ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1987, p. 1238–1242
0066-4804/87/081238-05$02.00/0

Vol. 31, No. 8

Antiherpesvirus Activity of 9-(4-Hydroxy-3-Hydroxymethylbut-1-yl)Guanine (BRL 39123) in Cell Culture

MALCOLM R. BOYD,* TERESA H. BACON, DAVID SUTTON, AND MARTIN COLE

Beecham Pharmaceuticals Research Division, Biosciences Research Centre, Epsom, Surrey KT18 5XQ, England

Received 29 December 1986/Accepted 22 May 1987

The activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) against several herpesviruses was compared with that of acyclovir (ACV). In plaque reduction tests with clinical isolates of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella-zoster virus, mean 50% inhibitory concentrations (IC_{50}s) (n = number tested) for BRL 39123 were 0.4 (n = 17), 1.5 (n = 13), and 3.1 (n = 5) μg/ml, respectively. Corresponding IC_{50}s for ACV were 0.2, 0.6, and 3.8 μg/ml. Cytomegalovirus was relatively resistant to BRL 39123 (IC_{50}, 51 μg/ml), but equid herpesvirus 1, bovid herpesvirus 2, and felid herpesvirus 1 were susceptible (IC_{50}s, 1.6, 1.2, and 0.9 μg/ml, respectively). BRL 39123 was inactive against an HSV-1 strain which does not express thymidine kinase activity, but a DNA polymerase mutant selected for resistance to ACV was sensitive to BRL 39123 (IC_{50}, 1.5 μg/ml). In contrast to the results from plaque reduction tests, BRL 39123 was more active than ACV against HSV-1 and of equal activity against HSV-2 in virus yield reduction assays in MRC-5 cells. After treatment of HSV-infected cultures for short periods, BRL 39123 was considerably more effective than ACV at reducing virus replication, and furthermore, after removal of extracellular BRL 39123, virus replication remained depressed for long periods, whereas such persistent activity was not observed with ACV. Neither compound significantly affected MRC-5 cell replication at 100 μg/ml, but at 300 μg/ml BRL 39123 was more inhibitory than ACV.

Since the discovery of acyclovir (ACV) and its introduction as an effective treatment for herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV) infections in humans, several laboratories have been searching for acyclic purine nucleosides with improved properties. ACV is a highly selective antiviral agent which is activated by the virus-induced thymidine kinase and inhibits susceptible viruses at concentrations which do not affect cell replication (9). Several other guanine derivatives have been described and evaluated as inhibitors of herpesviruses in cell culture and in animal infections (1, 10, 13, 14, 22–24). Many publications show that 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) is beneficial in the treatment of severe cytomegalovirus (CMV) infections in humans, but because of adverse toxicity its use in less serious infections may be limited (20).

Pandit et al. (18) first claimed to have synthesized 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine, but the compound was not fully characterized and no biological properties were given. The synthesis and isolation of pure compound have since been reported by Harnden and Jarvest (12), who found that the experimental conditions originally described provided a mixture of products.

In the present paper, the activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123; Fig. 1) is compared with that of ACV in plaque reduction assays against veterinary and human herpesvirus isolates, including recent clinical isolates of HSV and VZV and also two ACV-resistant strains of HSV-1. BRL 39123 and ACV have also been evaluated for their inhibitory effects on virus replication in virus yield reduction assays. The data in this report extend the observations made independently by Larsson et al. (15), MacCoss et al. (16), and Tippie et al. (24) on the biological activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine, the compound we have called BRL 39123.

* Corresponding author.

MATERIALS AND METHODS

Compounds. The guanine derivatives BRL 39123 (12) and ACV (19) were prepared within the chemical laboratories of Beecham Pharmaceuticals. Compounds were dissolved at 10 mg/ml in dimethyl sulfoxide and stored at −20°C prior to dilution in cell culture medium.

