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Broad-spectrum antiviral activity of PNU-183792, a 4-oxo-dihydroquinoline, against human and animal herpesviruses☆

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Abstract

We identified a novel class of 4-oxo-dihydroquinolines represented by PNU-183792 which specifically inhibit herpesvirus polymerases. PNU-183792 was highly active against human cytomegalovirus (HCMV, IC_{50} value 0.69 μM), varicella zoster virus (VZV, IC_{50} value 0.37 μM) and herpes simplex virus (HSV, IC_{50} value 0.58 μM) polymerases but was inactive (IC_{50} value >40 μM) against human alpha (α), gamma (γ), or delta (δ) polymerases. In vitro antiviral activity against HCMV was determined using cytopathic effect, plaque reduction and virus yield reduction assays (IC_{50} ranging from 0.3 to 2.4 μM). PNU-183792 antiviral activity against both VZV (IC_{50} value 0.1 μM) and HSV (IC_{50} ranging from 3 to 5 μM) was analyzed using plaque reduction assays. PNU-183792 was also active (IC_{50} ranging 0.1–0.7 μM) in cell culture assays against simian varicella virus (SVV), murine cytomegalovirus (MCMV) and rat cytomegalovirus (RCMV). Cell culture activity was compared with the appropriate licensed drugs ganciclovir (GCV), cidofovir (CDV) and acyclovir (ACV). PNU-183792 was also active against both GCV-resistant and CDV-resistant HCMV and against ACV-resistant HSV. Toxicity assays using four different species of proliferating mammalian cells indicated PNU-183792 was not cytotoxic at relevant drug concentrations (CC_{50} value >100 μM). PNU-183792 was inactive against unrelated DNA and RNA viruses indicating specificity for herpesviruses. In animals, PNU-183792 was orally bioavailable and was efficacious in a model of lethal MCMV infection.

Keywords: Herpesviruses; DNA polymerases; Antiviral activity; Dihydroquinoline; PNU-183792

1. Introduction

Herpesvirus infections including those caused by human cytomegalovirus (HCMV), herpes simplex virus type 1 and 2 (HSV-1, HSV-2), and

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varicella zoster virus (VZV) are common and are usually self-limiting in otherwise healthy individuals. However, in the immune impaired as well as the very young or old, infections with HCMV, HSV and VZV can often be much more severe and problematic. HCMV is a major opportunistic pathogen in both HIV and transplant patients and current management of disease is complex and often limited (de Jong et al., 1998; Cohen-Stuart et al., 1998). Currently available treatment options include the nucleoside antiviral ganciclovir (GCV), the pyrophosphate analogue foscarnet (PFA), and the nucleotide analogue cidofovir (CDV). Each of these drugs has limited use in the HCMV patient population due to poor bioavailability, poor penetration of drug in retinitis patients, and the development of drug related toxicity (discussed in de Jong et al., 1998). HCMV strains have also been identified which are resistant to one or more drugs targeted toward the viral polymerase. These include resistant mutants derived in the laboratory (Sullivan et al., 1993) and resistant virus isolated from drug treated patients (Chou et al., 1998; Jabs et al., 1998). Cross-resistance within the HCMV polymerase region has been investigated for GCV, CDV and PFA (Cihlar and Hitchcock, 1998).

Several currently licensed drugs are available for the treatment of HSV disease including CDV, PFA, and the nucleoside analogues ACV, famciclovir and valaciclovir. While drug resistant virus is rarely isolated from immunocompetant individuals having mucocutaneous HSV infections, HSV drug resistance to the nucleoside antivirals has been reported in the severely ill and in immunosuppressed individuals (discussed in Cohen-Stuart et al., 1998; Chatis and Crumpacker, 1992; reviewed in Field and Biron, 1994). Cross-resistance of HSV to the nucleoside antivirals has also been reported (Pelosi et al., 1998). The nucleoside antivirals, have also been used for the treatment of herpes zoster resulting from reactivation of VZV (discussed in Cohen-Stuart et al., 1998). Similar to resistance development in other human herpesviruses against nucleoside antivirals, ACV-resistant VZV has been identified in AIDS patients (Talarico et al., 1993; reviewed in Field and Biron, 1994).

