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Improved synthesis and biological evaluation of an acyclic thiosangivamycin active against human cytomegalovirus*

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Summary

We previously described the synthesis and in vitro antiviral activity of an acyclic thiosangivamycin analog (Gupta et al., 1989a). In order to extend these initial studies, a new, multi-gram synthesis of 4-amino-7-[(2-hydroxy-ethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (compound 229) was achieved in 5 steps from the known 5-amino-2-bromo-3,4-dicyanopyrrole in good overall yield. In plaque reduction assays with HCMV, compound 229 had an IC_{50} of 7 μM; in yield reduction assays the IC_{90} was 25 μM. The compound was less active against MCMV, HSV-1, HSV-2, and least active against VZV. Concentrations of compound 229 up to 32 μM did not affect the growth of KB cells for incubation periods up to 72 h. At 100 μM, a prolongation in population doubling time from 21 h (untreated) to 35 h was noted. This inhibition, however, was reversible upon removal of the compound suggesting the inhibition was cytostatic rather than cytotoxic. Flow cytometric studies with compound 229 in HFF cells revealed an accumulation of cells in S

phase and a concurrent loss of cells in G_2/M phase, suggesting an early S phase blockage. We conclude there is adequate separation between antiviral activity and cytotoxicity to merit further work with this class of pyrrolopyrimidines.

HCMV; Acyclic nucleosides; Ganciclovir; Cellular-DNA content

Introduction

Human cytomegalovirus is the cause of debilitating and often life or sight threatening opportunistic infections in patients infected with human immuno-deficiency virus and in other immunocompromised patients (Mills and Masur, 1990; Bowden, 1991). It also is a leading cause of birth defects as a result of congenital infection (Demmler, 1991). Currently, the drugs used to treat HCMV infections are ganciclovir (DHPG) and the newly approved foscarnet (PF). Both drugs, however, suffer from poor oral bioavailability and produce a number of adverse effects in both animals and humans (Shepp et al., 1985; Collaborative Study Group, 1986). In contrast, acyclovir is relatively non-toxic because it is phosphorylated to its corresponding monophosphate by a herpes virus encoded thymidine kinase (Elion et al., 1977; Fyfe et al., 1978). Unfortunately, acyclovir is not as effective as ganciclovir in inhibiting HCMV replication (Crumpacker et al., 1979; Tyms et al., 1981).

As part of our ongoing research with pyrrolo[2,3-*d*]pyrimidine nucleosides (Tolman et al., 1969), we have designed, synthesized, and evaluated a number of new analogs as potential antiviral agents (Townsend et al., 1991). Although the parent nucleoside antibiotics tubercidin, toyocamycin, and sangivamycin are active against HCMV, they are equally cytotoxic in uninfected cells (Turk et al., 1987). In an attempt to limit cytotoxicity, we initiated studies involving specific chemical modifications of the nucleoside antibiotics per se. For example, we have synthesized a number of acyclic pyrrolo[2,3-*d*]pyrimidine derivatives containing either the acyclovir or ganciclovir side chain as potential HCMV agents (Saxena et al., 1988; Pudlo et al., 1988; Gupta et al., 1989a; Gupta et al., 1989b; Pudlo et al., 1990). These studies identified a series of acyclic bromotubercidins with potent activity against HCMV both in vitro and in vivo (Pudlo et al., 1988, 1990; Nassiri et al., 1990, 1991b). The initial antiviral evaluation of other compounds also identified a thiosangivamycin analog (4-amino-7-[(2-hydroxyethoxymethyl)]pyrrolo[2,3-*d*]pyrimidine-5-thiocarbox-amide, compound 229[*]) as active against HCMV ($IC_{50} = 11$ μM) without the severe cytotoxicity observed with toyocamycin and sangivamycin (Gupta et al., 1989a). To further evaluate the antiviral activity and cytotoxicity of compound 229, sufficient quantities were required. However, the original synthetic scheme described by Gupta (1989a) was not suitable for supplying the multi-gram

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[*]Referred to as compound 11 by Gupta et al., 1989a.

quantities necessary for our more extensive studies. This prompted us to develop an alternate, multi-gram synthesis of compound 229 which facilitated a confirmation and extension of antiviral and cytotoxicity studies.