Cell cultures. EBTr, RK-13, and Vero cells (Flow Laboratories Ltd., Irvine, Scotland) were grown in Eagle minimal essential medium (MEM) with 10% newborn calf serum (NCS). BHK-21 cells (Flow) were grown in Glasgow MEM containing 10% NCS and 10% tryptose phosphate broth. MRC-5 cells (National Institute for Biological Standards and Control, Holly Hill, London, United Kingdom) and MDBK cells (Central Veterinary Laboratories, Weybridge, United Kingdom) were grown in Eagle MEM with 10% fetal calf serum (FCS). FEA cells (feline embryonic type A cells; provided by D. A. Harbour, University of Bristol) were grown in Glasgow MEM containing 10% tryptose phosphate broth and 5% FCS. All media and sera were purchased from Gibco Ltd., Paisley, Scotland. BHK-21, MRC-5, and Vero cells, which were used extensively in this work, were cultured in the absence of antibiotics and were free from mycoplasma contamination.

Viruses. Stocks of HSV-1 and HSV-2 strains and also bovid herpesvirus type 2 (BHV-2; bovine mammillitis), strain New York 1, were prepared in BHK-21 cells. BHV-1, strain Oxford 1964 (infectious rhinotracheitis), equid herpesvirus type 1 (EHV-1), strain Quai Hais (equine rhinopneumonitis), and felid herpesvirus type 1 (FHV-1), strain B927 (feline rhinotracheitis), were grown in MDBK, RK-13, and FEA cells, respectively. Stocks of CMV and VZV were prepared in MRC-5 cells. HSV-1 strain Cl(101) and its ACV-resistant variants were supplied by H. J. Field (University of Cambridge, Cambridge, United Kingdom) (11).

Plaque reduction assays. Confluent cell monolayers in 24-well multidishes (well diameter, 1.5 cm; Gibco, Paisley,

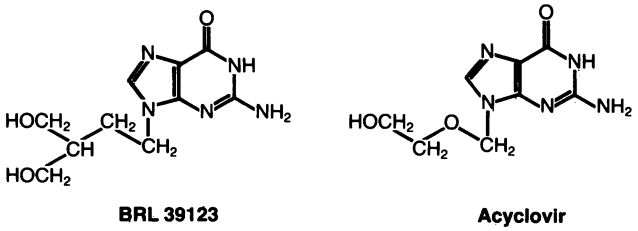

FIG. 1. Structures of BRL 39123 and ACV.

Scotland) were infected with about 50 PFU of virus in 100 μl of phosphate-buffered saline (PBS). After adsorption at room temperature for 1 h, residual inoculum was replaced with 0.5 ml of medium containing 5% NCS and 0.9% agarose (Indubiose A37; Uniscience Ltd., London, United Kingdom). Heat-inactivated FCS was used instead of NCS in plaque assays with VZV and CMV. Once the agarose had set, dilutions of the test compounds, which had been prepared in medium containing 5% NCS or FCS as appropriate, were added, each well receiving 0.5 ml of liquid overlay. The final concentrations of drugs achieved were 100, 30, 10, 3, 1, 0.3, 0.1, and 0.03 μg/ml. Infected cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air until plaques were clearly visible. In CMV plaque reduction assays, which were incubated for about 9 days, each well contained 1 ml of agarose and 1 ml of liquid overlay. Cell monolayers were fixed in formol saline and stained with carbol fuchsin, and plaques were counted with the aid of a stereomicroscope. By reference to the number of plaques observed in virus control monolayers (untreated cultures), the concentration of test compound which inhibited plaque numbers by 50% (IC_{50}) was calculated.

