Bioorganic & Medicinal Chemistry 20 (2012) 3710–3718

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and antiviral activity of certain second generation methylenecyclopropane nucleosides

John D. Williams [a,*], Atiyya R. Khan [a], Emma A. Harden [b], Caroll B. Hartline [b], Geraldine M. Jefferson [b], Kathy A. Keith [b], Mark N. Prichard [b], Jiri Zemlicka [c], Norton P. Peet [a], Terry L. Bowlin [a]

[a] Microbiotix, Inc., One Innovation Drive, Worcester, MA 01605, USA
[b] Department of Pediatrics, University of Alabama School of Medicine, 1600 6th Avenue South, Birmingham, AL 53233, USA
[c] Department of Chemistry, Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, 110 E. Warren Avenue, Detroit, MI 48201, USA

ARTICLE INFO

Article history:
Received 9 February 2012
Revised 17 April 2012
Accepted 25 April 2012
Available online 1 May 2012

Keywords:
Antiviral agents
Nucleoside analogs

ABSTRACT

A second-generation series of substituted methylenecyclopropane nucleosides (MCPNs) has been synthesized and evaluated for antiviral activity against a panel of human herpesviruses, and for cytotoxicity. Although alkylated 2,6-diaminopurine analogs showed little antiviral activity, the compounds containing ether and thioether substituents at the 6-position of the purine did demonstrate potent and selective antiviral activity against several different human herpesviruses. In the 6-alkoxy series, antiviral activity depended on the length of the ether carbon chain, with the optimum chain length being about four carbon units long. For the corresponding thioethers, compounds containing secondary thioethers were more potent than those with primary thioethers.

1. Introduction

The human herpesviruses (HHV) are a closely related family of enveloped, double-stranded DNA viruses that cause a range of human diseases.[1–3] There are eight distinct human herpesviruses, each of which is thought to be responsible for a set of human ailments, from oral and genital herpes (HHV-1 and HHV-2, respectively) to Kaposi's sarcoma (HHV-8). The human herpesviruses are highly prevalent in both urban and rural populations, can establish latent infections, and can have severe consequences, especially for immunocompromised populations such as AIDS patients, transplant recipients, neonates, and the elderly.

Although acyclovir[4] and its valine ester prodrug, valacyclovir,[5] (Fig. 1) have been extremely effective at treating herpes simplex (HSV or HHV-1/2) and varicella zoster (VZV or HHV-3) viruses, they are far less effective against the other members of the herpesvirus family. Other drugs, such as ganciclovir,[6] valganciclovir,[7] cidofovir,[8] and foscarnet[9] have also been used as anti-herpesvirus agents, particularly against human cytomegalovirus, but have relatively narrow therapeutic ranges because of their inherent toxicities. No drugs have been approved for use against HHV-6, HHV-7, or HHV-8.

Methylenecyclopropane nucleosides (MCPNs) are a series of acyclic nucleoside analogs that have showed promise against several different human herpesviruses.[10–13] A large series of mono-hydroxymethyl (first-generation) MCPNs with varying substituents at the purine 6-position has been synthesized (Fig. 2), and several SAR trends have been elucidated within that series.[14]

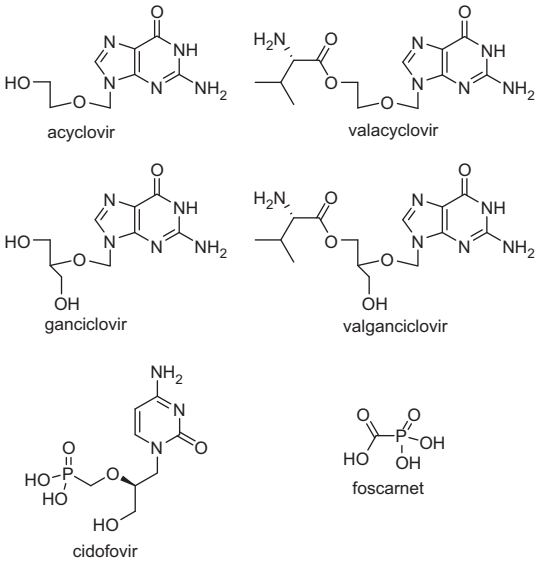


Figure 1. FDA-approved anti-herpes drugs

* Corresponding author.
E-mail address: jwilliams@microbiotix.com (J.D. Williams).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.04.049