Materials and Methods

Synthesis
General methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. TLC was run on silica 60F-254 plates (Analtech, Inc.). Detection of components on TLC was made by UV light absorption at 254 nm. Ultraviolet spectra were recorded on a Hewlett-Packard 8450 A spectrophotometer. IR spectra were taken on a Nicolet 5DXB infrared spectrometer. NMR spectra were determined at 270 or 360 MHz with a BRUKER WP 270/360 SY. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent DMSO-d_6 unless otherwise specified. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ and are within $\pm$ 0.4% of the theoretical values. E. Merck silica gel (230–400 mesh) was used for flash column chromatography, which was carried out as described by Still and Kahn (1978). Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature at 40°C. Acetonitrile was distilled from CaH$_2$.

1-[2-(acetoxyethoxy)methyl]-2-bromo-5-(ethoxymethylene)imino-pyrrole-3,4-dicarbonitrile (3). A mixture of 2-amino-5-bromo-3,4-dicyanopyrrole (Swayze et al., 1991) (1, 2.11 g, 10.0 mmol) and distilled triethylorthoformate (2.96 g, 20 mmol) in dry acetonitrile (75 ml) under argon was heated at reflux for 2 h, cooled to room temperature and the solvent evaporated in vacuo. The resulting residue was co-evaporated with toluene (2 × 100 ml) and rotary evaporated until a dry powder was obtained. The ^{1}H NMR spectrum of this compound agrees with structure 2: δ 13.5 (1H, s, NH), 8.40 (1H, s, CHN), 4.28 (2H, q, CH$_3$CH$_2$O, J = 7.1 Hz), 1.30 (3H, t, CH$_3$CH$_2$, J = 7.1 Hz). Crude 2 was dissolved in dry acetonitrile (100 ml) then treated with sodium hydride (60% w/w%, 0.60 g, 15 mmol) at room temperature. The mixture was stirred for 0.5 h, then (2-acetoxyethoxy)methyl bromide (Robins and Hatfield, 1982) (3.94 g, 20 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 0.5 h, then filtered. The filtrate was evaporated and the resulting oil subjected to flash column chromatography (CHCl$_3$) to yield a pale yellow oil. The compound was recrystallized from methanol to yield 2.12 g (55%) of 3: mp 102–103°; TLC, MeOH/CHCl$_3$ (5:95, v:v) Rf = 0.72; FTIR (KBr)ν_{max}2227, 1741, 1725, 1613, 1243, 1137, 1111, 1057, 637 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 8.39 (1H, s, CHN), 5.39 (2H, s, C-1′), 4.37 (2H, q, CH$_3$CH$_2$O, J = 7.1 Hz), 4.16 (2H, t, CH$_2$O$_2$, J = 4.7 Hz), 3.66 (2H, t, OCH$_2$, J = 4.7 Hz), 2.03 (3H, s, CO$_2$CH$_3$), 1.40 (3H, t, CH$_3$CH$_2$, J = 7.1 Hz); Anal calcd for C$_{14}$H$_{15}$N$_4$O$_4$Br: C, 43.88; H, 3.95; N, 14.62. Found: C, 43.71; H, 4.09; N, 14.80.