Virus yield reduction assays. Monolayers of MRC-5 cells prepared in microtiter plates (Nunc) were infected with HSV-1 or HSV-2 diluted in PBS, 50 μl per well. After adsorption for 1 h at room temperature, residual inoculum was removed and cell monolayers were washed twice with PBS. Test compounds which had been diluted in assay medium (Eagle MEM plus 5% NCS) were added to give 0.2 ml per well. Plates were incubated at 37°C, and at various times after infection supernatants were collected, clarified by centrifugation (Eppendorf microcentrifuge; 2 min at 12,000 rpm), and stored at −70°C. In experiments in which infected cell cultures were treated with BRL 39123 and ACV for various times and subsequently reincubated in drug-free medium, cultures were washed twice with PBS before fresh medium was added. All experiments were performed in triplicate. Supernatants from replicate cultures were titrated individually on Vero cells, and the geometric mean infectivity titer was calculated.

In one series of experiments, several strains of HSV-1 and HSV-2 were tested for susceptibility to BRL 39123 and ACV. The compounds were added 1 h after infection, and the yield of virus 24 h after infection was measured. By reference to the amount of virus produced by virus control monolayers (untreated cultures), the concentration of compound required to inhibit the virus control yield by 99% (IC_{99}) was calculated.

Cytotoxicity assay. Effects on cell proliferation were studied by the addition of compounds in the absence of dimethyl sulfoxide to actively replicating MRC-5 cells. Cell numbers were measured after incubation for 72 h in a Coulter counter (Coulter Electronics, Harpenden, United Kingdom).

Statistical analysis. The analysis of variance was used to compare IC_{50}s for clinical isolates of HSV-1, HSV-2, and VZV and also to compare IC_{99}s derived from virus yield reduction assays. Differences between BRL 39123 and ACV in cytotoxicity assays were evaluated by Student's t test. A value of $P < 0.05$ was considered statistically significant.

TABLE 1. Activity of BRL 39123 and ACV against different herpesviruses measured by the plaque reduction assay

Virus (strain)	Cells	IC_{50} (μg/ml)	
		BRL 39123	ACV
HSV-1 (HFEM)	MRC-5	0.5	0.3
HSV-2 (MS)	MRC-5	0.8	0.4
VZV (Ellen)	MRC-5	2.4	3.3
CMV (AD-169)	MRC-5	52	25
BHV-1 (Oxford 1964)	EBTr	100	>100
BHV-2 (New York 1)	BHK-21	1.2	25
EHV-1 (Quai Hais)	RK-13	1.6	2.6
FHV-1 (B927)	FEA	0.9	11

RESULTS

Spectrum of activity. Several laboratory strains of herpesviruses were tested for susceptibility to BRL 39123 and ACV by the plaque reduction assay (Table 1). BRL 39123 was up to twofold less active than ACV against HSV-1 and HSV-2 but slightly more active than ACV against VZV. Both compounds showed minimal activity against CMV. Epstein-Barr virus (EBV) was susceptible to BRL 39123 and ACV in a test which measured the inhibition of virus capsid antigen expression, activity being observed at 10 μg/ml (data not shown). Greater differences between the two compounds were observed among veterinary herpesviruses (Table 1). BRL 39123 was active at about 1 μg/ml against BHV-2 and FHV-1, while the IC_{50} of ACV for both viruses exceeded 10 μg/ml. BRL 39123 was slightly more active than ACV against EHV-1, but both compounds were inactive against BHV-1.

Clinical isolates of HSV-1, HSV-2, and VZV were screened for susceptibility to BRL 39123 and ACV by the plaque reduction assay (Table 2). These viruses have undergone limited passage in vitro since isolation. By this method, BRL 39123 was significantly less active than ACV against HSV-1 ($P = 0.02$) and HSV-2 ($P < 0.01$) and was of equal activity against VZV. HSV-1 was significantly more susceptible to BRL 39123 than HSV-2 ($P < 0.01$), and HSV-2 was more susceptible than VZV ($P < 0.01$).

