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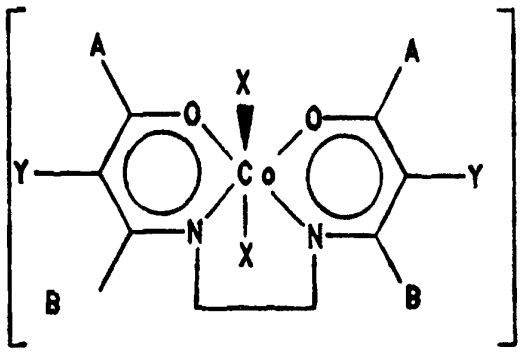
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(54) Title: ANTIVIRAL COMPOSITIONS AND METHOD FOR THEIR USE

(57) Abstract

The present invention provides a method for treating chorioretinal disease and viral diseases caused by human herpes virus type 6 by administering to a subject an anti-chorioretinal infection amount or an anti-HHV-6 virus infective amount of a compound having structure (II), wherein A is methyl or phenyl, Y is hydrogen or chlorine, Z is chlorine or bromine, X is ammonia or 2-methylimidazole, and B may be the same or different and each is hydrogen or an alkyl group.



(II)

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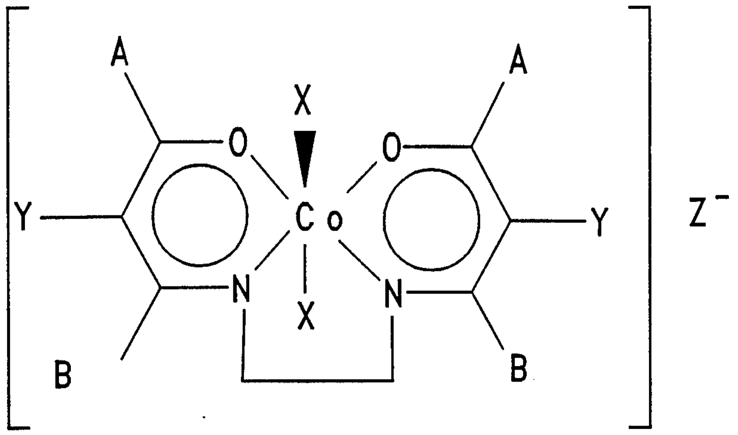
ANTIVIRAL COMPOSITIONS AND METHOD FOR THEIR USE

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. application Serial No. 07/803,259, filed December 5, 1991, which in turn, is a continuation-in-part of application Serial No. 502,294, filed March 30, 1990, which in turn is a continuation-in-part of U.S. application Serial No. 07/279,417, filed on December 2, 1988, now U.S. Patent 5,049,557 which, in turn, is a continuation-in-part of U.S. application Serial No. 07/147,713 and U.S. application Serial No. 07/147,714, both filed on January 25, 1988, now U.S. Patents 4,866,054 and 4,866,053, respectively, which, in turn, are continuation-in-parts of U.S. application Serial No. 06/862,804, filed on May 13, 1986, now abandoned. The contents of U.S. application Serial Nos. 502,294 and 06/862,804 and U.S. Patents 5,049,557, 4,866,054, and 4,866,053 are hereby incorporated by reference into the present disclosure.

BACKGROUND OF THE INVENTION

The present invention relates to metallo-organic cobalt compounds and their use in the treatment of subjects for conditions and diseases caused by viruses and viral infections. It has been discovered that certain conditions and diseases, e.g., inflammation, burns, wounds, and diseases caused by bacteria and fungi in mammalian species can be treated with certain complexes of cobalt having the structure:



I.

wherein each A may be the same or different and is an alkyl group, a phenyl group or a substituted derivative of a phenyl group;

wherein each Y may be the same or different and is hydrogen, an unbranched alkyl group, a halide or a group having the structure $R-\underset{\underset{O}{\|}}{C}-$ wherein R is hydrogen, an alkoxide group, an alkyl group, or OH;

wherein each B may be the same or different and each is hydrogen or an alkyl group;

wherein each X may be the same or different and each is a water soluble group having weak to intermediate ligand field strength; and

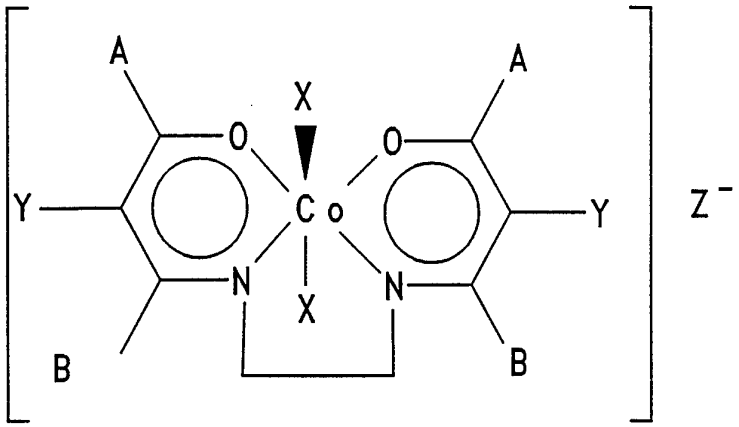
Z^- is a soluble, pharmaceutically acceptable negative ion.