Development of a new herpesvirus antiviral having broad-spectrum antiviral activity against the human herpesviruses and oral bioavailability, and an improved toxicity profile over existing HCMV therapies, would be of value as both a therapeutic agent and as a prophylactic agent for chronic use. We have recently reported on the anti-HCMV activity of napthalene carboxamide derivatives, which are non-nucleoside inhibitors of the viral polymerase (Vaillancourt et al., 2000). Additional chemical modification of this class by our laboratories has led to the discovery of the 4-hydroxyquinoline derivatives which have broad-spectrum antiviral activity against the polymerase of HCMV, HSV and VZV (Brideau et al., 2000; Oien et al., 2000). We now report on the antiviral activity of PNU-183792, a novel non-nucleoside inhibitor belonging to the 4-oxo-dihydroquinoline class which has potent cell culture activity against human and animal herpesviruses, and is orally bioavailable and therapeutically active in an animal model of murine cytomegalovirus (MCMV) infection.

2. Materials and methods

2.1. Polymerase assays

HCMV, HSV-1, VZV, human α, and human δ polymerases were expressed as c-terminal histidine-tagged proteins using the baculovirus expression system and purified by standard Ni-affinity chromatography. Human γ polymerase was obtained from W. Copeland, NIH, USA. PNU-183792 was evaluated against the purified polymerase of HCMV, VZV, HSV-1 and purified human α and γ polymerase using a scintillation proximity assay (SPA) as previously described (Vaillancourt et al., 2000). Briefly, SPA assay conditions were as follows, 6.4 mM HEPES (pH 7.5); 12 mM KCl; 25 mM NaCl; 2 mM CHAPS; 4.5 mM DTT; 5 mM $MgCl_2$; 5% glycerol; 5% DMSO; 46 μg/ml BSA; 10 nM oligo dT annealed to polydA homopolymer; and 0.1 μM ^{3}H-TTP. The reaction was terminated after 12 min by the addition of 200 μg strepavidin coated SPA beads and 133 nM EDTA (Amersham Pharmacia Bio-

tech, Piscataway, NJ, USA). Delta polymerase activity was determined using 10 μg/ml polyA-dT primer/template in a buffer containing 0.85 nM delta polymerase, 20 mM HEPES, 40 μg/ml BSA, 2 mM $MnCl_2$, 5% glycerol, 1 MM DTT, and 5% DMSO. The reaction was terminated after 10 min by the addition of 5% TCA. All polymerase reactions were performed at 27 °C. Cellular and viral polymerases were estimated to be $>50\%$ pure and had BSA added to them to provide stability during storage. Removal of all extraneous polymerases was demonstrated by repeating the purification process using recombinant baculovirus containing genes other than polymerases (data not shown). Polymerase assays were performed under conditions of first order kinetics.

2.2. Cells and viruses

HCMV strains Davis and Ad169, VZV strains Webster and Oka, and RCMV were obtained from the ATCC, Rockville, MD, USA. Drug resistant HCMV strains were obtained from the NIH reagent depository, Rockville, MD, USA and were originally described by Sullivan et al. (1993). MCMV (Smith strain) was originally obtained from DK Kelsey, University of Utah, Salt Lake City, UT, USA. HSV-1 (KOS strain) was obtained from FL Homa, HSV-2 (35D strain) was obtained from HE Renis, and Vesicular stomatitis virus (VSV) was obtained from DA Stringfellow (Upjohn Laboratories, Kalamazoo, MI USA). Vaccinia virus (VV) was originally obtained from PL Collins, NIH. Drug resistant HSV-1 mutants derived using the KOS parental strain were obtained from DM Coen, Harvard, Boston, MA. Simian varicella virus (SVV) was obtained from K Soike, Tulane Primate Center, Covington, LA, USA. Human foreskin fibroblasts (HFF) were prepared in our laboratories and were used to propagate HCMV, VSV, VZV and Vaccinia. Murine 10T1/2 cells were used to propagate MCMV, rat REF-5 cells were used to propagate RCMV, and monkey Vero cells were used to propagate HSV and SVV, and were obtained from the ATCC. All cells were maintained in Dulbecco's Modified Minimal Essential Medium (DMEM) containing 10% FBS, glutamine, and antibiotics.