While both BRL 39123 and ACV showed limited activity against an HSV-1 strain which did not synthesize viral thymidine kinase, Cl(101)TK^-, BRL 39123 was active

TABLE 2. Activity of BRL 39123 and ACV against clinical isolates of HSV-1, HSV-2, and VZV in MRC-5 cells measured by the plaque reduction assay

Virus	No. of isolates tested[a]	Mean IC_{50} (μg/ml) ± SD	
		BRL 39123	ACV
HSV-1	17	0.4 ± 0.1	0.2 ± 0.2
HSV-2	13	1.5 ± 0.4	0.6 ± 0.2
VZV	5	3.1 ± 0.8	3.8 ± 0.7

[a] Clinical isolates of HSV-1 and HSV-2 were kindly provided by H. Cummin (Epsom Public Health Laboratory, West Park Hospital, Epsom, Surrey, United Kingdom) and M. M. Ogilvie (Southampton General Hospital, Southampton, United Kingdom). VZV strains were supplied by E. V. Meurisse (Epsom Public Health Laboratory, West Park Hospital).

TABLE 3. Activity of BRL 39123 and ACV against ACV-resistant mutants of HSV-1 Cl(101)

Virus strain	Phenotype	IC_{50} (μg/ml)[a]	
		BRL 39123	ACV
Cl(101)	Wild type	1.1	0.4
Cl(101)TK$^-$	TK$^-$	87	58
Cl(101)P_2C_5	TK$^+$, DNA polymerase mutant (ACV^r)	1.5	21

[a] Plaque reduction assay in Vero cells.

against a DNA polymerase mutant, Cl(101)P_2C_5, which was resistant to ACV (Table 3).

Effects on HSV replication. BRL 39123 and ACV inhibited the replication of HSV-1 and HSV-2 in the virus yield reduction assay in MRC-5 cells; the extent of inhibition was related to the concentration of compound (Fig. 2). The IC_{99}s (concentrations required to reduce the yield of infectious virus obtained 24 h after infection by 99% relative to control cultures) derived from the data in Fig. 2 were 0.4 and 1.0 μg/ml for BRL 39123 and ACV, respectively, against HSV-1. The corresponding figures for HSV-2 were 0.7 μg/ml for BRL 39123 and 0.9 μg/ml for ACV. Several other clinical isolates have been tested in addition to the laboratory strains HSV-1 (HFEM) and HSV-2 (MS) (data not shown). The IC_{99}s were in good agreement with those quoted above. In virus yield reduction assays, BRL 39123 was thus more potent than ACV against HSV-1 strains ($P = 0.05$) and of equal activity against HSV-2 strains ($P = 0.77$).

In subsequent experiments the effect of varying the time of contact between the test compounds and cell monolayers infected with HSV-1 and HSV-2 was investigated. MRC-5 cells infected with HSV-2 (MS) were exposed to BRL 39123 and ACV at 30 μg/ml for various lengths of time beginning 2 h after infection, and cell-free virus titers were measured 25 h after infection (Table 4). Brief treatment with BRL 39123, for example between 2 and 6 h after infection, resulted in a considerably lower virus yield than equivalent treatment with ACV. The continuous presence of ACV was required to achieve maximum reduction in virus yield, whereas this was attained after 5 h of incubation with BRL 39123. Similar results were obtained with HSV-1 in MRC-5 cells.

TABLE 4. Effect of duration of treatment on the inhibition of HSV-2 (MS) replication in MRC-5 cells

Treatment period[a] (h postinfection)	Virus yield at 25 h[b] (PFU/0.1 ml)	
	BRL 39123	ACV
None	4.5×10^3	4.5×10^3
2–4	2.2×10^3	2.8×10^3
2–5	3.2×10^2	1.8×10^3
2–6	2.0×10^0	6.3×10^2
2–7	$<1.0 \times 10^0$	4.5×10^2
2–9	$<1.0 \times 10^0$	1.3×10^2
2–11	$<1.0 \times 10^0$	3.0×10^1
2–13	$<1.0 \times 10^0$	1.0×10^1
2–25	$<1.0 \times 10^0$	1.4×10^0

[a] Nucleoside analogs at 30 μg/ml were added 2 h after infection and replaced at various times with fresh assay medium.
[b] The MOI was 1.8 PFU per cell.