Today, virus infections are known to be significant causes of morbidity and mortality in human and veterinary medicine. Many of these diseases are untreatable or the available therapies are not entirely satisfactory and only provide

minimal clinical response. For the most part, it is known that viral diseases do not respond to therapy with conventional antibiotics. Despite some recent successes in the development of antiviral chemical therapeutic agents, new treatments for these diseases are needed to improve the management of viral infections in clinical medicine.

Parent application Serial No. 502,294, referred to hereinabove, discloses the use of the foregoing described compounds as antiviral agents.

Parent application Serial No. 07/803,259, discloses compounds having the structure II



II

wherein

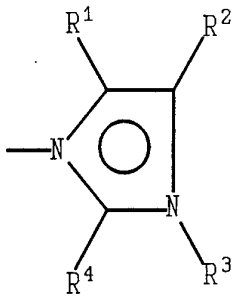
each A may be the same or different and is an alkyl group, a phenyl group or a substituted derivative of a phenyl group;

each Y may be the same or different and is hydrogen, an unbranched alkyl group, a halide or a group having the structure $R-\overset{\overset{O}{\|}}{C}-$ wherein R is hydrogen, an alkoxide group, an alkyl group, or OH;

each B may be the same or different and each is hydrogen or an alkyl group;

Z^- is a soluble, pharmaceutically acceptable negative ion, and

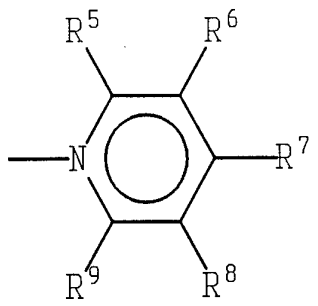
each X may be the same or different and is an axial ligand selected from the group consisting of moieties having the formula:



IIa

wherein R^1, R^2, R^3, and R^4 may be the same or different and may be selected from the group consisting of electron donating groups and electron withdrawing groups,

with the proviso that R^1, R^2, R^3, and R^4, are of a sufficiently small size so as not to prohibit the attachment of the axial ligand to the Co atom due to steric hindrance.



IIB

wherein R^5, R^6, R^7, R^8 and R^9 may be the same or different and may be selected from the group consisting of electron donating groups and electron withdrawing groups,

with the proviso that R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 are of a sufficiently small size so as not to prohibit the attachment of the axial ligand to the Co atom due to steric hindrance.

The above compounds have been found, as disclosed in the parent applications, to exhibit significant activity as antiviral agents. In particular, compounds of Formulas I and II can be used for treating viral infections as is or in a composition form, when combined with a pharmaceutically acceptable carrier. Depending upon the nature of the infection and the manner in which it manifests itself, the inventive antiviral compositions may be administered by using conventional modes of administration, e.g., oral, topical application, parenteral, intravitreal injection or intravitreal implant, and the like.

Research is ongoing to develop drugs having antiviral effects on newly discovered and/or recognized viruses and symptoms and diseases therefrom. For example, human herpesvirus type 6 (HHV-6) is a relatively newly discovered

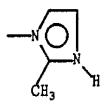
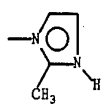
member of the herpesvirus family which includes cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus 1 and 2 (HSV-1,2), herpesvirus type-7 (HHV-7) and varicella zoster virus (VZV). It is known that primary infection with HHV-6 is a major cause of acute febrile illness (roseaola infantum) in young children (see Yamanishi et al, 1988, "Identification of human herpesvirus-6 as a causal agent for exanthem subitum", Lancet 1:1065-7; and Pruksananonda et al, 1992, "Primary human herpesvirus 6 infection in young children", New Engl. J.Med. 326(22:1445-1450). HHV-6 has been isolated from peripheral blood leukocytes of organ transplant patients as well as patients with acquired immunodeficiency syndrome (AIDS), and immunoproliferative disorders (see Agut et al, 1988, "Concomitant infection by human herpesvirus 6, HTLV-1 and HIV-2", Lancet I, 712; Carrigan et al, 1992, "Human herpesvirus 6 and bone marrow transplantation. IN: Biology Epidemiology, Molecular Biology, and Clinical Pathology of HHV-6", DV Ablashi and GRF Krueger eds. Amsterdam Elsevier Science Publishers, in press; Downing et al, 1987, "Isolation of human lymphotoropic herpesviruses from Uganda", Lancet II:390; Efstathiou et al, 1988, "DNA homology between a novel human herpesvirus (HHV-6) and human cytomegalovirus", Lancet II:63-64; Salahuddin et al, 1986, "Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders", Science 234:596-601; Tedder et al, 1987, "Novel lymphotrophic herpesvirus", Lancet II:390-392).

HHV-6 may be involved in lymphomas and sarcoidosis, in transient febrile illnesses, which occur in renal transplant patients having HHV-6 infections as well as with chronic fatigue syndrome (see Morris et al, 1989, "Human herpesvirus 6

infection in renal transplant recipients. Lancet 1:1560-1561; and Krueger et al, 1987, "Persistent fatigue and depression in patients with antibody to human B-lymphotrophic virus", Lancet 2:36).

SUMMARY OF THE INVENTION

We have now discovered that certain of the compounds disclosed in the parent application have significant effect in suppressing the replication and/or aborting the infective life cycle of HHV-6 virus and HCMV virus which can induce chorioretinal infection. In particular, we have discovered that compounds having the following structures may be effective for the treatment of these two viruses. In the following diagram, B is, in each case, methyl, and A, Y, X and Z- refer to those symbols as used in Structure II.