4-Amino-6-bromo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**4**). To 100 ml of saturated methanolic ammonia in a pressure bottle at 25°C was added compound **3** (2.60 g, 6.78 mmol). The bottle was sealed and the solution stirred for 24 h at room temperature. The resulting suspension was cooled to −78°C then filtered to yield 1.89 g (89%) of **4**: mp 235-236°(d); TLC, (40:9:1 EtOAc/MeOH/H_2O, v:v) Rf = 0.71: UV 1 max (ε_{mM}) (pH 1) 230 (15), 282 (16); (EtOH), 285 (16); (pH 11) 229 (11), 285 (16); FTIR (KBr)ν_{max} 3569, 3366, 3329, 3116, 2942, 2225, 1666, 1649, 1596, 1364, 1306, 1115, 1051, 792 cm^{-1}; ^{1}H NMR: δ 8.24 (1H, s, C-2), 7.00 (2H, s, NH_2, D_2O exchangeable), 5.62 (2H, s, C-1'), 4.66 (1H, t, OH, D_2O exchangeable, J = 5.2 Hz), 3.49–3.43 (4H, m, CH_2); Anal calcd for $C_{10}H_{10}N_5O_2Br$: C, 38.48; H, 3.23; N, 22.45. Found: C, 38.62; H, 3.35; N, 22.55.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**5**). Compound **4** (2.47 g, 8.0 mmol) was dissolved in a mixture of EtOAc and EtOH (2:1 v:v; 150 ml) containing 5 ml of 1 N NH_4OH. Palladium-carbon (10%; 593 mg) was added, and the reaction mixture was hydrogenated at 42 psi at room temperature for 12 h. The mixture was filtered, the filtrate evaporated in vacuo and the resulting solid adsorbed onto silica gel (7.5 g) then subjected to column chromatography in MeOH/$CHCl_3$ (5:95, v:v) to yield 1.19 g **5** (63%): mp 186–188°; TLC, MeOH/EtOAc/$CHCl_3$ (1:1:5, v:v) Rf = 0.28; ^{1}H NMR: δ 8.34 (1H, s, C-2), 8.23 (1H, s, C-6), 6.90 (2, br s, NH_2, D_2O exchangeable), 5.56 (2H, s, C-1'), 4.67 (1H, s, OH, D_2O exchangeable), 3.44 (4H, m, CH_2). All other spectroscopic data obtained for compound **5** was identical to that previously reported (Gupta et al., 1989a). A sample was recrystallized from EtOH for analysis: Anal calcd for $C_{10}H_{11}N_5O_2$: C, 51.49; H, 4.75; N, 30.03. Found: C, 51.46; H, 4.77; N, 30.08.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine-5-thiocarboxamide (**229**). Dry H_2S was passed through a solution of sodium methoxide (1.5 g, 28 mmol) in dry methanol (300 ml) for 0.5 h. The nitrile **5** (3.25 g, 13.9 mmol) was added in one portion, and the mixture was stirred in a sealed pressure vessel at 80°C for 1.5 h. The resulting solution was allowed to cool to room temperature, then adjusted to pH 7 with 1 N HCl. The solvent was rotary evaporated and the compound recrystallized from H_2O containing a small amount of EtOH to yield 1.68 g of **229** (45%): mp 199–200°; TLC, MeOH/$CHCl_3$ (5:95, v:v) Rf = 0.17; ^{1}H NMR: δ 9.49 and 9.52 (1 each, br s, $CSNH_2$, D_2O exchangeable), 8.14 (1H, s, C-2), 7.93 (2H, s, NH_2, D_2O exchangeable), 7.86 (1H, s, C-6), 5.54 (2H, s, C-1'), 4.69 (1H, s, OH, D_2O exchangeable), 3.47 (4H, m, CH_2). All other spectroscopic data obtained for compound **229** was identical to that previously reported (Gupta et al., 1989a); Anal calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 44.73; H, 4.79; N, 26.31.

Cell cultures

KB cells, an established human cell line derived from an epidermal oral carcinoma, were routinely grown in MEM with Hanks' salts [MEM(H)] supplemented with 10% calf serum. Diploid cell cultures of HFF cells were grown in MEM with Earle salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged by conventional procedures by using 0.05% trypsin and 0.02% EDTA in a HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-buffered salt solution (HBS) (Shipman et al., 1976). To increase the likelihood of detecting bacterial or mixed bacterial and mycoplasmal contamination, antibiotics were never used in the routine passage of cells (Hayflick, 1973). Cell lines were screened periodically and were mycoplasma-free.