MRC-5 cells infected with HSV-2 were treated with BRL 39123 and ACV for 18 h after infection, and thereafter incubation was continued in the absence of the compounds. The amount of virus produced by these cultures was measured for up to 6 days (Fig. 3). After the removal of BRL 39123 at 3 μg/ml, the infectivity titers of supernatants from these cultures declined. In contrast, the amount of virus produced by infected cells after treatment for 18 h with ACV at 30 μg/ml rapidly increased to give levels similar to those released by untreated cells. Persistent antiviral activity in MRC-5 cells was observed with concentrations of BRL 39123 as low as 1 μg/ml. Furthermore, persistent antiviral activity of BRL 39123 was also observed in Vero cells infected with HSV-1 and HSV-2, although the effect was not as great as that described for MRC-5 cells (data not shown).

Cytotoxicity. No morphological abnormalities were observed in uninfected, established cell monolayers treated with either BRL 39123 or ACV at 100 μg/ml during the plaque reduction assays. However, to determine whether cell replication was adversely affected by these guanine derivatives, subconfluent monolayers of MRC-5 cells were grown in 24-well plates for 72 h with various concentrations of either BRL 39123 or ACV. At 100 μg/ml, neither compound significantly affected cell proliferation. At 300 μg/ml,

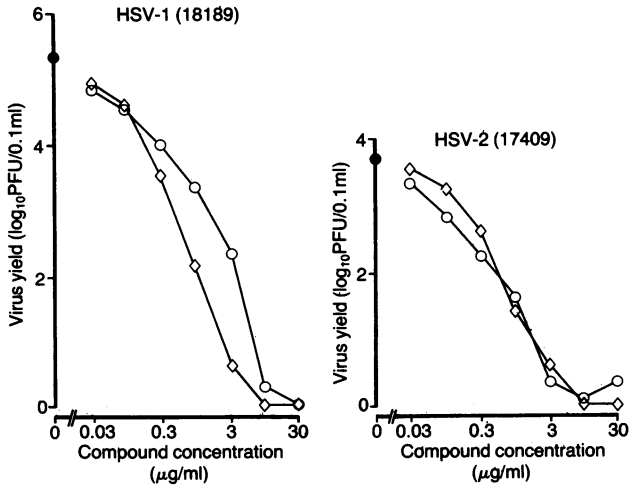

FIG. 2. Inhibition of replication of clinical isolates of HSV-1 and HSV-2 by the virus yield reduction assay in MRC-5 cells. Symbols: ◇, BRL 39123; ○, ACV; ●, virus control yield. The MOIs were 0.9 PFU per cell for HSV-1 and 0.4 PFU per cell for HSV-2. Nucleoside analogs were added 1 h after virus infection, and culture supernatants were harvested 24 h after infection.

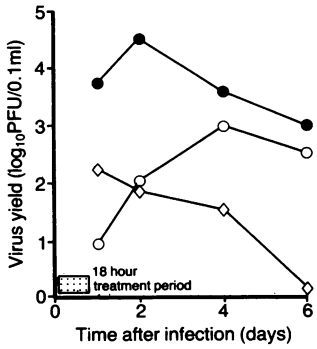

FIG. 3. Duration of antiviral activity after 18-h treatment of MRC-5 cells infected with HSV-2 (MS). Symbols: ◇, BRL 39123; ○, ACV; ●, untreated virus control. The concentrations of BRL 39123 and ACV were 3 and 30 μg/ml, respectively. The MOI was 0.7 PFU per cell. Compounds were added 1 h after infection and replaced 18 h later with fresh assay medium. Infectivity titers of culture supernatants collected at the times indicated were measured.

both compounds were significantly different from the control ($P < 0.05$), and BRL 39123 was more inhibitory than ACV ($P < 0.05$). The mean numbers of population doublings in cultures treated at 300 μg/ml were 2.3 for BRL 39123 and 3.0 for ACV, compared with 3.2 for control cultures.