2.3. Animals

BALB/c mice and Sprague–Dawley rats were obtained from Charles River Laboratories, Portage, MI, USA. Dogs were obtained from Marshall Farms, North Rose, NY, USA. Animals were used in compliance with the Animal Welfare Act (USA) and subsequent amendments and in accordance with the *Guide for Care and Use of Laboratory Animals* (NIH).

2.4. Antiviral assays

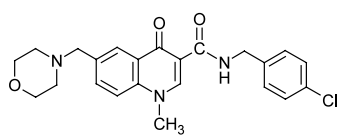
Viral plaque reduction assays were used for all viruses. Plaques were either counted using low power magnification or by eye following counter staining with crystal violet. Herpesviruses and VV were grown using carboxymethylcellulose overlays. VSV was grown using an agarose overlay. In some experiments, HCMV replication was measured in a conventional cytopathic effect assay (cpe) using an ELISA format as described (Tatarowicz et al., 1991) which used a mouse monoclonal antibody (ViroStat, Portland, ME, USA) directed against the 65 kDa HCMV viral protein. HCMV replication was also measured in a virus yield reduction assay (Pritchard et al., 1990) in which HCMV was harvested from monolayers of drug treated virus infected cells following 5 days of incubation, taken through one freeze thaw cycle, and then placed onto fresh HFF cells for titration. For plaque reduction assays, the multiplicity of infection (MOI) was generally 0.001–0.005 pfu per cell, for the cpe and virus yield reduction assays the MOI was 0.01–0.05 pfu per cell. Percent inhibition data for antiviral treatments was entered into an Excel software program used to calculate the IC_{50} and IC_{90} value. Assay samples were run in duplicate or triplicate, four to six dilutions were used in virological assays and eight to 16 dilutions were used in biochemical assays for each treatment to generate inhibition data for entry into a software program (EXCEL) which used linear regression analysis to generate IC_{50} and IC_{90} values.

2.5. Analysis of cell viability

The cytotoxicity of compounds on uninfected mammalian cells was determined using HFF, Vero, 10T1/2 and REF-5 cells seeded as subconfluent monolayers and treated with compound for 3 days. Each of these cell types was the same cell type used for growth of species specific animal and human herpesviruses. Cell viability determinations were performed using both microscopic evaluation and a quantitative neutral red dye uptake assay using an Enzyme Linked Immunosorbent Assay (ELISA) format as described (Lowik et al., 1993). An EXCEL software program was used to determine the cell cytotoxicity CC_{50} value.

2.6. Antiviral compounds

PNU-183792 (Fig. 1), a 4-oxo-dihydroquinoline, was prepared at Pharmacia Corporation, Kalamazoo, MI, USA. In cell culture studies, the compound was first diluted into 100% DMSO followed by dilution into culture medium to yield a final concentration of DMSO of 0.5% across all compound concentrations. Ganciclovir (Cytovene®) was obtained from a wholesale pharmacy (Bergen Brunswick, Lansing, MI, USA). CDV (Vistide®) was obtained from a local pharmacy. Acyclovir (ACV) (acycloguanosine powder) was obtained from Sigma Chemical, St. Louis, MS, USA.



PNU-183792

Fig. 1. Structure.

