Antiviral Research, 4 (1984) 231–243
Elsevier

AVR 00136

Screening for new compounds with antiherpes activity*

Balbino Alarcón[1], Juan Carlos Lacal[1], José María Fernández-Sousa[2] and Luis Carrasco[1]

[1]*Departamento de Microbiología, Centro de Biología Molecular, Universidad Autónoma, Canto Blanco, Madrid-34 and* [2]*Antibióticos S.A., c/ Bravo Murillo, 38, Madrid-3, Spain*

(Received 23 November 1983; accepted 15 February 1984)

Summary

A number of compounds have been tested for antiherpes activity. Actinobolin, amicetin, carrageenan, laspartomycin, megalomycin C, pleuromutilin, suramin and tetracenomycin C showed significant protection of HeLa cell monolayers infected with herpes simplex virus type 1. The action of these new antiherpes compounds was compared with those antiherpes agents that have been described previously. Actinobolin, amicetin and tetracenomycin C were also active against viruses other than herpes simplex.

herpesvirus; antiviral compounds; antibiotics; viral inhibitors

Introduction

The development of new compounds with antiviral activity in the last few years has given rise to the so-called second generation of antiviral compounds [5,6]. In addition to a high antiviral potency, some of these new agents show little, if any, toxicity. Thus, acycloguanosine and bromovinyldeoxyuridine (BVdUrd) are among the antiherpes drugs with the highest activity to toxicity ratio [2,7]. Ribavirin is another promising, broad-spectrum antiviral agent with little toxicity for experimental animals [11]. The need for a continuing search of antiviral agents is evident considering the fact that herpes simplex type 1 (HSV-1) mutants might arise during acycloguanosine treatment [4,8,13]. Also the search for a compound with a wider antiviral spectrum should be pursued.

In an attempt to identify new compounds with antiherpes activity we have tested a

* Presented at the International Symposium on Antiviral Compounds, Universitäts-Augenklinik Eppendorf, Hamburg, F.R.G., June 13–16, 1983.

number of natural and synthetic compounds, including antibiotics with a yet unknown mode of action [12,15]. The activity of previously described antiviral compounds is given for comparative purposes.

Materials and Methods

Cells and viruses

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% newborn calf serum (Gibco). HSV-1 (KOS) was grown in Vero cells and titered by the standard plaque assay method in Vero cells.

Measurement of protein synthesis

0.5 ml of methionine-free medium and 0.11 μCi of [^{35}S]methionine (The Radiochemical Centre, Amersham, 1100 Ci/mmol) were added to the cells for 1 h. The medium was then removed, and the cells were washed with PBS solution and precipitated with 5% trichloroacetic acid. After 5 min, the trichloroacetic acid was removed and the cell monolayer washed 3 times with ethanol, dried under an infrared lamp and dissolved with 250 μl 0.1 N NaOH plus 1% SDS. 125 μl were counted in an Intertechnique scintillation spectrometer.

Estimation of the cytopathic effect

HeLa cell monolayers were infected with HSV-1 (KOS) at 0.2–0.5 plaque-forming units (PFU) cell, in the presence of the indicated concentrations of the compound. After 48 h, the cytopathic effect was examined under a phase-contrast microscope.

Results and Discussion

Systems to evaluate the antiviral effects of compounds

Several systems have been developed to assay antiviral activity [10]. These systems make use of whole animals, explanted organs or tissues, culture cells, cell-free systems and even purified enzymes [3,9]. The most obvious approach in the search for new antiviral agents starts with their analysis in cultured cells, followed by a further exploration of their efficacy in experimental animals, their activity spectrum and mechanism of action.

We have used a cell culture system to detect new antiherpes compounds. The system is based on the infection of HeLa cell monolayers with HSV-1 at a low multiplicity of infection (0.2–0.5 PFU/cell). The test compound was added together with the virus and after 48 h of incubation the cytopathic effect was recorded upon examination with a phase-contrast microscope. The protein-synthesizing capacity of the cell monolayer was estimated by using a short pulse of [^{35}S]methionine as indicated in Materials and Methods.