Antiviral assays

The effect of compound 229 and DHPG on HCMV replication was determined by plaque and yield reduction assays. HCMV plaque reduction assays were performed with monolayer cultures of HFF cells. A plaque purified isolate, P_0, of the Towne strain of HCMV obtained from Dr. M.F. Stinski, University of Iowa, was used. Preparation of HCMV stocks and the plaque assay are detailed elsewhere (Turk et al., 1987). The virus inoculum contained approximately 100 PFU of HCMV. Selected concentrations of the compound were dissolved in the medium and added 1 h after addition of virus. At 10–12 days plaques were enumerated microscopically at a magnification of 30-fold. Yield (titer) reduction assays were performed as reported previously (Prichard et al., 1990). Monolayer cultures of HFF cells were infected at a multiplicity of infection of 0.5 PFU per cell and incubated in the presence of test compounds for 7–10 days. Following three cycles of freezing at $-76°C$ and thawing at $37°C$, the resulting lysates were diluted and the amount of infectious virus was quantitated on new cultures of HFF cells.

The activity of compounds against other herpes viruses was determined by plaque assay. The susceptibility of MCMV (Smith strain) to antiviral drugs was assayed by plaque assay in primary mouse embryo fibroblasts. The sensitivity of HSV-1 (E-377 strain), HSV-2 (MS strain), and VZV (Ellen strain) to compounds was determined in HFF cells by the plaque reduction assay. Confluent cell monolayers in 6-well plates were inoculated with 20–50 PFU of the appropriate virus and incubated at 37°C and 90% humidity for 1 h. Serial 5-fold dilutions of each drug were prepared in twice concentrated MEM(E), mixed with an equal volume of a 1% agarose and 2 ml of the mixture added to the monolayer cultures. An additional overlay mixture without drug (1 ml) was added on day 4 for MCMV and on days 3 and 6 for VZV. At the appropriate time (HSV, 3 days; MCMV, 7 days; VZV, 10 days) monolayers were stained with neutral red and plaques enumerated either visually or with the aid of a stereo dissecting microscope. Drug-treated cultures were compared to untreated control cultures and IC_{50} values were calculated using a dose–effect analysis software program (Elsevier-Biosoft, Cambridge, U.K.).

Cell growth rates

The inhibitory effect of compound 229 on KB cell growth was studied and population doubling time and cell viability were determined as detailed elsewhere (Nassiri et al., 1990). Briefly, 6-well plastic tissue culture dishes were seeded with 80,000 or 100,000 cells per well. Approximately 24 h later, the medium from the wells was aspirated and fresh medium with or without selected concentrations of drug was added. The incubation period was continued to 96 or 120 h at 37°C. Every 24 h, cultures were harvested by means of 0.05% trypsin plus 0.02% EDTA in HBS. Cells were enumerated by using a Coulter Counter (model ZF, Coulter Electronics, Hialeah, FL). Viability of untreated cultures were periodically checked by trypan blue dye exclusion. Control cells showed a viability of > 95%. Population doubling times were calculated by means of a least squares program fitting the exponential portion of the growth curve.

Plating efficiency

Plating efficiencies were carried out by procedures described earlier (Nassiri et al., 1991a). Briefly, KB cells were diluted with medium and planted in tissue culture dishes (150 × 25 mm, Falcon, Oxnard, CA) for a final concentration of 500–650 cells per dish. After an incubation period of 12–14 days, medium was removed and cells were rinsed with buffer solution then fixed with absolute methanol. After 2 min, the cells were stained with 0.1% crystal violet and clones > 1 mm in diameter were enumerated.

Flow cytometry

DNA-flow cytometry was carried out by procedures outlined earlier (Nassiri et al., 1990) with several modifications to be detailed elsewhere (Nassiri et al., manuscript in preparation). Briefly, untreated and drug-treated HFF cells were fixed in 70% ethanol for a minimum of 24 h. When ready for flow cytometric analysis, the cells were pelleted and resuspended in 500 μl of DNase (1 mg/ml of 2340 U/mg, Sigma, St. Louis, MO) per 10^6 cells and incubated at 37°C for 45 min. 100 μl of 1 mg/ml propidium iodide reagent (PI, Sigma, St. Louis, MO) was added to the suspension containing 1 × 10^6 cells which was chilled on ice water for 60 min. Propidium stained nuclei were analyzed on an EPICS V Flow Cytometer fitted with an argon-ion laser emitting at 488 nm (400 mW), a 515 nm long pass filter, a 560 nm dichroic filter and a 630 nm long pass filter (red, DNA). Chicken erythrocytes were added in amounts equal to 20% of each sample preparation as internal biological standards. In all samples, a total of 10,000 nuclei were analyzed. Percentage estimates of cells in G_0/G_1, S and G_2/M phases were made using an integration software analysis package provided with the flow cytometer for the Easy II computer (Coulter Electronics, Hialeah, FL).