COMPOUND	Y	X	Z	A
23	H	$-NH_3$	Cl	$-CH_3$
67	Cl	$-NH_3$	Cl	$-CH_3$
96	H		Br	$-CH_3$
102	H		Cl	C_6H_5

The present invention provides both a method and composition for the treatment of patients with HCMV-induced chorioretinal infection and HHV-6 infection.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph of intravitreal therapy and chorioretinal disease severity for compositions of the invention.

Fig. 2 is a bar chart of chorioretinal disease severity for compounds 67, 96, placebo and DHPG

DETAILED DESCRIPTION OF THE INVENTION

EXAMPLE 1 Safety/Toxicity Evaluation

The safety and/or toxicity of the above-noted compounds in pigmented rabbits was evaluated after intravitreal injection of the compounds. The safety/toxicity was evaluated by clinical fundus (indirect ophthalmoscopic) observation as well as histological evaluation of the chorioretina. In this example, all dosages were 100 μl injections of the amount of compound indicated in sterile water.

Twelve pigmented rabbits were evaluated by slit lamp and indirect ophthalmoscopy to determine normal ocular morphology and absence of pre-existing pathology. The animals were then divided into four groups of three rabbits each, and each group was dosed intravitreally with the compound. In Groups 1 through 3, each rabbit received intravitreal injections of the compound (Compound 23, Compound 96,

and Compound 102). The right eye was injected with 0.1 mg and the left eye was injected with 1 mg using three separate injections on day 1, day 3, and day 5.

The fourth group of three rabbits received intravitreal injections of sterile water for both eyes on the same days.

The animals were maintained in individual cages and were monitored on days 1, 3, 5 and 7 post inoculation. On day 7 post inoculation, all animals were sacrificed, eyes enucleated and tissue samples processed for histochemistry.

Results

Indirect ophthalmoscopic examination of eyes injected with 1 mg of the compounds demonstrated retinal areas of blanching (whitening of the retina), mild optic nerve head hyperemia and edema by day 5 post injection (after 2 mid-vitreal compound injections).

Indirect ophthalmoscopic examination of eyes injected with a concentration of 0.1 mg did not indicate any disruption of the retina and the retina and optic nerve head were normal.

Indirect ophthalmoscopic examination of eyes after injection of sterile water showed no adverse affects to the intraocular structures.

The histopathy for eyes receiving 1 mg of the compound demonstrated moderate to severe retinal and choroidal toxicity. This toxicity was evident as partial to complete destruction of the inner retinal layers. The photoreceptor layer (rods and cones) also demonstrated moderate to severe pathology characterized by edema, cellular hyperplasia, loss of cellular elements and partial to complete disruption of the normal retinal lamellar architecture. The pathology was diffuse and occupied approximately 80-95% of retina. The choroid demonstrated pathology included vascular cuffing of blood vessels, edema, blood vessel engorgement and congestion. The histological analysis of the choroid and retinal tissues after the injection confirmed the clinical indirect ophthalmoscopic observations.

Eyes receiving injections of 0.1 mg of the compounds showed no retinal or choroidal pathology. The histological sections demonstrated normal retinal and choroidal architecture with normal retinal layers and cellular elements.

Eyes receiving placebo sterile water injections exhibited no retinal or choroid alterations.

The foregoing results show that low dose mid-vitreal injection of the compounds did not induce retinal or choroidal pathology. In contrast, high dose injection was toxic to the retina and choroid.

EXAMPLE 2 Treatment of HCMV-induced Chorioretinal Infection

Thirty pigmented rabbits were used and evaluated by slit lamp and indirect ophthalmoscopy to determine normal ocular morphology and lack of pre-existing pathology. On day zero, all rabbits were inoculated by mid-vitreal injection of 10^5 PFU of HCMV strain AD 169. Animals were maintained in individual cages and monitored for developing chorioretinal HCMV disease on day 2 post inoculation. On this day, the inoculated animals were divided into six groups with matched chorioretinal disease scores and thereafter, received intravitreal therapy as follows (in all cases, the dosages were by injection of 100 μl of the amount of compound indicated in sterile water):

Group 1 5 animals, Compound 23, intravitreal therapy (0.1 mg) on days 3, 5, and 7 PI;

Group 2 5 animals, Compound 96 intravitreal therapy (0.1 mg) on days 3, 5 and 7 PI;

Group 3 5 animals, Compound 67 intravitreal therapy (0.1 mg) on days 3, 5 and 7 PI;

Group 4 5 animals, Compound 102 intravitreal therapy (0.1 mg) on days 3, 5 and 7 PI;

Group 5 5 animals, DHPG intravitreal therapy (0.2 mg) on days 3, 5, and 7 PI;

Group 6 5 animals, placebo intravitreal therapy on days 3, 5, and 7 PI.

All animals were monitored daily by indirect ophthalmoscopic examination, these examinations being performed independently by two readers, blind as to the therapy the animals were receiving.

On day 9 post inoculation, all animals were sacrificed. Chorioretina and iris tissues and vitreous samples were removed and processed for HCMV recovery by cell sonicate assay on HFF cell monolayers. Selected tissue samples were processed for histochemistry to demonstrate HCMV-induced ocural pathology.

Results

Placebo treated animals received a single injection of sterile saline 48 hours post inoculation with 10^5PFU of HCMV. The eyes developed mild chorio-retinal and vitreous diseases by 2 days post inoculation. The disease consisted of focal areas of retinal infiltration, optic nerve inflammation and redness and mild vitritis. The vitritis consisted of vitreous strands and peripheral cellular infiltrates and cloudiness. The placebo therapy did not arrest the development of chorioretinal disease and vitritis in these animals.