DISCUSSION

BRL 39123 is a selective inhibitor of herpesvirus replication, as demonstrated by the wide separation between the concentration which affected virus replication and that which affected cell proliferation. Our results show that BRL 39123 was active against herpesviruses expressing thymidine kinase, which implies that BRL 39123, like ACV, is converted by this enzyme to the monophosphate. Information on the biochemical mode of action of BRL 39123 is currently being obtained. Larsson et al. (15) recently reported that this compound is a substrate with high affinity for the viral thymidine kinase and a potent inhibitor of viral DNA synthesis.

We used two methods to obtain information on the relative potency and properties of BRL 39123 and ACV, the plaque reduction assay and the virus yield reduction assay (6). The results of plaque reduction assays indicate that BRL 39123 and ACV have a similar spectrum of activity but that there are differences in relative potency. Our independent assessment of the properties of BRL 39123 is in accord with that of others (15, 16, 24) and shows that the compound is a potent inhibitor of HSV-1 and HSV-2. In addition, we report that BRL 39123 is active against VZV. In plaque reduction assays against a range of clinical isolates, BRL 39123 was less active than ACV against HSV-1 and HSV-2, of equal activity against VZV, and active against three veterinary viruses, BHV-2, EHV-1, and FHV-1. As reported previously (15, 16, 24), our results indicate that in plaque reduction assays BRL 39123 is less active against HSV-2 than HSV-1. MacCoss et al. (16), however, reported 50% effective doses of 25 to 50 μM for HSV-1 and 200 μM for HSV-2, which are much higher than our values and those of others (15, 24). The reason for this discrepancy is unclear.

ACV inhibits EBV DNA replication in P3HR-1 cells and reduces the percentage of cells expressing capsid antigen (5). Our observations suggest that the expression of EBV capsid antigen was equally susceptible to inhibition by BRL 39123 and ACV. In accord with the results of Tippie et al. (24), we also found that BRL 39123 had limited activity against CMV. In contrast, DHPG, which is structurally related to BRL 39123, is very active against CMV (4, 17). The mechanism of activity of DHPG against CMV is poorly understood, since CMV is not known to express thymidine kinase, but it has been suggested that a cell enzyme could perform the initial phosphorylation of DHPG (3). Whatever the mechanism of action of DHPG against CMV, it does not appear to be the same as that of BRL 39123.

There was no evidence of intrinsic resistance to BRL 39123 among the clinical strains of HSV or VZV tested, which were from several sources. Similar findings have been reported for ACV (2, 8). However, it was noted that BRL 39123 inhibited an ACV-resistant strain of HSV-1, Cl(101)P_2C_5, which carries a mutation in the DNA polymerase gene. This suggests that either the two compounds interact with the DNA polymerase at different sites or that the affinity of the enzyme for BRL 39123 differs from that for ACV.

In virus yield reduction assays under conditions of relatively high multiplicity of infection (MOI) (about 1 PFU per cell), it was observed that BRL 39123 was more active than ACV against HSV-1 strains and equally active against HSV-2 strains. Results from yield reduction assays thus change the assessment of the relative potency of these two compounds compared with data obtained by plaque reduction assays. Two important differences between the tests concern the MOI and the nature of the endpoint; a low virus challenge was used in the plaque reduction assay, whereas cells were infected at a high MOI in the virus yield reduction assay, and in the latter assay infectious virus production was measured rather than plaque formation. Antiviral agents are often ranked according to their performance in plaque reduction tests (2, 7), but it may be of greater relevance to chemotherapy to test their ability to inhibit the production of infectious virus, as in the virus yield reduction assay.

Two further interesting phenomena emerged during our investigations of the activity of BRL 39123 against HSV. First, maximum inhibition of virus replication was achieved following comparatively short treatment with BRL 39123, whereas the presence of ACV was required throughout the incubation period to obtain the same inhibition. Second, once inhibition of virus replication was achieved by treatment with BRL 39123, virus replication remained depressed for long periods after extracellular compound had been removed from the cultures. In contrast, virus replication rapidly resumed after removal of extracellular ACV. These two findings may reflect the efficient trapping of the phosphorylated form of BRL 39123 in the infected cell. In addition, BRL 39123 may be phosphorylated more rapidly than ACV, and this might account for the faster generation of the antiviral state in cultures treated with BRL 39123. For example, Field et al. (10) found that in crude extracts of HeLa cells infected with HSV-1, the rate of phosphorylation of DHPG was much faster than that of ACV.