Data analysis

Dose–response relationships were used to quantitate drug effects. These were

constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. The 50% inhibitory (IC_{50}) concentrations were calculated from the regression lines using the methods described by Goldstein (1964). Ganciclovir (DHPG; kindly provided by Syntex Corporation, San Francisco, CA) was used as a positive control in all antiviral assays.

Results

Chemistry

The original synthesis of compound 229 (Gupta et al., 1989a) was not suitable for multi-gram synthesis and the first step in the reaction scheme itself resulted in an isomeric mixture of alkylated products. We have utilized the approach of Ramasamy (1986) which fixes the site of alkylation on the pyrrole and have also reduced the total steps in the synthesis of compound 229 (Fig. 1). The alkylated pyrrole **3** was obtained in 55% yield by treatment of the known pyrrole (Swayze et al., 1991) (**1**) with triethylorthoformate followed by the addition of sodium hydride and (2-acetoxyethoxy)methyl bromide (Robins and Hatfield, 1982). Ring annulation and removal of the protecting group was achieved with saturated methanolic ammonia in a pressure bottle at room temperature to give **4** in excellent yield (89%). Debromination of **4** via catalytic hydrogenation gave **5** which, when treated with methanolic sodium hydrosulfide generated in situ, gave 229 in a two-step yield of 28%. The overall yield for the new synthesis of compound 229 was 14% compared to the original value of 0.6% (Gupta et al., 1989a). Subsequent runs on a smaller scale have given compound 229 in yields of approximately 40% from **1**.

Biological evaluation

Antiviral activity. In our initial studies, compound 229 was moderately active against HCMV in plaque-reduction assays with an IC_{50} of 11 μM (Gupta et al., 1989a). In three separate experiments in the current study the average activity

TABLE 1

Activity of compound 229 against selected herpesviruses

Virus	Cell line[a]	50% inhibitory concentration (μM)[b]		
		Compound 229	Ganciclovir	Acyclovir
HCMV	human foreskin fibroblasts	7	10	—
MCMV	mouse embryo fibroblasts	16	2.6	—
HSV-1	human foreskin fibroblasts	21	—	1.3
HSV-2	human foreskin fibroblasts	30	—	3.6
VZV	human foreskin fibroblasts	46	—	9.3