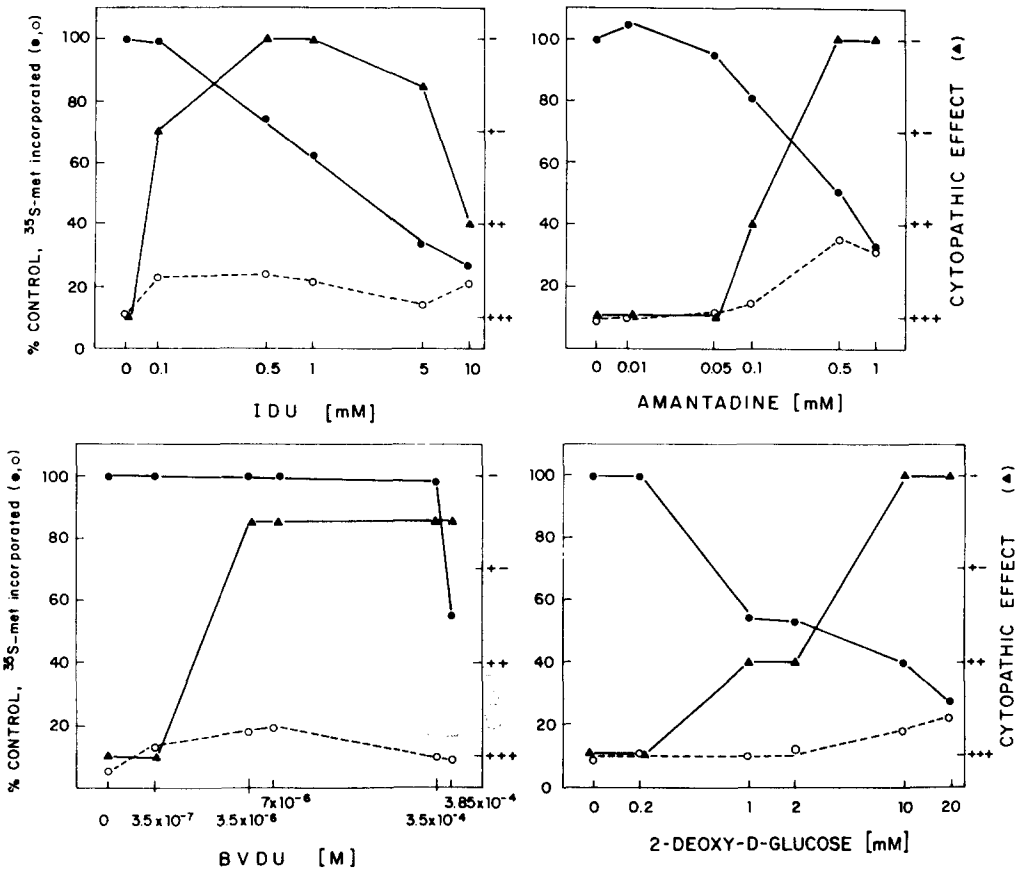

Fig. 1. Cytopathic effect (CPE) in HSV-1-infected HeLa cells, 48 h p.i. (▲). –, +–, ++, +++ represent CPE on a progressive scale, varying from no CPE (–) to maximum CPE (+++). Protein synthesis was measured as indicated in Materials and Methods, in HSV-1-infected cells (○) and uninfected control cells (●). BVDU, (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine); IDU, 5-iodo-2′-deoxyuridine (idoxuridine).

Effect of known antiviral agents

For comparative purposes we first analyzed the protective effects of several known antiviral agents on HeLa cells infected with HSV-1. These included iododeoxyuridine (IDU), amantadine, bromovinyldeoxyuridine (BVDU), 2-deoxy-D-glucose, glycyrrhizic acid, phosphonoformate, ribavirin, 5-trifluoromethyl-2′-deoxyuridine, acycloguanosine and vidarabine. The results are shown in Figs. 1 and 2. Some of these compounds showed a deleterious effect on uninfected control cells at the same concentrations that exhibited an antiviral effect. Thus, amantadine, 2-deoxy-D-glucose and vidarabine blocked translation in uninfected control cells at concentrations that protected the cell monolayer against HSV-1 infection. However, other compounds inhibited viral cytopathogenicity at concentrations which were not inhibitory to protein synthesis in uninfected cells. Table 1 presents the concentrations of these