The chorioretinal disease increased to the 2.5 to 3+ level by day 4 to 5 post inoculation. The chorioretinal disease developed into confluent areas of cellular infiltration with hemorrhage of the retinal veins and occasional retinal necrosis and detachment. Vitritis in these HCMV infected eyes developed to moderate to severe levels by day 5 post inoculation. After day 5 post inoculation, the vitritis obscured comprehensive evaluation of retinal and choroidal disease.

Histological evaluation of placebo-treated eyes demonstrated that HCMV infection had progressed from the inner retinal areas to involve the photoreceptor layer and occasionally resulted in retinal detachment. Histology demonstrated areas

of retinal edema, mixed cellular infiltration and occasional retinal detachment. Areas of extensive retinal HCMV disease involvement were next to areas of normal retina. Histology demonstrated moderate to extensive involvement of the choroid and retina.

HCMV was recovered from 2 out of 8 chorioretinal samples on day 9 post inoculation. The average titer of HCMV recovered from these chorioretinal samples was 10^1 to 10^2 PFU HCMV. The titers of HCMV recovered from these chorioretinal samples on day 8 post inoculation were relatively low compared to the input HCMV titer. HCMV recovery did correlate with the degree of chorioretinal disease that was observed clinically (e.g., the more severe chorioretinal disease state demonstrated high titers of HCMV in cell sonicate cultures).

The control therapy using DHPG began on day 3.5 post inoculation. All of the treated eyes had chorioretinal disease at a 0.5 to 1.25 level at the time of initiation of therapy. The dosage level was the conventional dosage of 0.2 mg. By day 5 post inoculation, the average involvement of HCMV chorioretinal disease in 70% of the treated eyes was stabilized and in some instances, was beginning to resolve. The chorioretinal disease remained predominantly focal with areas of retinal necrosis. The choroid remained congested through day 9 post inoculation. Vitritis in these eyes remained at moderate levels in 6 eyes and at higher levels (3-4+) in the remaining 4 eyes.

Histological evaluation confirmed the clinical indirect ophthalmoscopic examinations that moderate areas of the retina were involved in HCMV-induced

disease. The pathology was characterized by the presence of large areas of immune cell infiltrates, occasional retinal detachment and occasional hemorrhage. Hematoxylin and eosin staining demonstrated retinal destruction to the level of the mid-retina. The vitritis was moderate and was characterized by a mixed cellular infiltrate.

For Groups 1 through 4, administration of Compounds 23, 67, 96, and 102, respectively, were effective in reducing the development of HCMV-induced chorioretinitis and vitritis compared to animals with placebo therapy.

Compound 67 and 96 therapy appeared to be somewhat more effective than therapy with compounds 102 and 23, being comparable to DHPG intravitreal therapy. Histological evaluation of the Compound 67 and 96 treated chorioretinal samples confirmed the clinical indirect ophthalmoscopic observations. HCMV induced disease in the retina was restricted to focal sites of inflammatory cells and edema in the inner retinal areas. The destruction of the retina was confined predominantly to the area of the inner retina with non-involved areas of the retina being clearly demarcated from the infectious sites. The vitritis was mild with occasional areas of a more heavy vitritis and vitreous strands.

In contrast, chorioretinal disease treated with Compounds 102 and 23 did not remain focal and confined but was more geographic with greater optic nerve head redness and inflammation. Vitritis was moderate and remained in the 1.5 to 2.0+ range. Chorioretinal HCMV induced disease with these compounds increased in severity through day 5 post inoculation and then began to resolve.

The histological analysis of the eyes treated with Compounds 23 and 102 confirmed the clinical impressions of disease development and resolution.

HCMV recovery by cell sonicate assay is shown in Table 1. All single agent intravitreal therapies reduced the recovery of HCMV by cell sonicate culture on day 9 post inoculation compared to placebo therapy. It is noted that the recovery from normal placebo treated eyes on day 8 post inoculation is routinely low and in the range of 10 to 35% positive.

TABLE 1

THERAPY	HCMV-RECOVERY (% POSITIVE)
Compound 23	3/8
Compound 102	3/8
Compound 67	1/8
Compound 96	2/8
DHPG	1/8
Placebo	4/8

No ocular toxicity for any of these compounds including the DHPG (ganciclovir) was observed at these concentration. The threshold for the inventive compounds for ocular compound toxicity was in the range of 1 mg and was demonstrated only after a cumulative intravitreal dose of 3 mg (three separate intravitreal injections) had been administered. Thus, the therapeutic level for the inventive compounds is a minimum of 10 to 30 fold below the threshold toxic concentration.

It should also be noted that animals receiving therapy with inventive compounds by intravitreal injection exhibited a decreased level of vitritis (vitritis being the accumulation of host immune cells within the vitreous). This was not observed with the DHPG-treated and placebo-treated animals.

This indicates that the inventive compound therapy has an effect on the host immune response, i.e., an anti-inflammatory effect that decreases the vitritis. This is similar to the response observed with the same compounds for stromal disease in HSV corneal infection. The effect of these compounds on the inflammatory host immune response demonstrates a second mode of action in addition to the reduction in HCMV titers and clinical disease development. This immune response alteration was shown not only by a decrease in the levels of vitritis, but also, as retinal sparing. As this anti-inflammatory effect was not observed with the DHPG therapy, these compounds are advantageous for treatment of chorioretinal HCMV infection.

