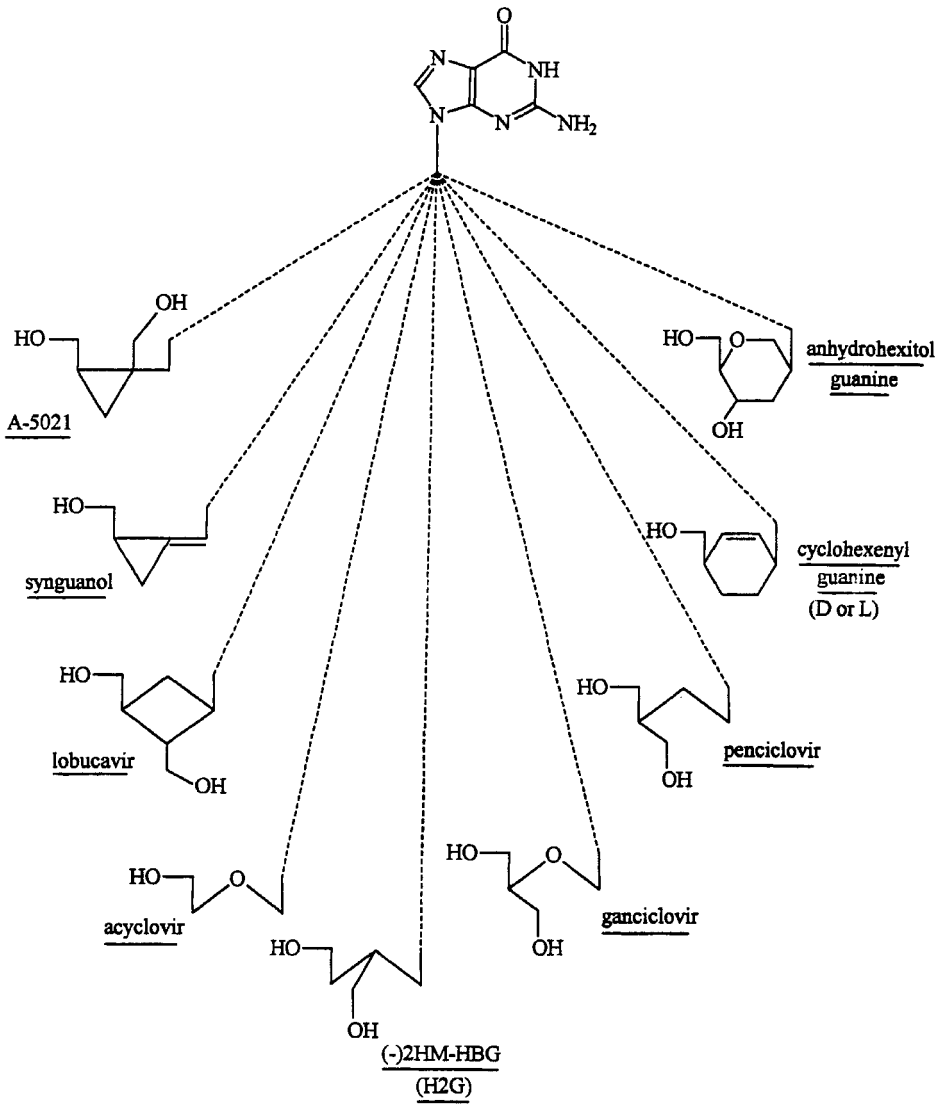


FIG. 1. Synoptic view of carbocyclic and acyclic guanosine analogues.

against HSV-1, HSV-2 and VZV.[20] H2G has been found to suppress simian varicella virus infection in African green monkeys at a dose as low as 1 mg/kg/day[21] and has been pursued for the treatment of VZV infections in humans.