Table 1
In vitro inhibition of viral polymerase using PNU-183792

Viral polymerase	PNU-183792 IC_{50} (μM)[a]
HCMV	0.69 ± 0.17
HSV-1	0.58 ± 0.09
VZV	0.37 ± 0.06
Cellular polymerase	
Alpha (α)	>40
Gamma (γ)	>40
Delta (δ)	>40

[a] IC_{50}, the concentration of drug required to reduce enzyme activity by 50%; each value represents the mean ± S.D. from five experiments.

2.7. Animal studies

PNU-183792 was formulated as a aqueous solution, using 25 mM methanesulfonic acid at pH 2.6 to aid solubility, for use in rodent IV pharmacokinetic (PK) and in oral efficacy studies. For dogs, PNU-183792 was prepared as a suspension using 0.12 M methanesulfonic acid with 0.4% Tween 80 and administered PO in hard gelatin capsules. For rats and dogs, serial blood samples were obtained from individual study animals. In mouse studies three mice were sacrificed per time point for PK analysis. All blood samples were collected into EDTA tubes, centrifuged and clear plasma was stored frozen. Prior to HPLC drug analysis of PNU-183792, plasma proteins were precipitated with acetonitrile, and then chromatographed by reversed-phase chromatography with UV detection at 320 nm.

In infection studies, mice were challenged with an intraperitoneal (i.p.) dose (approximately 20 000 PFU) of MCMV pre-determined to induce 70–100% mortality as described previously (Brideau and Wolcott, 1985). All compounds were administered PO in a volume of 0.2 ml. Data from efficacy studies was analyzed using a non-parametric test (Kruskal–Wallis Analysis of Variance(ANOVA)) to determine P values.

3. Results

3.1. Inhibition of viral polymerase

Specificity of PNU-183792 for viral herpesvirus polymerases was determined in vitro measuring incorporation of ^{3}H-nucleotide into primer/template by purified polymerases. Table 1 summarizes inhibition of the HCMV, HSV-1 and VZV poly-

Table 2
Inhibition of human cytomegalovirus in cell culture using PNU-183792

Assay[a]	IC_{50}/IC_{90} (μM)[b]	
	PNU-183792	Ganciclovir
Cytopathic effect	2.4/n.d.[c]	1.6/n.d.
Plaque reduction	0.3/3.0	0.9/3.2
Virus yield reduction	0.8/3.3	0.7/2.4

[a] Virus yield reduction and the cytopathic effect assay used a multiplicity of infection of 0.01–0.05 and the plaque reduction assay used a multiplicity of infection of 0.001–0.005 using the Davis HCMV strain.

[b] IC_{50}, the concentration of drug required to reduce viral plaques, viral titers or cytopathic effect by 50%; IC_{90}, the concentration of drug required to reduce viral plaques or viral titers by 90%; data from three to four assays using HFF cells.

[c] n.d., not determined due to lack of linearity in the inhibition curve using the cytopathic effect assay.

merase. PNU-183792 had an IC_{50} value against each of these viral polymerases below 1 μM with the most sensitive viral polymerase being VZV. PNU-183792 was inactive against human α, γ, or δ polymerase (IC_{50} value >40 μM) using similar in vitro assay conditions (Table 1).

3.2. Broad antiviral activity of PNU-183792 in cell culture

Initial studies focused on the inhibition of HCMV. Table 2 summarizes the antiviral activity of PNU-183792 compared with the licensed drug GCV using three different anti-HCMV antiviral assays. In the cpe assay, in which the extent of viral infection of HFF monolayers is quantitated using an ELISA format employing an antibody to the viral matrix protein, both PNU-183792 and GCV exhibited equivalent levels of activity (IC_{50} ranging from 1 to 2 μM). Using a viral plaque reduction assay both PNU-183792 and GCV had an IC_{50} value below 1.0 μM. Using the virus yield reduction assay which utilizes a higher MOI, we found the antiviral activity against HCMV for both PNU-183792 and GCV was below 1.0 μM (IC_{50}). Both PNU-183792 and GCV had a similar IC_{90} value in the plaque reduction and virus yield reduction assay.