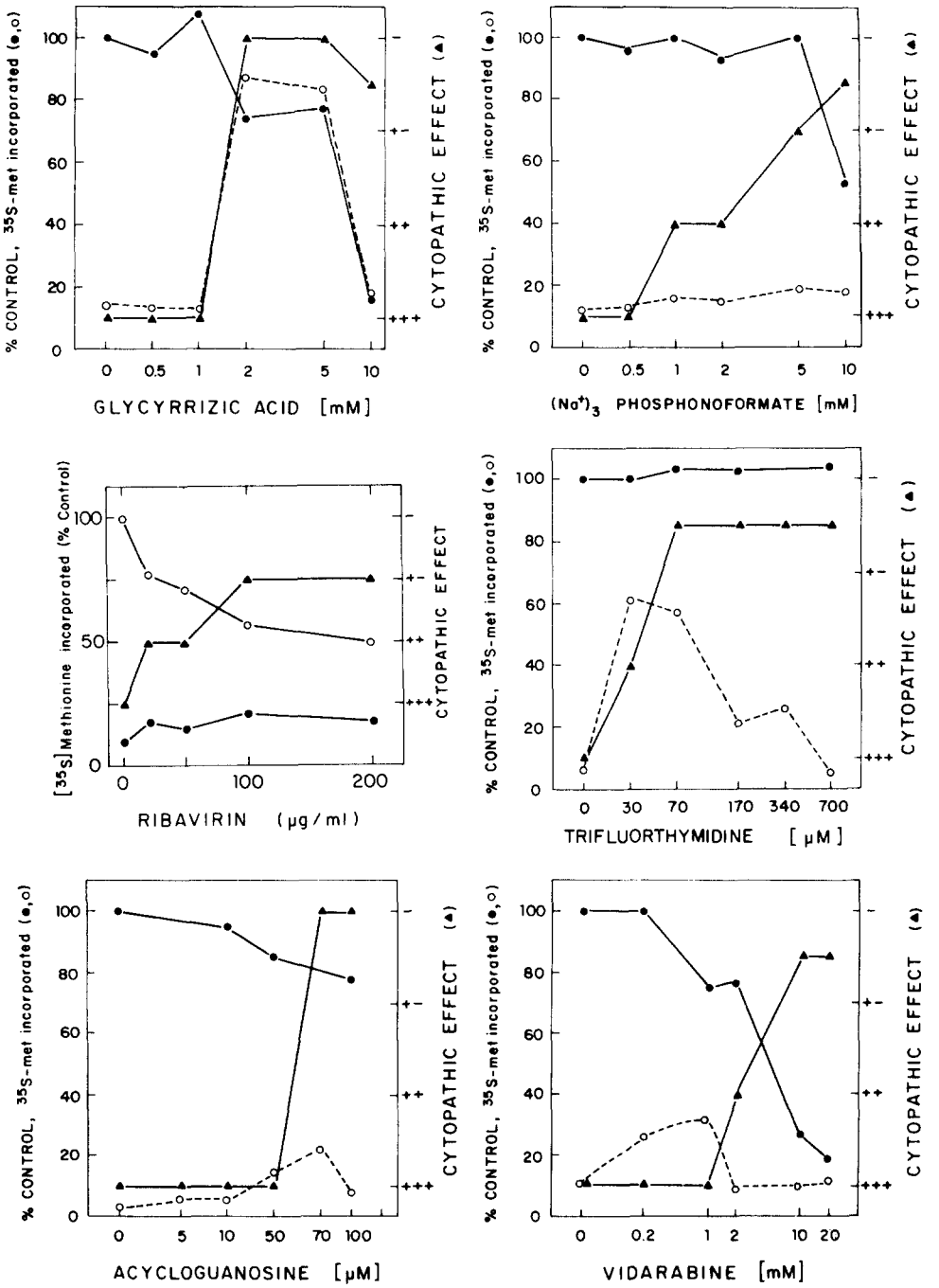

Fig. 2. Cytopathic effect in HSV-1-infected HeLa cells, 48 h p.i. Viral CPE and protein synthesis were determined as indicated in the legend to Fig. 1.

TABLE 1

Reference compounds with antiherpetic activity

Name	TOX 50 (mM)	CPE 50 (mM)
Acycloguanosine	10	0.06
Amantadine	0.5	0.2
BVdUrd	0.4	0.001
Cytarabine	20	5
2-Deoxy-D-glucose	5	5
Glycyrrhizic acid	8	1.5
IDU	5	5
Phosphonoformate	10	4
Ribavirin	0.8	0.3
Trifluorothymidine	0.7	0.05
Vidarabine	5	5

TOX 50, Concentration of the compound that caused a 50% inhibition of protein synthesis in uninfected HeLa cells after 48 h incubation.
CPE 50, Concentration of the compound that conferred a 50% protection of the cytopathic effect induced by HSV-1 infection after 48 h incubation.

compounds that conferred a 50% protection of the cytopathic effect, as well as the concentration that caused a 50% inhibition of protein synthesis in uninfected control cells. BVdUrd and acycloguanosine had the highest selectivity index. BVdUrd showed activity at 0.001 mM and acycloguanosine was active at a 60-fold higher concentration.

Screening of new compounds with antiherpes activity

In an attempt to find new compounds with antiherpes activity we analyzed a number of natural substances for their protective effect of HeLa cell monolayers against HSV-1 infection. The toxic effects for uninfected control HeLa cells were also recorded. The compounds that did not exhibit protection and the concentration at which they were tested are listed in Table 2. Some compounds previously reported to possess antiviral activity, such as fatty acids, sinefungin, prostaglandins and glucosamine, did not show a significant inhibition of viral cytopathic effects in our assay. Many of these agents proved quite cytotoxic but none showed antiviral activity at non-toxic concentrations. It is not excluded that some of the compounds listed in Table 2 and devoid of significant antiherpes activity could exhibit an antiviral effect if assayed in a different system or with a different virus.

The compounds which we found active against HSV-1 in our screening system are listed in Table 3. Some of them, such as aabomycin, actinobolin, aquayamycin, formycin A and P3355, showed protection at concentrations that clearly blocked protein synthesis in uninfected cells. Others showed a rather interesting selectivity since they were active at concentrations that were not harmful to the control cells (Table 3).

Figures 3 and 4 show the effect of amicetin, megalomycin C, laspartomycin, atropine, carrageenan, tetracenomycin, suramin, and 9-methylstreptimidone on pro-

TABLE 2

Compounds which proved inactive against HSV-1 in our screening system

Name	Range of concentrations tested	TOX 50
A 19009	10 - 200 µg/ml	> 200 µg/ml
AB 74	0.1 - 500 µg/ml	100 µg/ml
Aceclidine	10 - 400 µg/ml	300 - 400 µg/ml
Acetomycin	10 - 200 µg/ml	10 - 20 µg/ml
Aconitine	5 - 100 µg/ml	25 µg/ml
Acromycin	10 - 200 µg/ml	<20 µg/ml
Actinonin	10 - 200 µg/ml	-
Adenomycin	10 - 200 µg/ml	> 200 µg/ml
Adonitol	10 - 200 µg/ml	> 200 µg/ml
ADP-S	0.1 - 1 mM	>1 mM
Ajmaline	10 - 200 µg/ml	-
Aldgamycin	10 - 200 µg/ml	> 200 µg/ml
Allopurinol	10 - 200 µg/ml	100 µg/ml
Althiomycin	10 - 200 µg/ml	
D-Altrose	10 - 200 µg/ml	> 200 µg/ml
Amiclenomycin	10 - 200 µg/ml	200 µg/ml
4-Amino-4-deoxytrehalose	10 - 400 µg/ml	> 400 µg/ml
o-Aminophenyl β-D-glucuronide	10 - 200 µg/ml	> 200 µg/ml
Amipurimycin	0.1 - 100 µg/ml	1 µg/ml
Amphetamine sulfate	10 - 200 µg/ml	-
Amphotericin B	10 - 200 µg/ml	25 µg/ml
AMP-S	0.1 - 1 mM	>1 mM
Angolamycin	0.1 - 100 µg/ml	100 µg/ml
Anguidine	10 - 200 µg/ml	<10 µg/ml
Angustimycin A	5 - 200 µg/ml	> 200 µg/ml
Anhydroerythromycin A	10 - 100 µg/ml	> 200 µg/ml
Anisomycin	10 - 200 µg/ml	-
Antiamoebin	10 - 200 µg/ml	20 - 50 µg/ml
Apramycin	10 - 200 µg/ml	> 200 µg/ml
D-Arabitol	10 - 200 µg/ml	> 200 µg/ml
Arachidonic acid	10 - 200 µg/ml	50 - 100 µg/ml
Arecoline	10 - 200 µg/ml	-
Argininosuccinic acid	10 - 200 µg/ml	> 200 µg/ml
DL-Arterenol	10 - 200 µg/ml	75 µg/ml
Aspartocin	10 - 200 µg/ml	-
ATP-S	0.01 - 1 mM	>1 mM
Aureofungin	10 - 200 µg/ml	<10 µg/ml
Axenomycin B	1 - 100 µg/ml	> 100 µg/ml
Axenomycin D	0.1 - 200 µg/ml	0.5 µg/ml
Baciphelacin	0.1 - 100 µg/ml	2 µg/ml
Bacitracin	10 - 200 µg/ml	-
Berberine hydrochloride	10 - 200 µg/ml	<10 µg/ml
Berninamycin	10 - 200 µg/ml	-
Betulin	10 - 200 µg/ml	-
Bicyclomycin	2 - 200 µg/ml	> 200 µg/ml
Bleomycin A_2	0.1 - 200 µg/ml	> 200 µg/ml