EXAMPLE 3

A series of experiments was carried out to evaluate the toxicity of compounds 23, 67, 96, and 102 on Molt-3 cells, as well as to determine the effect of the compounds on infectivity on HHV-6 *in vitro* and the replication of HHV-6 *in vitro*. Stock drug solutions of each of Compounds 23, 67, 96 (1 mg/ml), and 102 (0.25 mg/ml) were dissolved in water by stirring at room temperature (Compounds 67 and 102 required overnight stirring). Concentrations were confirmed by refractometer. The drug solutions were sterilized passing through a microfilter and used to make RPMI media with 10% fetal bovine serum. The stock media was serially

diluted in RPMI without drugs to obtain a final concentration as used below. Media pH with drugs was between 7.9 and 8.0.

The evaluation of the toxicity of the compounds was carried out by incubating 3 x 10^5 Molt-3 cells per well in triplicate in media with the drug concentrations at 35°C in a humidified CO_2 incubator. The effect of the compounds on the infectivity of HHV-6 was carried out by incubating 1.2 ml of HHV-6 (2 x 10^5 PFU/0.4 ml) with the indicated drug concentration at 37°C for 30 minutes. After incubation, 0.4 ml of drug treated virus was mixed with 3 x 10^5 Molt-3 cells in triplicate and incubated for 3 hours. After rehydration of the cultures with 4 ml of growth media containing the specific drug concentration, incubation was continued at 37°C in a humidified CO_2 incubator.

The effect of the compounds on the replication of HHV-6 *in vitro* was carried out by mixing 3 x 10^5 Molt-3 cells in triplicate with 0.4 ml of untreated virus (2 x 10^5 PFU/0.4 ml) followed by incubation for 3 hours. The cultures were rehydrated with 4 ml of growth media containing the appropriate drug concentration, and incubation continued at 37°C in a humidified CO_2 incubator.

All of the cultures were monitored microscopically to assess the cytopathic effect (CPE). The percentage of infected cells in each culture was also estimated microscopically.

At days 3 and 6, aliquots from each well were removed, cells collected by centrifugation, and plated onto clean coverslips and fixed in acetone. The fixed cells were subjected to immunofluorescence staining and polyclonal antisera to HHV-6. One hundred cells were counted for each coverslip and antigen positive cells were recorded as percent infected cells. The data for the evaluation of toxicity is set forth herein in Table 2. The results from the determination of the infectivity of HHV-6 are set forth in Table 3 and the results from the determination of the effect of the compounds on replication on HHV-6 are set forth in Table 4. The percentages reported are an average of triplicate values.

TABLE 2

EFFECT OF COMPOUNDS ON THE VIABILITY OF MOLT-3 CELLS IN CULTURE

Transition Metal Compound	% of Viable Molt-3 Cells											
	1.0 mg/ml		0.5 mg/ml		0.25 mg/ml		0.1 mg/ml		0.01 mg/ml		0.001 mg/ml	
	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6
Compound 23	0	0	0	0	10	0	50	0	95	95	95	95
Compound 67	0	0	0	0	0	0	25	0	50	30	95	95
Compound 96	0	0	0	0	10	0	50	0	95	95	95	95
Transition Metal Compound	0.125 mg/ml		0.05 mg/ml		0.025 mg/ml		0.01 mg/ml		0.001 mg/ml		0.0001 mg/ml	
	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6
Compound 102	0	0	50	50	95	95	95	95	95	95	95	95

Non drug treated cells were 95-100% viable throughout the incubation period

TABLE 3

EFFECT OF COMPOUNDS OF THE INFECTIVITY OF HHV-6 (VIRUCIDAL)

Transition Metal Compound	% of Infected Cells*											
	1.0 mg/ml		0.5 mg/ml		0.25 mg/ml		0.1 mg/ml		0.01 mg/ml		0.001 mg/ml	
	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6
Compound 23	0	0	0	0	10	0	0	0	30	40	75	80
Compound 67	0	0	0	0	0	0	0	0	0	0	30	40
Compound 96	0	0	0	0	0	0	0	0	50	70	75	80
Transition Metal Compound	0.125 mg/ml		0.05 mg/ml		0.025 mg/ml		0.01 mg/ml		0.001 mg/ml		0.0001 mg/ml	
	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6	day 3	day 6
Compound 102	0	0	50	50	80	90	95	95	95	99	99	99

Non drug treated virus infected cells showed 99% CPE at day 3 and day 6.

* % of infected cells was determined by immunofluorescence staining using polyclonal antisera to HHV-6

TABLE 4

EFFECT OF COMPOUNDS ON THE REPLICATION OF HHV-6 (ANTIVIRAL ACTIVITY)

Transition Metal Compound	% of Infected Cells *					
	0.5 mg/ml		0.1 mg/ml		0.001 mg/ml	
	day 3	day 6	day 3	day 6	day 3	day 6
Compound 23	0	0	0	0	60	70
Compound 67	0	0	0	0	30	30
Compound 96	0	0	0	0	50	60
Transition Metal Compound	0.05 mg/ml		0.01 mg/ml		0.001 mg/ml	
	day 3	day 6	day 3	day 6	day 3	day 6
Compound 102	50	50	85	90	90	95

Non drug treated virus infected cells showed 99% CPE at day 3 and day 6.