Additionally, we determined the antiviral activity of PNU-183792 against other human and animal herpesviruses. PNU-183792 had broad cell culture antiviral activity against both human (HCMV, HSV, VZV) and animal (MCMV, RCMV, SVV) herpesviruses. Table 3 summarizes inhibition using the plaque reduction assay and compares PNU-183792 to the licensed drugs GCV, CDV, and ACV for the various human and animal herpesviruses. PNU-183792 had the most potent activity against human VZV. The IC_{50} value for PNU-183792 (0.1 μM) against VZV was markedly low in comparison to each of the licensed drugs. This potent inhibition against human Varicella was also extended to monkey Varicella for PNU-183792 (IC_{50} value 2.7 μM).

Table 3
Antiviral activity of PNU-183792 against other herpesviruses

Treatment	Plaque reduction IC_{50} (μM)[a]					
	Varicella[b]		Herpes simplex[c]		Rodent cytomegalovirus[d]	
	Human	Monkey	HSV-1	HSV-2	Mouse	Rat
PNU-183792	0.1	2.7	3.3	4.6	0.7	0.2
Ganciclovir	19	18			3.7	24
Cidofovir	1.8	11			0.04	0.5
Acyclovir	14	>25	3.9	5.6		

[a] IC_{50}, the concentration of drug required to reduce viral plaques by 50%; data from at least two experiments.

[b] Varicella zoster virus was grown on HFF cells; simian varicella virus was grown on Vero cells.

[c] Herpes simplex viruses were grown on Vero cells.

[d] Murine cytomegalovirus was grown on mouse 10T1/2 cells; rat cytomegalovirus was grown on rat REF cells.

Table 4
Lack of activity of PNU-183792 against unrelated viruses and mammalian cells

Virus	IC_{50} (μM)[a]
Vaccinia	76.2 ± 8.9
Vesicular stomatitis	>100
Cells	**CC_{50} (μM)[b]**
HFF (human)	>100
Vero (monkey)	>100
REF-5 (rat)	>100
10T1/2 (mouse)	>100

[a] IC_{50}, the concentration of drug required to reduce viral plaques on HFF cells by 50%; each value represents the mean ± S.D. from at least two experiments.

[b] $CC_{50,}$ the concentration of drug required to reduce neutral red dye uptake by each cell type by 50%; each value represents the mean of two experiments.

GCV, CDV, and ACV were much less active against monkey Varicella (IC_{50} above >10 μM). The antiviral activity of PNU-183792 was also compared with ACV in cell culture against the HSVs. Both PNU-183792 and ACV had comparable activity (IC_{50} ranging from 3 to 6 μM) against HSV-1 and HSV-2 (Table 3). In the next series of experiments, we compared antiviral activity of PNU-183792 to both GCV and CDV using the rodent cytomegaloviruses. Also shown in Table 3, PNU-183792 and CDV each had an IC_{50} value below 1.0 μM against both MCMV and RCMV. However, GCV was less active against MCMV in culture than either PNU-183792 or CDV and was inactive against RCMV. PNU-183792 had broad cell culture antiviral activity against both human (HCMV, HSV, VZV) and animal (MCMV, RCMV, SVV) herpesviruses.

Using plaque reduction assays we have also determined that PNU-183792 was inactive against both an unrelated DNA (VV) and RNA (VSV) virus. Table 4 summarizes the lack of inhibition of PNU-183792 against VV and VSV ($IC_{50} > 75$ μM). Testing by an independent contract laboratory has also determined that PNU-183792 was inactive ($IC_{50} > 100$ μM) against the following unrelated viruses: coxsackie-B, adenovirus type 2, hepatitis B virus, influenza A, and HIV (data not shown). Table 4 also summarizes the lack of cell toxicity with PNU-183792 using four different mammalian cells including human, monkey, rat and mouse cells. Each of these cell types was also used in the antiviral assays described previously to support replication of human and animal herpesviruses. PNU-183792 did not inhibit cell proliferation ($CC_{50} > 100$ μM). In addition, microscopic evaluation of PNU-183792 treated non-infected monolayers of mammalian cells did not reveal morphological changes at compound treatment levels below 100 μM (data not shown).