A-5021

A new class of compounds, based on the cyclopropylmethyl entity as the sugar substitute,

TABLE 1. Comparative potency of different antiherpetic compounds against different herpesviruses

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

§E. De Clercq, unpublished data.
*-, if EC_{50} > 100 μM.
+, if EC_{50} = 10-100 μM
++, if EC_{50} = 1-10 μM
+++, if EC_{50} = 0.1-1 μM
++++, if EC_{50} = 0.01-0.1 μM
+++++, if EC_{50} = 0.001-0.01 μM
( ) if only borderline, or if not accurately determined.

namely A-5021 or (1'*S*,2'*R*)-9-{[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl}guanine, has been recently identified as potent and selective inhibitors of HSV-1, HSV-2 and VZV.[9] 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.[22] 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.[23]

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);[10] it has also marked activity against Epstein-Barr virus (EBV) but only limited potency against HSV-1, HSV-2 and VZV.[10] 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.[24] 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).[25] Synguanol, as well as other methylenecyclopropane analogues of nucleosides, have proven effective in the treatment of murine cytomegalovirus (MCMV) infections in mice.[26]

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,[11] 5-iodouracil,[11] 5-ethyluracil[12] or 5-trifluoromethyluracil[27] 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.

D- AND L-CYCLOHEXENYL G

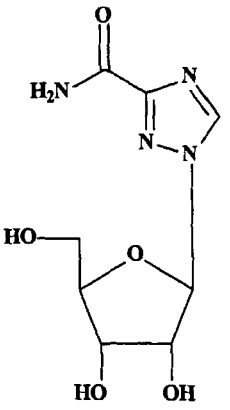
The cyclohexene nucleoside analogues can be considered as analogues of the natural furanose nucleosides where the ring oxygen has been replaced by a double bond. D-cyclohexenyl G and L-cyclohexenyl G were found to possess similar potency[13] against HSV-1, HSV-2, VZV and CMV (Table 1). This is not the case for the D- and L-anhydrohexitol nucleoside analogues where only the D-analogues demonstrated antiviral activity.[28] As the activity of D- and L-cyclohexenyl G was lower against TK-deficient (TK^-) HSV-1 strains than 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.[29] 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.[29]

MYCOPHENOLATE MOFETIL

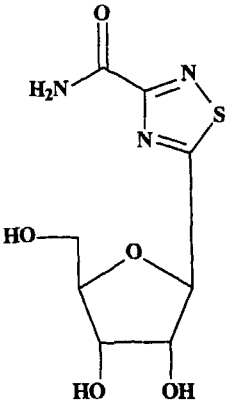
Mycophenolate mofetil (which is currently used as an immunosuppressant in kidney transplant recipients) is the morpholinoethyl ester of mycophenolic acid (Fig. 2). The latter is known to be a potent inhibitor of IMP dehydrogenase.[30] Like other IMP dehydrogenase inhibitors (Fig. 2), such as ribavirin, tiazofurin, EICAR[31] and VX-497,[32] 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 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 2) and *in vivo*;[33] and a similar marked enhancement was noted for the activity of H2G against HSV-1, HSV-2 and both TK^+ and TK^- VZV.[34] 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.[35] 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.[36] 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.

COMBINED GENE THERAPY/CHEMOTHERAPY

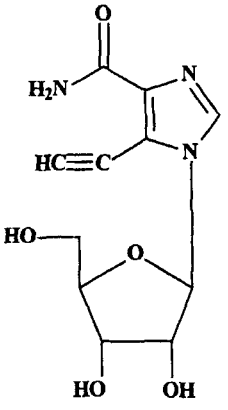
As originally demonstrated 15 years ago,[37] transformation of tumor cells, i.e. murine mammary FM3A carcinoma cells, with the HSV-1 thymidine kinase (TK) gene makes them highly sensitive to the cytostatic action of all those anti-herpesvirus agents (including ganciclovir) that for their antiviral activity 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.[38] Like