[a]Used to propagate the virus and assess visual cytotoxicity.
[b]Virus strains and plaque assays described in the text, average of two or more experiments.
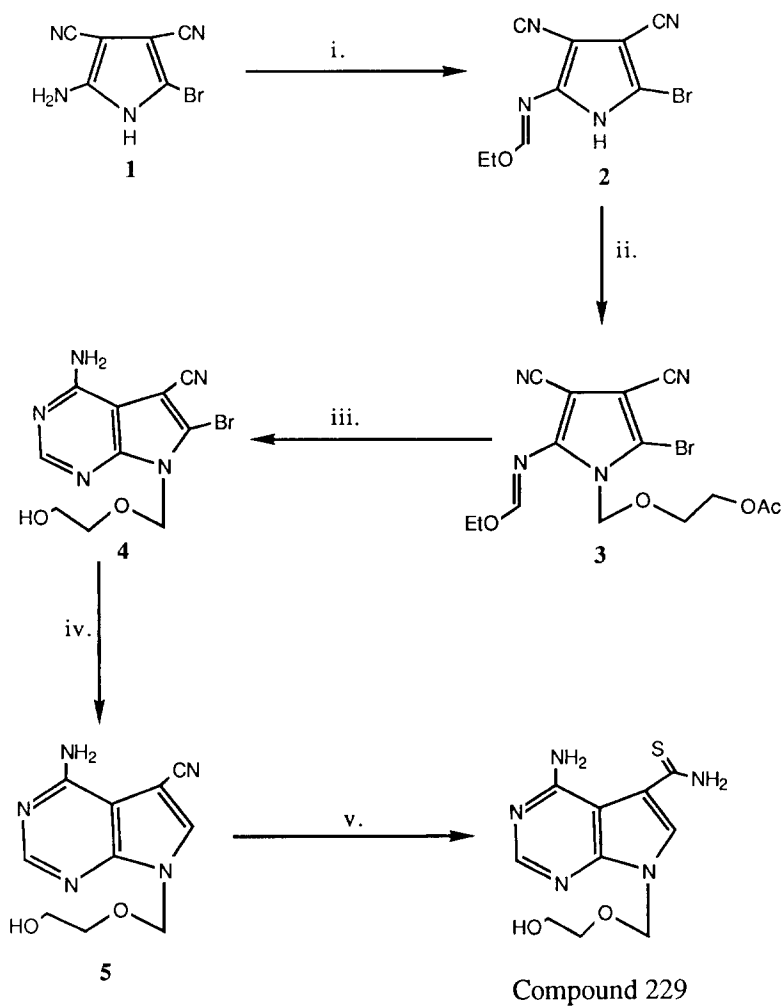
Fig. 1. Synthesis of 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (compound 229). Numbered reactants are as follows: i. CH(OEt)₃, CH₃CN ii. NaH, (2-acet-oxyethoxy)methyl bromide iii. NH₃, MeOH iv. Pd/C (10%), H₂ v. NaOMe, MeOH and H₂S.

against HCMV in a plaque reduction assay was 7 μM for compound 229 and 10 μM for ganciclovir (Table 1). Compound 229 was somewhat less active than

TABLE 2

Population doubling times of KB cells treated with compound 229[a]

Concentration of compound 229 (μM)	0	10	32	100	100 (reversal)
Population doubling time (h)	21.0	21.5	23.5	34.7	23.9

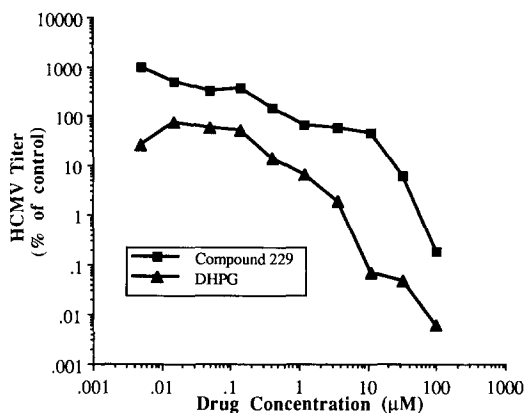[a]Results are from duplicate cultures. Experiment was performed two times.

Fig. 2. Effect of compound 229 and DHPG on the replication of HCMV. Subconfluent monolayers of HFF cells were infected at a multiplicity of infection of 0.5 PFU per cell and incubated in the presence of test compounds for 7 days. Infectious virus present at that time was quantitated by plaque assay on HFF cells as described in the text. Average data from two experiments are presented.

ganciclovir against MCMV and was least active against HSV-1, HSV-2 and VZV (Table 1). In previous work (Gupta et al., 1989a), we found slight activity ($\sim 30\%$ inhibition) against HSV-1 at 100 μM. In more extensive evaluations,
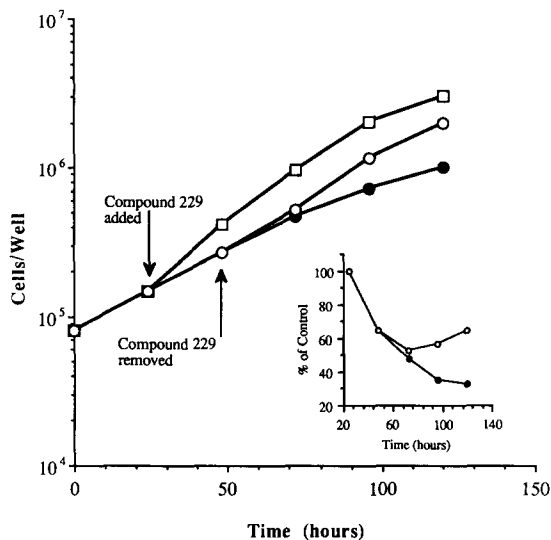