3.3. Activity of PNU-183792 against drug resistant virus

The 759^r D-100 and $GDGp53^r$ mutant HCMV strains have dual resistance against both GCV and CDV (Sullivan et al., 1993) and were used to test for antiviral activity of PNU-183792. PNU-183792 was a potent inhibitor against both the 759^r D-100 mutant strain and $GDGp53^r$ mutant strain of drug-resistant virus with IC_{50} below 2 μM (Table 5, top). In these experiments, the parental wild-type Ad169 HCMV strain was also included. PNU-183792, GCV and CDV were all active against wild-type Ad169. GCV and CDV were less potent against the resistant HCMV strains as expected.

A panel of six ACV-resistant HSVs were tested for sensitivity to PNU-183792 in cell culture (Table 5, bottom). PNU-183792 was active against both the parental wild-type HSV-1 (KOS strain) as well as the ACV-resistant strains (IC_{50} ranging from 0.5 to 3.3 μM). ACV was active against the parental wild-type HSV1 KOS strain but inactive against the ACV-resistant strains as expected.

3.4. Pharmacokinetics of PNU-183792 in animals

Table 6 summarizes the PK profile following oral dosing including the C_{max} and $\%F$ (bioavailability) and the rate of drug clearance following IV dosing of PNU-183792 in mice, rats and dogs. PNU-183792 had good oral bioavailability in mice, rats and dogs with moderate rates of clearance in each of these species.

Further PK studies in un-infected mice showed that blood levels of PNU-183792 could be achieved above the cell culture antiviral IC_{90} (4.2 μM), following an oral dose of 25 mg/kg (Fig. 2). No change in blood levels of drug was observed after 5 days of consecutive dosing. These preliminary PK data suggested that PNU-183792 would be active in an animal model of MCMV.

3.5. Efficacy of PNU-183792 in a MCMV infection model

In initial animal experiments we determined the efficacy of PNU-183792 against MCMV challenge of BALB/c mice using a prophylactic drug treatment schedule. Table 7 summarizes the potency of PNU-183792 following oral dosing 1 h prior to a

Table 5
Activity of PNU-183792 against drug resistant virus in cell culture

Virus HCMV	Mutation[a]	IC_{50}/IC_{90} (μM)[b]		
		PNU-183792	Ganciclovir	Cidofovir
Ad169 WT[c]		0.8/8.9	0.9/19	0.3/3.7
759[r] D-100	pol (V)	1.4/7.6	13/77	2.6/36
GDG p53[r]	pol (V)	1.1/5.9	4.3/49	1.5/16
Virus HSV-1		IC_{50}/IC_{90} (μM)[a]		
		PNU-183792	Acyclovir	
KOS WT[c]		3.3/8.5	3.9/12.7	
AraA[r]9	N961K(V)	3.3/8.1	>10/>50	
AraA[r]13	V813M(III)	0.5/2.0	>10/>50	
PAA[r]5	R842S(III)	1.5/3.0	>10/>50	
F891C	F891C(I)	1.0/3.4	>10/>50	
TsD9	E597K(A)	2.5/7.1	>10/>50	
PFA[r]2	R605V(A)	0.9/2.6	>10/>50	

[a] The location of the mutation in the polymerase (pol) gene for each HCMV or HSV-1 resistant virus is listed. The region in the pol gene is included in parentheses.

[b] $IC_{50,}$ the concentration of drug required to reduce viral plaques by 50%; $IC_{90,}$ the concentration of drug required to reduce viral plaques by 90%; data from two to three experiments.

[c] WT, wild-type parental virus; [r], resistant.