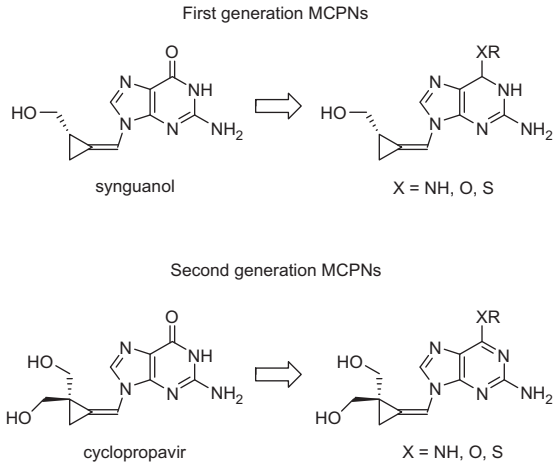


Figure 2. First and second generation MCPNs. SAR is well-defined for first generation compounds; SAR is little explored for second generation compounds.

Several first-generation MCPNs containing alkylamine, ether, and thioether substituents at the 6-position of the purine were potent inhibitors of HCMV (HHV-5), while the ether-containing analogs were generally more potent against HHV-6 and HHV-8.[15]

The synthesis and subsequent development of these first-generation compounds have been complicated, however, by the presence of a chiral center in the molecule, which requires a more complicated synthesis to execute with good enantiomeric excess. A second generation of MCPNs that eliminate the chiral center by adding a second hydroxymethyl group to the cyclopropyl ring has been subsequently synthesized and tested for antiviral activity.[10,12,16] Several of these second-generation MCPNs were found to be potent inhibitors of several different human herpesviruses. Most notably, the guanine analog, cyclopropavir, was found to be a potent inhibitor of HCMV both in vitro and in vivo; it also has moderate activity against EBV, HHV-6 and HHV-8. Cyclopropavir is currently undergoing preclinical development as a HCMV therapeutic.[17]

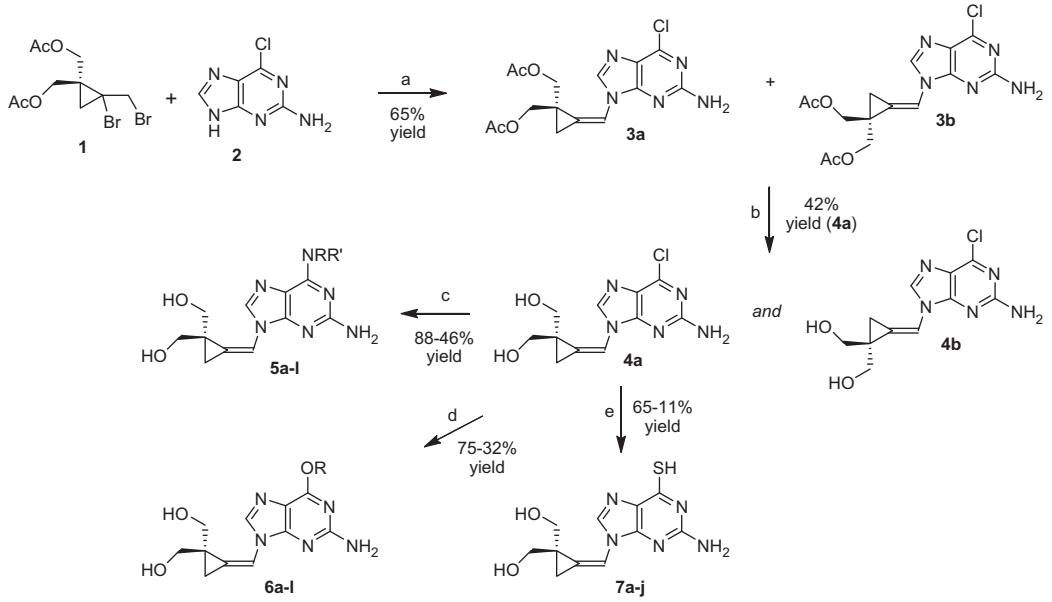
To date, far fewer examples of second-generation MCPNs have been synthesized and tested for antiviral activity than the corresponding first-generation compounds; given the limited number of second-generation compounds, SAR trends are difficult to establish. Herein we describe the synthesis of a range of second-generation MCPNs, and the conclusions that can be drawn from the different analogs.