Our results concerning the persistent activity of BRL 39123 are similar to those of Cheng et al. (4), who found that treatment of HSV-1-infected cultures with DHPG up to 8 h after infection was as effective as having the compound present throughout the incubation period. It was also shown (21) that DHPG triphosphate in HSV-2-infected cells had greater stability than ACV triphosphate. A similar trapping mechanism has been proposed for buciclovir [(*R*)-9-(3,4-dihydroxybutyl)guanine] (15). If also true for BRL 39123, this could be clinically advantageous when a drug is rapidly removed from the circulation or site of administration; effective treatment with BRL 39123 may not be dependent on maintenance of antiviral drug concentrations in the bloodstream.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of C. Patience and thank M. R. Harnden, R. A. Vere Hodge, and D. N. Planterose for helpful discussion.

LITERATURE CITED

1. **Ashton, W. T., L. F. Canning, G. F. Reynolds, R. L. Tolman, J. D. Karkas, R. Liou, M.-E. M. Davies, C. M. DeWitt, H. C. Perry, and A. K. Field.** 1985. Synthesis and antiherpetic activity of (*S*)-, (*R*)-, and (±)-9-[(2,3-dihydroxy-1-propoxy)methyl]-guanine, linear isomers of 2′-nor-2′-deoxyguanosine. J. Med. Chem. **28**:926–933.
2. **Biron, K. K., and G. B. Elion.** 1980. In vitro susceptibility of varicella-zoster virus to acyclovir. Antimicrob. Agents Chemother. **18**:443–447.
3. **Cheng, Y.-C., S. P. Grill, G. E. Dutschman, K. Nakayama, and**

K. F. Bastow. 1983. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl)guanine, a new anti-herpes virus compound, in herpes simplex virus-infected cells. J. Biol. Chem. **258**:12460–12464.