Table 6
Pharmacokinetics of PNU-183792

Species	PO dosing[a]					i.v. Dosing[b]		
	Dose (mg/kg)	C_{max} (μM)	T_{max} (h)	AUC (μM × h)	%F	Dose (mg/kg)	CL (L/h per kg)	$T_{1/2}$ (h)
Mouse	25	12	0.5	28	83	10	1.7	1.1
Rat	15	6.3	0.5-1	20	104	5	1.8	1.1
Dog	15	3.8	1.0	21	49	5	0.8	3.0

[a] PO dosing parameters include C_{max}, maximum concentration of drug in blood; $T_{max,}$ time of peak blood levels of drug; AUC, area under the curve;%F, percent bioavailable drug in blood after oral dosing.

[b] I.V. dosing parameters include CL, clearance of drug from blood; $T_{1/2}$, half life of drug in blood.

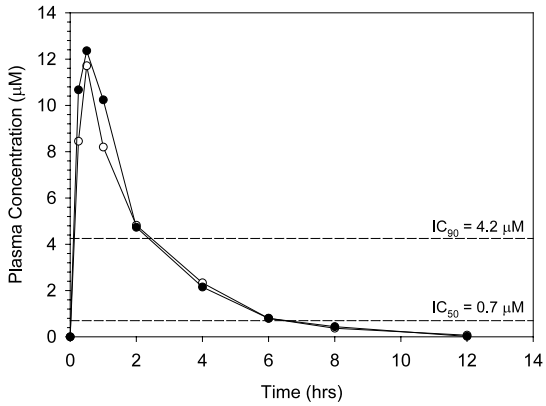


Fig. 2. Pharmacokinetic profile in mice following oral treatment with PNU-183792. Groups of three mice were sacrificed and blood was collected at each time point shown. Open circles represent plasma concentration of PNU-183792 following a single day of dosing. Closed circles represent plasma concentration of PNU-183792 following 5 days of single daily dosing. The dotted lines represent the IC_{50} and IC_{90} for PNU-183792 against MCMV in cell culture.

moderate MCMV virus challenge (75–90% mortality). In each of three studies shown in Table 7, PNU-183792 was able to protect 100% of the mice, from lethal MCMV challenge ($P < 0.001$). A dose of compound as low as 12.5 mg/kg (BID) protected 100% of the mice (Study 2).

A more rigorous challenge dose of MCMV, which typically results in 100% mortality in placebo treated mice, was used to test the therapeutic activity of PNU-183792 when treatment was initiated 24 or 48 h after challenge. Table 8 summarizes the potency of PNU-183792 against MCMV infection and includes treatment of mice with GCV as a comparator. In these studies 100% of the vehicle treated MCMV-infected animals expired by day 7. PNU-183792 was 100% protective when the first drug treatment was administered 24 h post MCMV as was GCV (P value < 0.001 for both treatments). PNU-183792 protected 90% of the mice (50 mg/kg dose) when administered at 48 h after MCMV challenge ($P < 0.001$) and GCV protected 100% of the mice (25 mg/kg dose) when the first drug treatment was administered 48 h post MCMV challenge ($P < 0.001$). A dose of 25 mg/kg of PNU-183792 protected 50% of the mice and significantly increased the average day of death ($P < 0.001$) when drug treatment was started 48 h following MCMV challenge.

4. Discussion

We have analyzed the antiviral potency of PNU-183792, a 4-oxo-dihydroquinoline that was found to have broad-spectrum antiviral activity, against both human and animal herpesviruses. PNU-183792 is a non-nucleoside inhibitor targeted against the viral polymerase of HCMV, VZV and HSV. PNU-183792 did not inhibit human α, γ, or

Table 7
Murine CMV infection in mice

Group	Dose (mg/kg)[a]	% Mortality		
		Study 1	Study 2	Study 3
Vehicle[b]	–	87 (6.1)	75 (7.8)	90 (6.3)
PNU-183792	100	0*	–	–
PNU-183792	50	0*	0*	–
PNU-183792	25	0*	0*	–
PNU-183792	12.5	–	0*	0*
PNU-183792	6.2	–	–	100 (6.6)
PNU-183792	3.1	–	–	80 (7.7)

Values in parentheses includes the mean day of death for those groups with expired mice. * P value < 0.001 compares each treatment group to vehicle group.