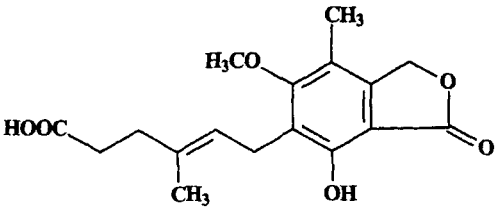
Ribavirin



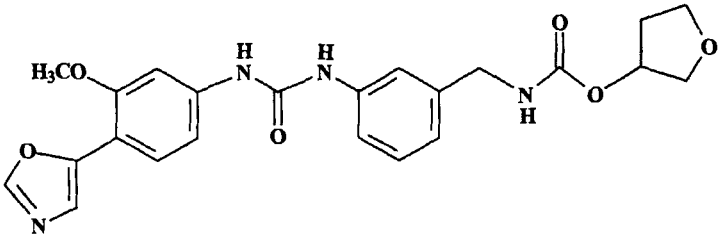
Tiazofurin



EICAR



Mycophenolic acid



VX-497

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

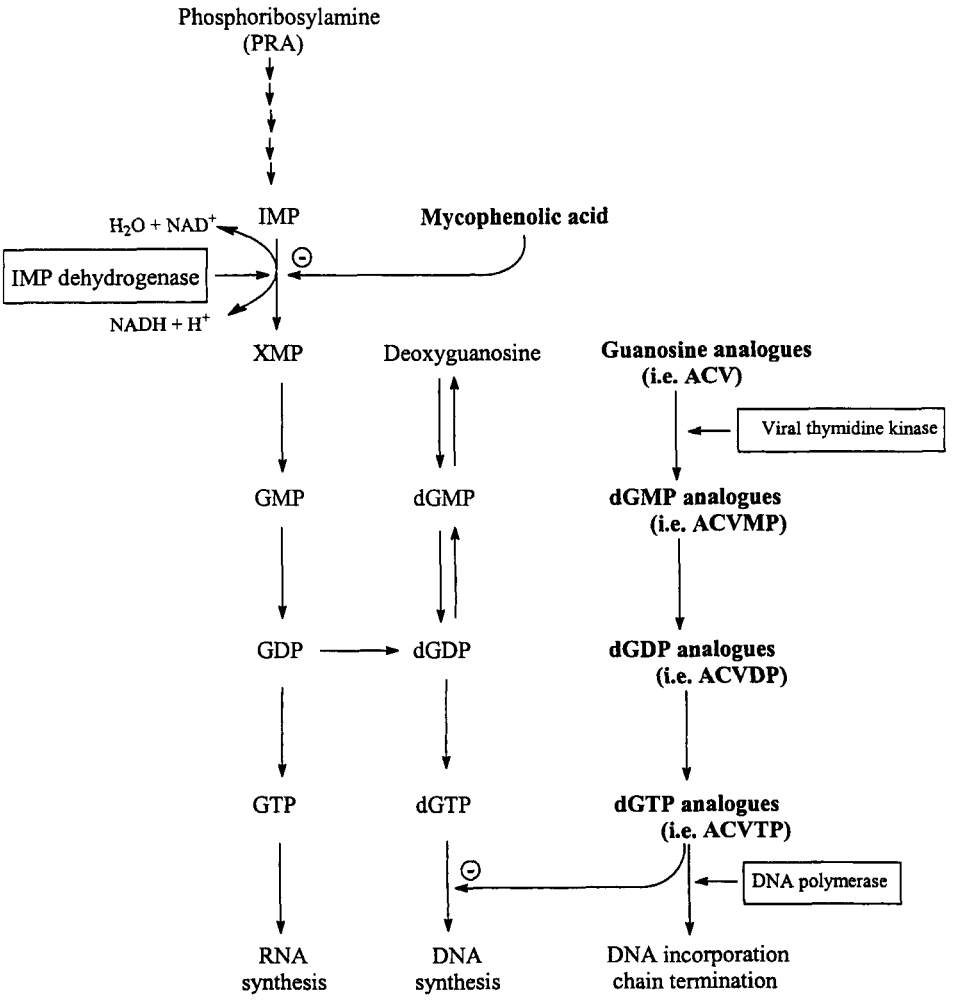


FIG. 3. Interaction of mycophenolic acid (*via* inhibition of IMP dehydrogenase) with inhibitory effects of the guanosine analogues (triphosphates) at the DNA polymerase level.

ganciclovir, various other guanosine analogues also become significantly more cytostatic to tumor cells after these cells have been transfected by the HSV-1 or HSV-2 TK gene (Table 3).[39] This observation, which has been extended to human osteosarcoma cells transfected by the HSV-1 TK/GFP fusion gene (Table 3),[40] can be considered as a paradigm for cancer chemotherapy provided the tumor cells are engineered such that they express the viral TK. 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 proved able to kill up to 90% of the cells of a population that contained only 10% viral TK gene-transfected tumor cells.[40]