TABLE 2

Compounds which proved inactive against HSV-1 in our screening system

Name	Range of concentrations tested	TOX 50
Boldine	10 - 200 µg/ml	-
Borneol	10 - 200 µg/ml	-
Brucine sulfate	10 - 200 µg/ml	-
BU-1709 A_1	0.1 - 100 µg/ml	> 100 µg/ml
BU-1709 A_2	0.1 - 100 µg/ml	> 100 µg/ml
BU-1709 E_1	0.1 - 100 µg/ml	10 µg/ml
Caffeine	10 - 200 µg/ml	50 - 100 µg/ml
Endo-3-bromo-D-camphor	10 - 200 µg/ml	50 - 100 µg/ml
Carboxymethylcellulose	10 - 200 µg/ml	-
Cardiolipin	8 - 160 µg/ml	> 160 µg/ml
Carminomycin	1 - 200 µg/ml	<1 µg/ml
β-Carotene	10 - 200 µg/ml	> 200 µg/ml
Celesticetin	1 - 200 µg/ml	50 µg/ml
α-Cellulose	10 - 200 µg/ml	-
Cellulose phosphate	10 - 200 µg/ml	-
Ceramides from cerebrosides	10 - 200 µg/ml	> 200 µg/ml
Ceramides from sphingomyelin	10 - 200 µg/ml	200 µg/ml
Chaetocin	10 - 200 µg/ml	200 µg/ml
Chalcomycin	10 - 200 µg/ml	-
Chartreusin	10 - 200 µg/ml	-
Chitin	10 - 200 µg/ml	> 200 µg/ml
Chlorogenic acid	10 - 200 µg/ml	-
Chlorothricin	10 - 200 µg/ml	100 µg/ml
Chlorotetracycline	0.1 - 10 mM	0.1 mM
Cholesterol arachidate	10 - 200 µg/ml	150 µg/ml
Cholesterol hemisuccinate	10 - 200 µg/ml	> 200 µg/ml
Cholesterol methylcarbonate	10 - 200 µg/ml	> 200 µg/ml
Chondroitin sulfate type C	10 - 200 µg/ml	-
Cinchonidine	10 - 200 µg/ml	-
Clindamycin	1 - 100 µg/ml	> 100 µg/ml
Corticosterone	1 - 10 µg/ml	>10 µg/ml
Cortisone	1 - 10 µg/ml	>10 µg/ml
Crotonic acid	10 - 200 µg/ml	> 200 µg/ml
Cyanein	10 - 200 µg/ml	<10 µg/ml
Cyclandelate	10 - 400 µg/ml	20 - 100 µg/ml
Cycloheximide	10 - 200 µg/ml	100 µg/ml
DAN 1701	10 - 200 µg/ml	-
Danomycin	1 - 100 µg/ml	> 100 µg/ml
Decoyinin	5 - 200 µg/ml	200 µg/ml
Desdanine	10 - 200 µg/ml	-
Deoxyerythromycin B	10 - 200 µg/ml	-
Dextomycin A	0.01 - 0.1 mM	>0.1 mM
Digitonin	0.1 - 2 µg/ml	0.15 µg/ml
Dihydroxyantraquinone	10 - 200 µg/ml	-
Dihydroerythromycin A	10 - 200 µg/ml	-
1,3-Dipalmitin	10 - 200 µg/ml	50 - 100 µg/ml
Diumycin A	0.1 -1000 µg/ml	1000 µg/ml