[a] Oral treatment was begun 30–60 mins prior to virus challenge. Animals received drug or vehicle treatment twice daily on days 0–4; study 1 and 2 had eight animals per group and study 3 had 10 animals per group.

[b] Vehicle consisted of an aqueous solution containing 25 mM methanesulfonic acid at pH 2.6.

Table 8
Therapeutic treatment with PNU-183792 against MCMV

Group	Dose[a] mg/kg	First dose	% Mortality	Average day of death[b]	*P* value[c]
Vehicle[d]		+24 h	100	6.3	
PNU-183792	25	+24 h	0	>14	<0.001
	50	+24 h	0	>14	<0.001
Ganciclovir	25	+24 h	0	>14	<0.001
Vehicle		+48 h	100	6.2	
PNU-183792	25	+48 h	50	11.1	<0.001
	50	+48 h	10	13.1	<0.001
Ganciclovir	25	+48 h	0	>14	<0.001

[a] Oral treatment was begun 24 or 48 h following virus challenge, was administered twice daily and continued through day 4; 10 mice per treatment group.
[b] Average day of death includes all animals per group and assigns day 14 to those which survived.
[c] *P* value compares each treatment group to vehicle group.
[d] Vehicle consisted of an aqueous solution containing 25 mM methanesulfonic acid at pH 2.6.

δ polymerase. PNU-183792 is part of a class of antiviral compounds which have been shown to be competitive inhibitors of herpesvirus polymerases (Oien et al., 2000). In cell culture, PNU-183792 had antiviral activity equivalent to GCV against HCMV. The activity against VZV using primary HFF cells was superior to the licensed drugs GCV and CDV, which are nucleoside and nucleotide polymerase inhibitors, respectively. PNU-183792 had comparable activity to that found for ACV, a licensed nucleoside analogue, against HSV-1 and HSV-2 laboratory strains. Human and rodent (MCMV and RCMV) cytomegaloviruses were also inhibited in cell culture further demonstrating the broad-spectrum anti-herpesvirus activity of PNU-183792.

An important feature of any current antiviral under investigation is activity against drug resistant virus strains. PNU-183792 remained active against GCV- and CDV- resistant HCMV strains. PNU-183792 was also active against ACV-resistant strains of HSV1. The ability of PNU-183792, a 4-oxo-dihydroquinoline, to inhibit drug resistant virus suggests this class of polymerase inhibitors may have utility in the treatment of clinical isolates resistant to the current nucleoside analogs. Ongoing studies are addressing development of PNU-183792-resistant herpesviruses generated in the laboratory and mapping the critical resistance domains in the polymerase gene of these mutants (Homa et al., 2001).

In animal studies PNU-183792 had good oral bioavailability with moderate rates of clearance following IV dosing. In mice, PNU-183792 blood levels exceeded the cell culture IC_{50} and IC_{90} value (for MCMV), and prophylactic oral administration of PNU-183792 induced a protective response to lethal MCMV challenge. PNU-183792 was also active therapeutically against MCMV and remained beneficial when administered as late as 48 h following virus challenge. A reduction in mortality and a delay in progression to death was found for PNU-183792 treated animals whether the compound was administered prior to or following MCMV challenge. The availability of a safe, orally active anti-herpesvirus agent with broad-spectrum activity against HCMV, VZV and HSV would offer a significant benefit over currently licensed therapies. In a preliminary 14 day rodent toxicology study, PNU-183792 was well tolerated at doses 10× the efficacious dose observed in the mouse studies (data not shown).

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