Time (hours)

Fig. 3. Reversibility of inhibition of KB cell growth by compound 229. Cells were seeded at 80,000 cells per well and incubated for 24 h at which time 100 μM compound 229 was added to two sets of cultures (O, ●) and incubated for an additional 24 h. At that time, medium was removed from one set of drug-containing cultures (O) and following a rinse with medium, the cultures were incubated with drug free medium. Cells were subsequently harvested at selected time periods up to 120 h and enumerated with a Coulter counter. Inset: plot (as percent of cells in control cultures) of total KB cells in cultures continually treated with 100 μM compound 229 (●) and cultures from which drug was removed after 24 h (O). Similar results were obtained in two separate experiments.
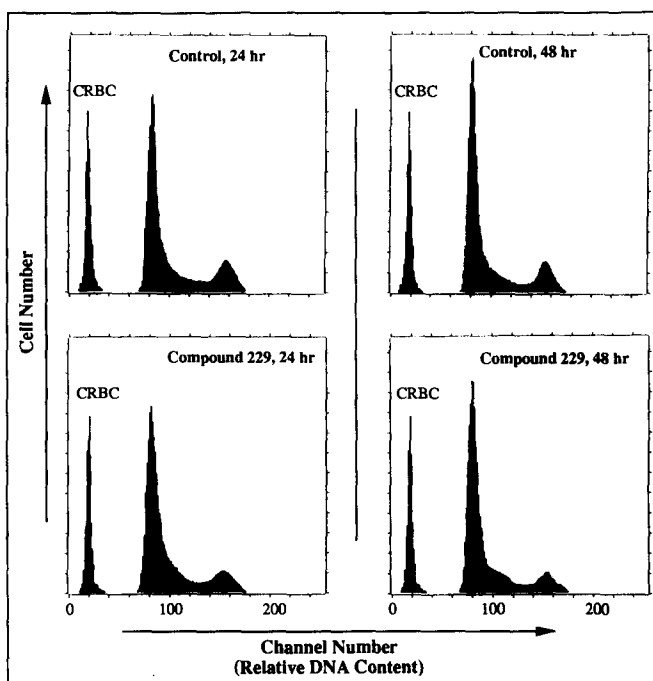
Fig. 4. Flow histograms illustrating the response of HFF cells to 100 μM compound 229 for 24 and 48 h. Percentages of G_0/G_1, S and G_2/M were determined as described in the text: Control, 24 h: G_0/G_1, 54; S, 27; G_2/M, 19. Compound 229, 24 h: G_0/G_1, 53; S, 33; G_2/M, 14. Control 48 h: G_0/G_1, 64; S, 24; G_2/M, 12. Compound 229 48 h: G_0/G_1, 65; S, 28; G_2/M, 7.

compound 229 was less active than ganciclovir in yield reduction experiments (Fig. 2) (IC_{90} = 25 μM vs. 1 μM, respectively). Compound 229 reduced HCMV titer by more than three log_{10} units (> 1000-fold) at 100 μM compared to a greater than four log_{10} reduction by ganciclovir.

Effects on uninfected cells. The effect of compound 229 on the growth of uninfected KB cells was examined as a measure of drug cytotoxicity. Compound 229 slightly suppressed the growth of KB cells at 10 and 32 μM. Population doubling times increased from 21 h in the control to 21.5 h with 10 μM and 23.5 h with 32 μM (Table 2). Cells continued to grow at concentrations up to 100 μM, where the population doubling time increased to 34.7 h. Cells did not grow in the presence of 320 μM.