TABLE 2

Compounds which proved inactive against HSV-1 in our screening system

Name	Range of concentrations tested	TOX 50
Diumycin B	0.1 -1000 µg/ml	>1000 µg/ml
Doxycycline	0.01 - 0.1 mM	>0.1 mM
Efrotomycin	5 - 200 µg/ml	20 µg/ml
Elaiofilin	10 - 200 µg/ml	<10 µg/ml
Enduracidin	10 - 200 µg/ml	-
Enterocin	2 - 100 µg/ml	> 100 µg/ml
Ergosterol	10 - 200 µg/ml	> 200 µg/ml
Ergotamine tartrate	10 - 200 µg/ml	-
Eritronolide B	10 - 200 µg/ml	-
Erythromycin A	10 - 200 µg/ml	> 200 µg/ml
Erythromycin B	10 - 200 µg/ml	> 200 µg/ml
Erucic acid	10 - 200 µg/ml	75 µg/ml
β-Escin	10 - 200 µg/ml	-
Esculin	10 - 200 µg/ml	-
Everninomycin C	10 - 200 µg/ml	200 µg/ml
Flammulin	5 - 200 µg/ml	30 µg/ml
Formycins	5 - 200 µg/ml	<5 µg/ml
Fortimycin A	5 - 200 µg/ml	> 200 µg/ml
Fosfomycin	10 - 200 µg/ml	200 µg/ml
Fumagillin	5 - 200 µg/ml	20 µg/ml
Funiculosin	0.1 - 200 µg/ml	0.5 µg/ml
Fusidic acid	10 - 200 µg/ml	-
G-52	1 - 200 µg/ml	> 200 µg/ml
G-418	0.1 - 200 µg/ml	> 200 µg/ml
Gangliosides	10 - 200 µg/ml	> 200 µg/ml
Gardimycin	5 - 200 µg/ml	> 200 µg/ml
Gelonin	0.1 - 10 µg/ml	0.5 - 1 µg/ml
Gossypol	0.001- 0.1 mM	0.01 mM
D-Glucosamine	0.1 - 20 mM	5 mM
D-Glucuronic acid	10 - 200 µg/ml	200 µg/ml
Griseofulvin	10 - 200 µg/ml	75 µg/ml
Griseoviridin	10 - 200 µg/ml	-
7-Methylguanosine	50 µM- 5 mM	>5 mM
7^{m}-GTP	50 µM- 5 mM	>5 mM
Herbicidin A	1 - 100 µg/ml	100 µg/ml
Hexamethylene tetraamine	10 - 200 µg/ml	-
Hikizimycin	10 - 200 µg/ml	-
Hydrastine	10 - 400 µg/ml	10 - 20 µg/ml
8-Hydroxyquinoline-glucuronide	10 - 200 µg/ml	-
6-Hydroxyuridine	10 - 200 µg/ml	200 µg/ml
Isoniazid	10 - 200 µg/ml	
JI-20A	1 - 100 µg/ml	> 100 µg/ml
JI-20B	1 - 100 µg/ml	> 100 µg/ml
Josamycin	10 - 200 µg/ml	-
Kirromycin	10 - 100 µg/ml	-
Kitasamycins	5 - 200 µg/ml	20 µg/ml
Largomycin F-II	0.1 - 200 µg/ml	5 µg/ml

TABLE 2

Compounds which proved inactive against HSV-1 in our screening system

Name	Range of concentrations tested	TOX 50
Lincomycin	1 - 100 µg/ml	> 100 µg/ml
Linoleic acid	10 - 200 µg/ml	100 - 200 µg/ml
Linolenyl alcohol	10 - 200 µg/ml	25 µg/ml
Linoleyl alcohol	10 - 200 µg/ml	25 µg/ml
Lividomycin A	10 - 200 µg/ml	200 µg/ml
Lividomycin B	10 - 200 µg/ml	200 µg/ml
LL-BM-123α	10 - 200 µg/ml	100 µg/ml
α-L-Lysophosphatidylcholine dodecyl	10 - 200 µg/ml	150 µg/ml
M-4365 A_1	10 - 200 µg/ml	-
Melezitose	10 - 200 µg/ml	-
Metixene chlorohydrate	10 - 200 µg/ml	-
Metronidazole	10 - 200 µg/ml	-
6-MFA	0.1 -1500 µg/ml	250 µg/ml
Minimycin	0.1 -1000 µg/ml	100 µg/ml
Mitogillin	0.1 - 1 mM	1 mM
Monoolein	10 - 200 µg/ml	75 µg/ml
Monopalmitin	10 - 200 µg/ml	150 µg/ml
Myomycin	10 - 200 µg/ml	> 200 µg/ml
Nebramycin	10 - 200 µg/ml	> 200 µg/ml
Negamycin	0.1 - 200 µg/ml	20 µg/ml
Neohesperidine dihydrochalcone	10 - 200 µg/ml	-
Neospiramycin	10 - 200 µg/ml	-
Nicotine	10 - 200 µg/ml	-
Nifitricin A	10 - 200 µg/ml	20 µg/ml
Nifitricin B	10 - 200 µg/ml	<10 µg/ml
Nikkomycin	10 - 200 µg/ml	> 200 µg/ml
Nisaplin	10 - 200 i.u./ml	-
Nisin	10 - 200 µg/ml	> 200 µg/ml
2-Nitroimidazole	10 - 200 µg/ml	-
Nocardicin	2 - 100 µg/ml	> 100 µg/ml
Nogalamycin	10 - 200 µg/ml	<10 µg/ml
Novobiocin	10 - 200 µg/ml	10 µg/ml
Nucleocidin	10 - 100 µg/ml	75 µg/ml
Oleandomycin	10 - 200 µg/ml	200 µg/ml
Ouabain	1 - 200 µg/ml	<1 µg/ml
Oudenone	10 - 200 µg/ml	200 µg/ml
Oxamicetin	0.1 - 500 µg/ml	500 µg/ml
Oxytetracycline	0.1 - 10 mM	0.1 mM
Paromomycin sulfate	10 - 200 µg/ml	-
Pentazocine	10 - 200 µg/ml	-
α-L-Phosphatidylcholine dilauroyl	10 - 200 µg/ml	> 200 µg/ml
α-L-Phosphatidylethanolamine	10 - 200 µg/ml	> 200 µg/ml
α-L-Phosphatidyl L-serine	10 - 200 µg/ml	> 200 µg/ml
Phospho L-arginine	10 - 200 µg/ml	> 200 µg/ml
Pikromycin	0.25 - 100 µg/ml	1 - 10 µg/ml
Pipemidic acid	10 - 200 µg/ml	200 µg/ml
Pirrolnitrin	10 - 200 µg/ml	-