* % of infected cells was determined by immunofluorescence staining using polyclonal antisera to HHV-6

Each of the four compounds exhibited activity on the replication and infectivity of HHV-6, Z-29.

Compound 67 was observed to be the most potent on cellular growth and replication and infectivity. Compound 102 was the least effective, the order of activity being 67 > 96 > 23 > 102).

The estimated IC_{50} values are set forth below:

Drug	IC_{50}
Compound 23	0.01 mg/ml (28.5 μM)
Compound 67	0.001 mg/ml (2.5 μM)
Compound 96	0.01 mg/ml (18.1 μM)
Compound 102	0.05 mg/ml (82.6 μM)

With respect to the IC_{50} values for ACV, GCV and PFA were 59, 25 and 49, respectively, when tested on HHV-6 GS strain grown in HSB2 cells (T-lymphocytes) (Antimicrobial Agents and Chemotherapy, Dec. 1990, p. 2417). The CTC compounds were tested on HHV-6 Z-29 strain grown in Molt-3 cells (T-lymphocytes).

It is noted at 28.5 μM drug concentrations, the antivirals ACV, GCV and PFA, Compound 23 and Compound 96 had no toxic effects on uninfected Molt-3 and HSB2 cells.

EXAMPLE 4

A study was carried out to compare the antiviral efficacy of Compounds 23 and 96 as compared to GCV, ACV and PFA during HHV-6 infections. For this purpose, samples of HHV-6 (Z-29 and GS strain) were incubated with a 28.5 μM concentration of the drug for six hours and overnight at 37°C. Triplicate samples of the drug-treated and untreated virus were absorbed to Molt-3 (Z-29) or HSB2 (GS) cells in the presence of the drugs in the incubation media for four hours at 37°C. After rehydration with growth media containing the compounds, the incubation was continued at 37°C. At the 3rd and 6th days, aliquots were plated on clean coverslips and fixed in acetone. Cells fixed on the coverslips were subjected to immunofluorescence staining using polyclonal antisera to HHV-6 to determine the percentages of infected cells. The results from these experiments are summarized in Tables 5 and 6.

TABLE 5

EFFECT OF COMPOUNDS ON THE INFECTIVITY OF HHV-6* *in vitro* (VIRUCIDAL)

Drug	6 hr Preincubation of HHV-6 with Drug (28.5 μM)	O.N. Preincubation of HHV-6 with Drug (28.5 μM)
No drug	95%	95%
Compound 23	20%	3%
Compound 96	30%	9%
ACV	95%	95%
GCV	15%	3%
PFA	15%	5%

* (HHV-6 strain Z29 in Molt-3 cells) O.N. = overnight

TABLE 6

EFFECT OF COMPOUNDS ON THE INFECTIVITY OF HHV-6* *in vitro* (VIRUCIDAL)

Drug	6 hr Preincubation of HHV-6 with Drug (28.5 μM)	O.N. Preincubation of HHV-6 with Drug (28.5 μM)
No drug	95%	95%
Compound 23	5%	2%
Compound 96	5%	2%
ACV	85%	80%
GCV	4%	1%
PFA	5%	4%

* (HHV-6 strain GS strain in HSB2 cells) O.N. = overnight

From the foregoing results, Compound 23 was shown to be about as effective as GCV against HHV-6. The sequence of activity against this virus is as follows:

$$GCV \approx 23 > PFA > 96 > ACV$$

It is noted that overnight preincubation of the HHV-6 in drugs was more effective than 6 hours preincubation.

Compounds 23 and 96 exhibit the capability of preventing HHV-6 virus from infecting healthy cells in the vicinity of the virus. Clinically, this is of significance. These compounds, unlike GCV and ACV, are not nucleoside analogs and thus less likely to produce drug-resistant mutants. Compound 23 has been shown to be effective in resolving HSV-1 induced epithelial disease and stromal keratitis in rabbits as well as preventing the progression of experimental arthritis in mice.

EXAMPLE 5

An experiment was carried out to evaluate the effect of increased incubation time on the virucidal activity of Compounds 23 and 96. For this study, the virus was incubated at 37°C with the appropriate drug concentration (0.05, 0.03 and 0.01 mg/ml) for time periods of 6 hours and overnight. Triplicate samples of drug-treated and untreated virus was absorbed to Molt-3 cells in the absence and presence of the compounds in the incubation media for four hours at 37°C. After rehydration with growth media with and without the compounds, incubation was continued at 37°C. At the third and sixth days, aliquots were plated onto clean coverslips and fixed in acetone. The acetone-fixed cells were subject to immunofluo-

rescence staining using polyclonal antisera to HHV-6 to determine the percentages of infected cells. The results are shown in Table 7.

It is noted that compound concentrations of 0.03 mg/ml and 0.05 mg/ml were toxic to the cells. However, 0.01 mg/ml exhibited no toxic effect, even up to day 6. As shown, Compounds 23 and 96 each drastically reduce the HHV-6 infectivity after preincubation in the presence of 0.01 mg/ml of the respective compound. At the six hours preincubation time, the virus retains 10 to 20% infectivity. However, overnight preincubation completely abolishes HHV-6 infectivity when the compound is present during adsorption and incubation. Similar tests with Acyclovir showed 90 - 95% HHV-6 infectivity after overnight preincubation at day 6.