4. **Cheng, Y.-C., E.-S. Huang, J.-C. Lin, E.-C. Mar, J. S. Pagano, G. E. Dutschman, and S. P. Grill.** 1983. Unique spectrum of activity of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine against herpes viruses *in vitro* and its mode of action against herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA **80**:2767–2770.
5. **Colby, B. M., J. E. Shaw, G. B. Elion, and J. S. Pagano.** 1980. Effect of acyclovir [9-(2-hydroxyethoxymethyl)guanine] on Epstein-Barr virus DNA replication. J. Virol. **34**:560–568.
6. **Collins, P., and D. J. Bauer.** 1977. Relative potencies of antiherpes compounds. Ann. N.Y. Acad. Sci. **284**:49–59.
7. **Collins, P., and N. M. Oliver.** 1985. Comparison of the *in vitro* and *in vivo* antiherpes virus activities of the acyclic nucleosides, acyclovir (Zovirax) and 9-[(2-hydroxy-1-hydroxymethylethoxy)methyl]guanine (BWB759U). Antiviral Res. **5**:145–156.
8. **Crumpacker, C. S., L. E. Schnipper, J. A. Zaia, and M. J. Levin.** 1979. Growth inhibition by acycloguanosine of herpesviruses isolated from human infections. Antimicrob. Agents Chemother. **15**:642–645.
9. **Elion, G. B., P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, and H. J. Schaeffer.** 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. USA **74**:5716–5720.
10. **Field, A. K., M. E. Davies, C. DeWitt, H. C. Perry, R. Liou, J. Germershausen, J. D. Karkas, W. T. Ashton, D. B. R. Johnston, and R. L. Tolman.** 1983. 9-[[2-Hydroxy-1-(hydroxymethyl) ethoxy]methyl]guanine: a selective inhibitor of herpes group virus replication. Proc. Natl. Acad. Sci. USA **80**:4139–4143.
11. **Field, H. J., G. Darby, and P. Wildy.** 1980. Isolation and characterization of acyclovir-resistant mutants of herpes simplex virus. J. Gen. Virol. **49**:115–124.
12. **Harnden, M. R., and R. L. Jarvest.** 1985. An improved synthesis of the antiviral acyclonucleoside 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine. Tetrahedron Lett. **26**:4265–4268.
13. **Larsson, A., S. Alenius, N.-G. Johansson, and B. Oberg.** 1983. Antiherpetic activity and mechanism of action of 9-(4-hydroxybutyl)guanine. Antiviral Res. **3**:77–86.
14. **Larsson, A., B. Öberg, S. Alenius, C.-E. Hagberg, N.-G. Johansson, B. Lindborg, and G. Stening.** 1983. 9-(3,4-Dihydroxybutyl)guanine, a new inhibitor of herpesvirus multiplication. Antimicrob. Agents Chemother. **23**:664–670.
15. **Larsson, A., K. Stenberg, A.-C. Ericson, U. Haglund, W.-A. Yisak, N.-G. Johansson, B. Öberg, and R. Datema.** 1986. Mode of action, toxicity, pharmacokinetics, and efficacy of some new antiherpesvirus guanosine analogs related to buciclovir. Antimicrob. Agents Chemother. **30**:598–605.
16. **MacCoss, M., R. L. Tolman, W. T. Ashton, A. F. Wagner, J. Hannah, A. K. Field, J. D. Karkas, and J. I. Germershausen.** 1986. Synthetic, biochemical and antiviral aspects of selected acyclonucleosides and their derivatives. Chem. Scripta **26**: 113–121.
17. **Mar, E.-C., Y.-C. Cheng, and E.-S. Huang.** 1983. Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine on human cytomegalovirus replication in vitro. Antimicrob. Agents Chemother. **24**:518–521.
18. **Pandit, U. K., W. F. A. Grose, and T. A. Eggelte.** 1972. A new class of nucleoside analogues. Synthesis of N_1-pyrimidinyl- and N_9-purinyl-4′-hydroxy-3-(hydroxymethyl)butanes. Synth. Commun. **2**:345–351.
19. **Schaeffer, H. J., L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, and P. Collins.** 1978. 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature (London) **272**:583–585.
20. **Shepp, D. H., P. S. Dandliker, P. de Miranda, T. C. Burnette, D. M. Cederberg, L. E. Kirk, and J. D. Meyers.** 1985. Activity of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine in the treatment of cytomegalovirus pneumonia. Ann. Intern. Med. **103**:368–373.
21. **Smee, D. F., R. Boehme, M. Chernow, B. P. Binko, and T. R. Matthews.** 1985. Intracellular metabolism and enzymatic phosphorylation of 9-(1,3-dihydroxy-2-propoxymethyl)guanine and acyclovir in herpes simplex virus-infected and uninfected cells. Biochem. Pharmacol. **34**:1049–1056.
22. **Smee, D. F., J. C. Martin, J. P. H. Verheyden, and T. R. Matthews.** 1983. Antiherpesvirus activity of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine. Antimicrob. Agents Chemother. **23**:676–682.
23. **Smith, K. O., K. S. Galloway, W. L. Kennell, K. K. Ogilvie, and B. K. Radatus.** 1982. A new nucleoside analog, 9[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine, highly active in vitro against herpes simplex virus types 1 and 2. Antimicrob. Agents Chemother. **22**:55–61.
24. **Tippie, M. A., J. C. Martin, D. F. Smee, T. R. Matthews, and J. P. H. Verheyden.** 1984. Antiherpes simplex virus activity of 9-[4-hydroxy-3-(hydroxymethyl)-1-butyl]guanine. Nucleosides Nucleotides **3**:525–535.