TABLE 2

Compounds which proved inactive against HSV-1 in our screening system

Name	Range of concentrations tested	TOX 50
Platenomycin A_1	10 - 200 µg/ml	-
Platomycin A	0.1 - 100 µg/ml	> 100 µg/ml
Platomycin B	0.1 - 100 µg/ml	> 100 µg/ml
Pokeweed antiviral protein	0.1 - 100 µg/ml	50 µg/ml
Polygalacturonic acid	10 - 200 µg/ml	-
Poly-L-glutamic acid	10 - 200 µg/ml	-
Polioxin complex	10 - 200 µg/ml	150 µg/ml
Polymyxin B	10 - 200 µg/ml	-
Potato starch	10 - 200 µg/ml	-
Prostaglandin A_1	0.5 - 4 µg/ml	>4 µg/ml
Prostaglandin E_1	0.5 - 4 µg/ml	1.5 µg/ml
Pyrazofurin	1 - 200 µg/ml	100 µg/ml
Quinine hydrochloride	10 - 200 µg/ml	-
Raffinose	10 - 200 µg/ml	-
Reserpine	10 - 200 µg/ml	-
Restrictocin	0.1 - 1 mM	>1 mM
Retinal (*trans*)	10 - 200 µg/ml	100 µg/ml
Retinol (*trans*)	10 - 200 µg/ml	100 µg/ml
α-Sarcin	5 - 100 µg/ml	<5 µg/ml
Sarkomycin	10 - 200 µg/ml	-
Seldomycin F-1	0.1 - 100 µg/ml	> 100 µg/ml
Seldomycin F-2	0.1 - 100 µg/ml	100 µg/ml
Showdomycin	10 - 200 µg/ml	-
Sinefungin	10 - 200 µg/ml	100 µg/ml
Siomycin A	10 - 200 µg/ml	-
Sodium palmitate	10 - 200 µg/ml	50 - 100 µg/ml
Sordarin	10 - 200 µg/ml	200 µg/ml
(-)-Sparteine sulfate	10 - 200 µg/ml	-
Spectinomycin	10 - 200 µg/ml	-
Spiramycin III	1 - 100 µg/ml	> 100 µg/ml
Steffimycin B	10 - 200 µg/ml	-
Streptovitacin A	10 - 200 µg/ml	-
Talaron	0.1 - 100 µg/ml	5 µg/ml
Taurocholic acid	10 - 200 µg/ml	> 200 µg/ml
Telomycin	10 - 200 µg/ml	-
Termorubin	10 - 200 µg/ml	100 - 200 µg/ml
Tetracycline	0.1 - 10 mM	5 mM
D-Tetranoline	10 - 200 µg/ml	-
Thiocillin II	0.25 - 100 µg/ml	0.25 µg/ml
Thiocillin III	0.1 - 100 µg/ml	0.25 µg/ml
Thiopeptin A_1	10 - 200 µg/ml	-
Thiopeptin B	10 - 200 µg/ml	-
Thioproline	10 - 200 µg/ml	200 µg/ml
4-Trehalosamine	10 - 200 µg/ml	200 µg/ml
Trichomycin	10 - 200 µg/ml	10 µg/ml
Trimethoprim	25 - 300 µg/ml	50 - 100 µg/ml
TSK-VI	10 - 200 µg/ml	-

TABLE 2

Compounds which proved inactive against HSV-1 in our screening system

Name	Range of concentrations tested	TOX 50
Tsushimycin	10 - 200 µg/ml	-
Turimycin h complex	10 - 200 µg/ml	-
Tuberactin	10 - 200 µg/ml	< 10 µg/ml
Tubercidin	10 - 200 µg/ml	200 µg/ml
Undecylenic acid	10 - 200 µg/ml	-
Venturicidin	0.1 - 100 µg/ml	0.75 µg/ml
Verdamycin	10 - 200 µg/ml	> 200 µg/ml
Vernamycin A	10 - 200 µg/ml	-
Vincamine	10 - 200 µg/ml	> 200 µg/ml
Vindoline	10 - 200 µg/ml	-
Virginiamycin	10 - 200 µg/ml	-
Viridogrisein	10 - 200 µg/ml	-
Xerosin	10 - 200 µg/ml	> 200 µg/ml
Ya-56-X	10 - 200 µg/ml	200 µg/ml
Ya-56-Y	10 - 200 µg/ml	200 µg/ml
Yohimbine hydrochloride	10 - 200 µg/ml	-

TOX 50: Concentration of the compound that caused a 50% inhibition of protein synthesis in uninfected HeLa cells after 48 h incubation.

Note: For details of the structural formulae and origin of the antibiotics listed, the reader is referred to Refs. 12 and 15.