EFFECT OF COMPOUNDS ON THE INFECTIVITY OF HHV-6 *IN VITRO* (Virucidal)

DRUG	6hr. Preincubation of HHV-6 with drug (0.01 mg/ml)		6hr. Preincubation of HHV-6 with media only		O.N. Preincubation of HHV-6 with drug (0.01 mg/ml)		O.N. Preincubation of HHV-6 with media only	
	+D	-D	+D	-D	+D	-D	+D	-D
23	15%	70%	50%	95%	0%	60%	50%	95%
96	30%	80%	50%	99%	10%	60%	50%	95%

+D = drug was present during adsorption and incubation and incubation

-D = drug was not present during adsorption and incubation

O.N. = overnight

CLAIMS:

1. A method for treating a subject having a chorioretinal infection induced by HCMV virus comprising administering to the subject an anti-chorioretinal infection effective amount of Compound 96, 67, 23, or 102.

2. A method for treating a subject having a disease caused by HHV-6 virus comprising administering to the subject an anti-HHV-6 virus effective amount of Compound 23, 67, 96, or 102.

3. A composition for the treatment of chorioretinal infection induced by HCMV virus comprising an anti-chorioretinal infection effective amount of Compound 96, 67, 23 or 102, and a pharmaceutically acceptable carrier.

4. A composition for the treatment of disease caused by HHV-6 virus comprising an anti-HHV-6 virus infection effective amount of Compound 23, 67, 96, or 102, and a pharmaceutically acceptable carrier.