The inhibition observed at 100 μM was reversible upon removal of the drug following a 24 h incubation period (Fig. 3). After the removal of compound 229, cell growth recovered to a population doubling time of 23.9 h (Table 2). This is more obvious in the inset which shows that the percentage of KB cells treated with 100 μM compound 229 increased after the removal of the compound, whereas the number of cells in the treated cultures continued to decline.

The ability of KB cells to attach to a substrate and clone in the presence of selected concentrations of compound 229 and DHPG was evaluated. We have found plating efficiency to be a technique often more sensitive than cell growth to evaluate cytotoxicity. Compound 229 had an IC_{50} value of 23 μM compared to an IC_{50} of 51 μM for ganciclovir (data not shown).

As an additional and detailed measure of cytotoxicity, perturbations in the cell cycle caused by compound 229 were measured by utilizing DNA flow cytometry (Barlogie et al., 1983; Gray and Coffino, 1979; Nassiri et al., 1990). Incubation of 100 μM compound 229 for 24 and 48 h with HFF cells resulted in an accumulation of cells in early S phase with a concurrent reduction of cells in G_2/M phase (Fig. 4). These data are consistent with an early S phase blockage and with the cell growth studies which showed inhibition of cell growth at 100 μM. The effect of 100 μM compound 229 on the cell cycle of KB cells was essentially the same as HFF cells at 12, 36, 48 and 72 h of treatment (data not shown). Concentrations of 10 and 32 μM over similar time periods showed no significant perturbations in the cycle of KB cells compared to control (data not shown). In other flow cytometry studies, we found that ganciclovir had a minor effect on the KB cell cycle at 100 μM. There appeared to be a 4% decrease in the total number of cells in G_0/G_1 with a similar increase in the number of cells in S phase. These effects, however, were not statistically significant by Student's t-test.

Discussion

In the present study, we have described the multi-gram synthesis of an acyclic analog of thiosangivamycin; examined or re-examined the activity against selected herpesviruses including HCMV, MCMV, HSV-1, HSV-2 and VZV; and evaluated the cytotoxicity of the compound in uninfected cells using cell growth studies, plating efficiency and DNA flow cytometry. In cell growth experiments, the inhibitory effect of this compound was not immediate and did not dramatically affect the growth of KB cells up to 100 μM. But even this effect was reversible, suggesting that compound 229 is acting as a cytostatic rather than a cytotoxic agent. This raises the possibility that the compound may inhibit DNA polymerase rather than be a substrate and act as a chain terminator.

Flow cytometry results from two separate cell lines suggest that compound 229 may be interfering with the progression of cells through S phase since there is an increase in the percentage of cells S phase coupled with a loss of cells in G_2/M. The overall cell cycle perturbation was modest. Interestingly, two other acyclic 4-amino-5-substituted pyrrolo[2,3-d]pyrimidines act as S phase blockers (Nassiri et al., 1990). These compounds are 5-bromotubercidin analogs which differ from compound 229 in that one is an acyclovir analog (4-amino-5-bromo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine, compound 102) and the other a ganciclovir analog (4-amino-5-bromo-7-[(1,3-dihydroxy-2-

propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine, compound 183). Like compound 229, the effects of compound 102 on uninfected cells were reversible upon removal of the compound, whereas the removal of compound 183 did not reverse growth inhibition. Furthermore, cells treated with compound 229 morphologically resembled those treated with compound 102, not compound 183. Unlike compound 229, compound 102 blocked the progression of the cell cycle at mid S phase whereas, like compound 229, compound 183 blocked cells at late G_0/G_1 and early S phase. The inhibitory effects of both compounds 102 and 183, though, were observed at 10 μM compared to 100 μM for compound 229. Based upon these comparisons, compound 229 may be acting by a cellular mechanism which is different from either compound 102 or 183 even though all three are pyrrolo[2,3-*d*]pyrimidine analogs. The exact mechanism underlying this phenomenon is under investigation.

Although compound 229 was not as efficacious as ganciclovir in yield reduction assays against HCMV and was slightly more cytotoxic, this study provides insights into a new class of pyrrolo[2,3-*d*]pyrimidines which are worthy of further investigation.

Acknowledgements

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