TABLE 3

Compounds which showed antiherpetic activity in our screening system

Name	TOX 50	CPE 50
Aabomycin A	<15 µM	23 µM
Actinobolin	167 µM	67 µM
Amicetin	78 µM	16 µM
Aquayamycin	43 µM	51 µM
Atropine	>1.3 mM	0.5 mM
Bamicetin	>331 µM	25 µM
Carrageenan	>200 µg/ml	<10 µg/ml
Formycin A	54 µM	217 µM
Laspartomycin	>110 µM	44 µM
Megalomycin C	100 µM	70 µM
Pleuromutilin	>530 µM	40 µM
P3355	36 µM	216 µM
Sodium alginate	>200 µg/ml	75 µg/ml
9-Methyl-streptimidone	195 µM	49 µM
Suramin	>140 µM	17 µM
Tetracenomycin C	212 µM	32 µM
Trypan blue	-	15 µM

CPE 50 and TOX 50 are as described in the footnote to Table 1.

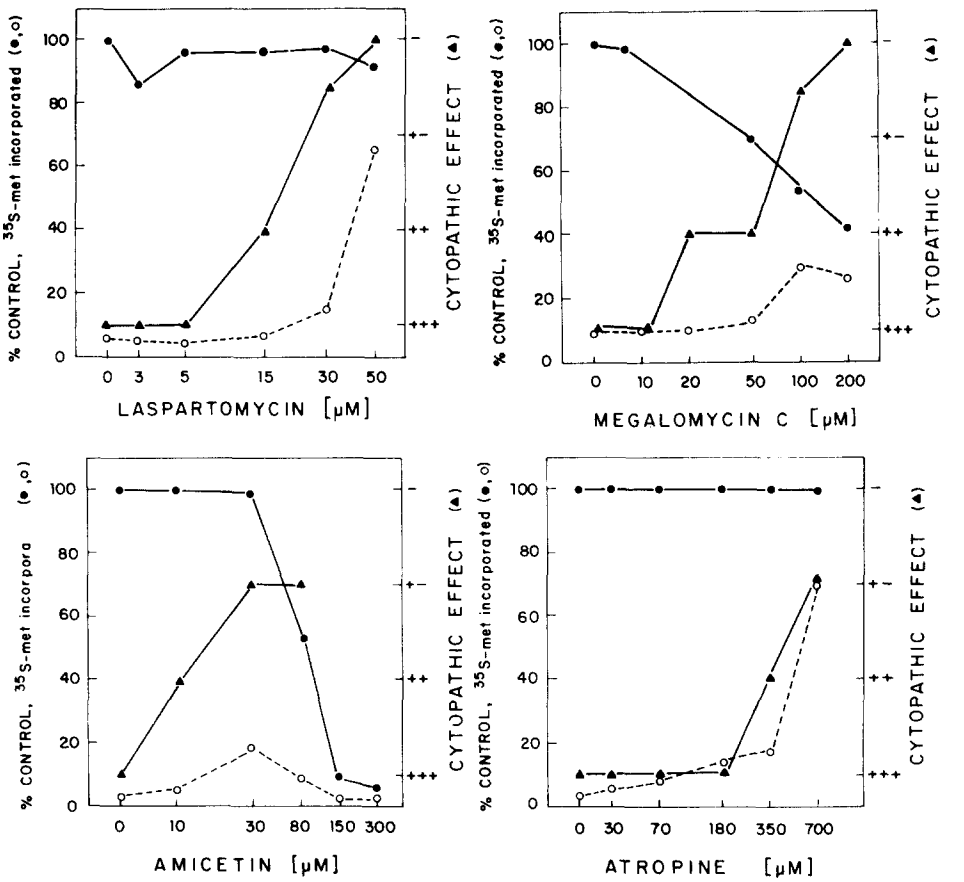

Fig. 3. Cytopathic effect in HSV-1-infected HeLa cells, 48 h p.i. Viral CPE and protein synthesis were determined as indicated in the legend to Fig. 1.

tein synthesis in uninfected control cells and on the cytopathic effects of HSV-1 for Hela cells, following the assay methods described in Materials and Methods. Again, for several compounds, i.e., amicetin, laspartomycin, carrageenan and suramin, a reduction of viral cytopathogenicity is observed at concentrations not affecting cellular protein synthesis.

Antiviral spectrum of the new antiherpes agents

Preliminary studies on the antiviral spectrum of some of the new antiherpes agents have begun. Table 4 illustrates that these compounds acted on both DNA and RNA viruses, including HSV-1, VSV (vesicular stomatitis virus), SFV (Semliki Forest virus), polio and EMC (encephalomyocarditis) virus. Some compounds were active against poliovirus but inactive against EMC virus and vice versa. More detailed studies on the antiviral spectrum of these compounds are being carried out at present. The molecular mechanism of action of these compounds is also subject to further study.