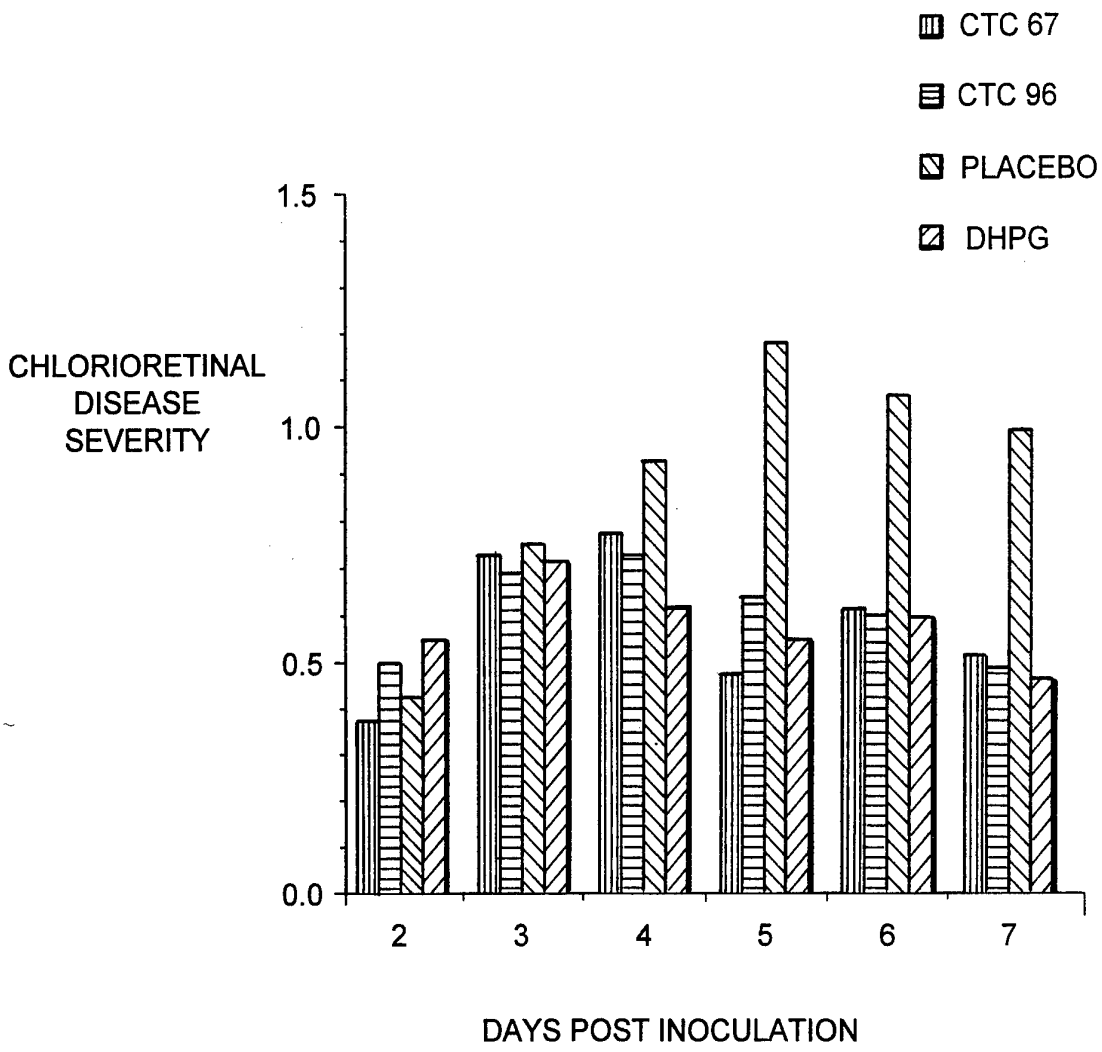

CTC 67
CTC 96
PLACEBO
DHPG
CHLORIORETINAL DISEASE SEVERITY
1.5
1.0
0.5
0.0
2
3
4
5
6
7
DAYS POST INOCULATION

FIG. 1

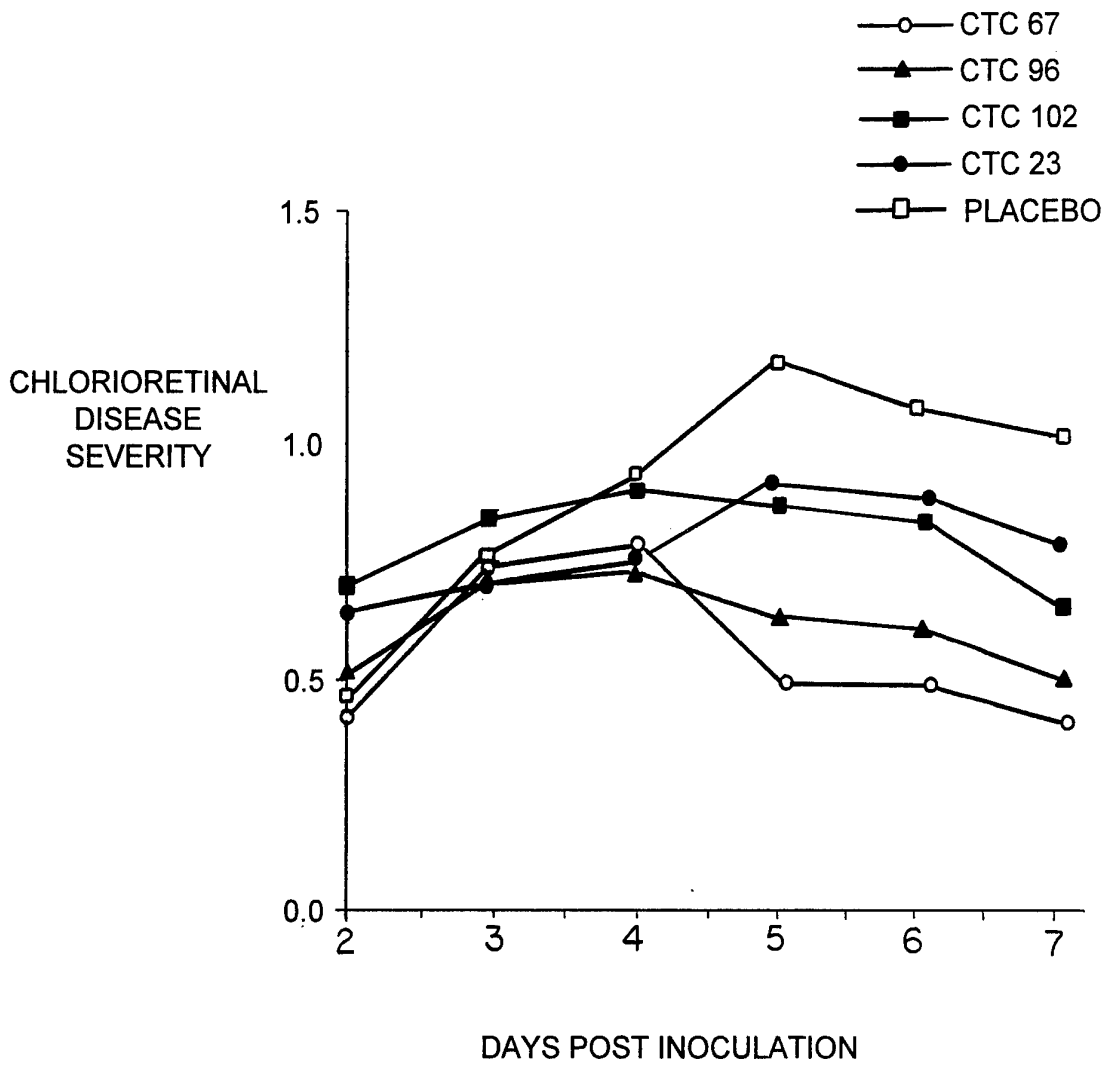

CTC 67
CTC 96
CTC 102
CTC 23
PLACEBO
CHLORIORETINAL DISEASE SEVERITY
1.5
1.0
0.5
0.0
2
3
4
5
6
7
DAYS POST INOCULATION

FIG. 2

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US93/12164

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/555; C07F 15/06
US CL :514/185; 556/146
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/185; 556/146

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Polish Journal of Chemistry, Vol. 64, No. 1-6, issued June 1990, C. Varhelyi et al., "NEW MIXED SCHIFF'S BASE COMPLEXES OF Co(III) WITH ETHYLENEDIIMINO-BIS-BENZOYLACETONE AND PYRIDINE DERIVATIVES", pages 305-316, especially Tables 1 & 2.	1-4
X	US, A, 5,049,557 (Dori et al.) 17 September 1991, entire document, especially Column 4, line 24 to Column 5, line 17 and Column 7, lines 21-62.	1-4
X	US, A, 5,106,841 (Scheer) 21 April 1992, entire document, especially Column 4, line 45 to Column 5, line 27, Tables 1 & 3 and claim 11.	1-4

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 FEBRUARY 1994	Date of mailing of the international search report 16 MAR 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL B. HYDORN Telephone No. (703) 308-1235

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,866,054 (Dori et al.) 12 September 1989, entire document.	1-4
A	US, A, 4,866,053 (Dori et al.) 12 September 1989, entire document.	1-4