TABLE 2. Effect of mycophenolic acid (MPA) on the anti-herpesvirus activity of guanosine analogues

Compound	EC_{50} (μg/ml)[a]					
	HSV-1		HSV-2		TK⁻ HSV-1	
	- MPA	+ MPA	- MPA	+ MPA	- MPA	+ MPA
Acyclovir	5.3 ± 2.6	0.1 ± 0.0	2.6 ± 2.0	0.04 ± 0.03	56 ± 6	0.3 ± 0.2
Penciclovir	6.6 ± 2.2	0.5 ± 0.3	5.3 ± 1.8	0.7 ± 0.2	> 100	2.6 ± 2.3
Ganciclovir	1.0 ± 0.5	0.01 ± 0.003	1.4 ± 0.7	0.05 ± 0.05	18 ± 3	0.4 ± 0.3
Lobucavir	1.8 ± 0.4	0.03 ± 0.004	0.7 ± 0.1	0.004 ± 0.001	11 ± 0.3	0.06 ± 0.003
H2G	5.3 ± 3.4	0.2 ± 0.2	11 ± 3	0.2 ± 0.2	> 100	0.08 ± 0.01
A-5021	1.3 ± 1.0	0.006 ± 0.001	1.5 ± 0.7	0.08 ± 0.02	≥ 70	1.3 ± 0.3

[a]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 33, 34 and 36; and unpublished data (J. Neyts and E. De Clercq).

TABLE 3. Inhibitory effects of different guanosine analogues on the proliferation of HSV thymidine kinase (TK) gene-transfected tumor cells

Compound	EC_{50} (μM)[a]				
	FM3A/TK⁻	FM3A/TK⁻/ HSV-1 TK⁺	FM3A/TK⁻/ HSV-2 TK⁺	OST/TK⁻	OST/TK⁻ HSV1 TK (GFP)⁺
Acyclovir	148 ± 28	62 ± 28	4.5 ± 2.6	73 ± 29	0.059 ± 0.015
Penciclovir	315 ± 12	5.1 ± 1.3	0.88 ± 0.42	231 ± 13	0.013 ± 0.0022
Ganciclovir	188 ± 45	1.0 ± 0.25	0.26 ± 0.08	44 ± 22	0.001 ± 0.0005
Buciclovir	304 ± 15	4.0 ± 1.5	1.1 ± 0.18	173 ± 67	0.006 ± 0.000
Lobucavir	72 ± 3	34 ± 6	2.5 ± 0.3	18 ± 0.4	0.008 ± 0.00081

[a]Concentration required to inhibit cell proliferation by 50%. FM3A/TK⁻: murine mammary carcinoma cells deficient in cytosol TK; OST/TK⁻: human osteosarcoma cells deficient in cytosol TK; FM3A/TK⁻/HSV-1 TK⁺, FM3A/TK⁻/HSV-2 TK⁺ and OST/TK⁻ HSV-1 TK(GFP)⁺: FM3A/TK⁻ and OST/TK⁻ cells transfected by the HSV-1 TK gene, HSV-2 TK gene or HSV-2 TK GFP (green fluorescence protein) fusion gene, respectively. Data taken from references 39 and 40.

CONCLUSION

A large variety of acyclic and carbocyclic guanosine analogues have been described. The most novel congeners contain either a 3- or 6-membered sugar substitute: *viz.* the cyclopropylmethyl derivative A-5021 and the D- and L-cyclohexenyl derivatives. These new guanosine analogues should be further explored for their therapeutic potential as antiviral and anticancer agents. In particular, these compounds should be further examined for their activity against a broad range of herpesviruses (including EBV, HHV-6, HHV-7 and HHV-8). Their *in*

vivo antiviral efficacy and their pharmacokinetic profile should be determined. It should also be assessed whether combination with mycophenolic acid (MPA) potentiates the antiviral activity of these new guanosine analogues, as it does with the older congeners (acyclovir, ganciclovir, penciclovir). Given their dependence on phosphorylation by the virus (i.e. HSV-1)-encoded thymidine kinase (TK), all these guanosine analogues should also be pursued from a cancer chemotherapy viewpoint, based on the premise that transfection of the tumor cells by the viral TK would make them particularly sensitive to the cytostatic action of these antiviral compounds.

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