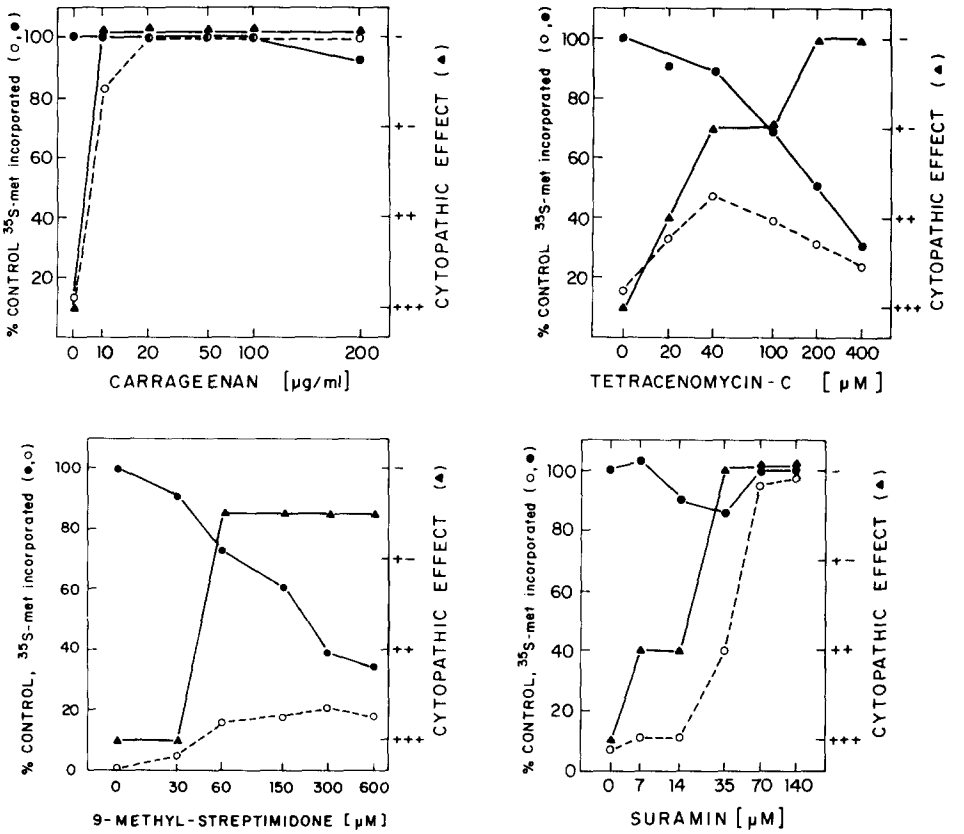

Fig. 4. Cytopathic effect in HSV-1-infected HeLa cells, 48 h p.i. Viral CPE and protein synthesis were determined as indicated in the legend to Fig. 1.

TABLE 4

Antiviral spectrum of some compounds

Compound	HSV-1	VSV	SFV	polio	EMC
Actinobolin	+	+	+	+	+
Amicetin	+	+	+	+	+
Atropine	+	+	+	+	-
Carrageenan	+	-	+	-	+
Formycin A	+	+	+	-	-
Laspartomycin	+	nd	+	-	nd
Megalomycin C	+	+	+	-	-
Pleuromutilin	+	+	-	-	-
Suramin	+	+	+	-	+
Tetracenomycin C	+	+	+	+	+

+, antiviral effect; -, no effect; nd, not determined.

Acknowledgements

The expert technical assistance of M.J. Norte, M.E. González and M.A. Ramos is gratefully acknowledged. This work was supported by grants from CAICYT and Plan Concertado de Investigación.

References

1 Bauer, D.J. (1977) The Specific Treatment of Virus Diseases. University Park Press, Baltimore, MD.
2 Bauer, D.J. (1980) Laboratory studies on acyclovir. In: Developments in Antiviral Therapy. Collier, L.H. and Oxford, J. (eds.), Academic Press, London, pp. 43–51.
3 Chandra, P., Ebener, U. and Gericke, D. (1979) Molecular mechanisms for control of RNA tumor viruses. In: Antiviral Mechanisms in the Control of Neoplasia. Chandra, P. (ed.), Plenum Press, New York, NY, pp. 523–537.
4 Darby, G., Field, H.J. and Salisbury, S.A. (1981) Altered substrate specificity of herpes simplex virus thymidine kinase confers acyclovir-resistance. Nature 289, 81–83.
5 De Clercq, E. (1981) Nucleoside analogues as antiviral agents. Acta Microbiol. Acad. Sci. Hung. 28, 289–306.
6 De Clercq, E. (1982) Specific targets for antiviral drugs. Biochem. J. 205, 1–13.
7 De Clercq, E., Descamps, J., De Somer, P., Barr, P.J., Jones, A.S. and Walker, R.T. (1979) (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine: a potent and selective anti-herpes agent. Proc. Natl. Acad. Sci. U.S.A. 76, 2947–2951.
8 Field, H.J. (1982) Development of clinical resistance to acyclovir in herpes simplex virus-infected mice receiving oral therapy. Antimicrob. Agents Chemother. 21, 744–752.
9 Goswami, B.B., Crea, R., Van Boom, J.H. and Sharma, O.K. (1982) 2′,5′-linked oligo(adenylic acid) and its analogs. A new class of inhibitors of mRNA methylation. J. Biol. Chem. 257, 6867–6870.
10 Grunert, R.R. (1979) Search for antiviral agents. Annu. Rev. Microbiol. 33, 335–353.
11 Hillyard, I.W. (1980) The preclinical toxicology and safety of ribavirin. In: Ribavirin, a Broad Spectrum Antiviral Agent. Smith, R.A. and Kirkpatrick, W. (eds.), Academic Press, London, pp. 59–71.
12 Korzybski, T., Kowszyk-Gindifer, Z. and Kurylowicz, W. (1978) Antibiotics: Origin, Nature and Properties. American Society for Microbiology, Washington, DC.
13 Parris, D.S. and Harrington, J.E. (1982) Herpes simplex virus variants resistant to high concentrations of acyclovir exist in clinical isolates. Antimicrob. Agents Chemother. 22, 71–77.
14 The Merck Index (1976) Windholz, M. (ed.), Merck & Co., Rahway, NJ.
15 Umezawa, H. (1978) Index of Antibiotics from Actinomycetes. Japan Scientific Societies Press, Tokyo, and University Park Press, Baltimore, MD.