2. Materials and methods

2.1. Chemistry

The chemistry used to synthesize the second-generation MCPNs has been adapted from the previous work of Zemlicka.[12,14] Thus, we began by synthesizing a versatile intermediate (**4a**) that could be scaled to multi-gram batches and used in the subsequent reactions without resorting to repetitious procedures (Scheme 1). To this end, commercially available 2-amino-5-chloropurine (**2**) was treated with dibrominated diester **1** in a two-stage reaction with base. In the initial low-temperature stage, the base is alkylated by **1**; after additional DBU is added, the temperature is raised, and dehydrohalogenation produces a mixture of the protected (*Z*)- and (*E*)-nucleosides **3a** and **3b** (1:1 mixture). Because it is impractical to separate the isomers in large batches at this stage, the subsequent saponification step was performed on the mixture. The resulting diols **4a** and **4b** are readily separated by crystallization, wherein the (Z)-isomer **4a** was then used for subsequent reactions.

Displacement of the 6-chloro group on the purine was readily achieved with amines, alkoxides, or thiolates (Scheme 1). Treating the intermediate **4a** with an excess of amine in ethanol at 70 °C provided the corresponding 2,6-diaminopurine analogs **5a–l** in 88–46% yield. Using a solution of alkoxide in DMF at 70 °C, the corresponding 2-amino-6-alkoxypurines **6a–l** were also synthesized in 75–32% yield. Reaction of thiolates and **4a** at elevated temperatures gave complex mixtures of products containing large amounts of a product containing two equivalents of thiol, as determined by ^{1}H NMR and LC–MS (data not shown), presumably from a second equivalent of thiolate adding to the double bond of the methylenecyclopropane moiety. Fortunately, we found that the thiolates were sufficiently nucleophilic to displace the 6-chloro group on the purine even at lower temperature; **4a** was thus reacted at room temperature with a series of thiolates, generated in situ from the



Scheme 1. Synthesis of 6-amino, 6-alkoxy, and 6-alkylthio MCPNs. Reagents and conditions: (a) DBU, DMF, 25–75 °C; (b) K_2CO_3, H_2O/MeOH; (c) amine, EtOH, 70 °C; (d) alcohol, NaH, DMF, 70 °C; (e) thiol, 1.0 M NaOH/H_2O, DMF, 25 °C.

corresponding thiols and aqueous sodium hydroxide, to provide a series of 2-amino-6-alkylthiopurines **7a–j** in 65–11% yield.

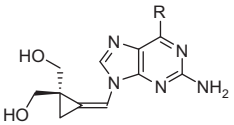
The ^{1}H of all the target compounds, and the ^{13}C NMR of representative compounds **5b**, **6a**, and **7a** corresponded very well with the analogous 1st generation methylenecyclopropane nucleosides,[14] as well as the limited number of known 2nd generation compounds.[18] Interestingly, addition of the second hydroxymethyl substituent in the methylenecyclopropane moiety resulted in some signals which were much more indistinct in the ^{13}C NMR spectra than in the corresponding 1st generation mono-hydroxymethyl compounds. Although the indistinct peaks complicated the identification of the compounds, we are unsure of the implication this phenomenon has to the biological activity of the compounds.

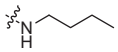
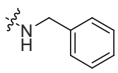
2.2. Biological evaluation

The methylenecyclopropane nucleosides were tested against a range of human herpesviruses, including herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), two variants of human herpes 6 (HHV-6A and HHV-6B), and human herpesvirus 8 (HHV-8). Cytotoxicity was also determined for the compounds in each of the cell lines used for the antiviral evaluations using the same cell number and duration of compound exposure. None of the compounds tested exhibited significant activity against HSV-1, HSV-2. One of the new 2,6-diaminopurine analogs (**5d**; see Table 1) demonstrated modest activity against HCMV, but the spectrum of activity was quite limited, with little or no activity against EBV, VZV, HHV-6, or HHV-8. This result was unexpected given the potent and relatively broad-spectrum antiviral activity of the analogous first-generation MCPNs, which show potent activity against all of these pathogens.

In contrast to the diaminopurine analogs, compounds containing ether and thioether substituents (see Table 2 and Table 3) at the 6-position had much broader antiviral profiles, very much like the first-generation MCPNs. In particular, certain members of the

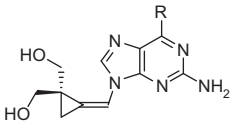
Table 1
Antiviral activity and cytotoxicity for 6-alkylamino-substituted MCPN analogs

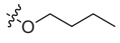
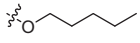
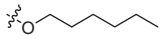
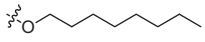
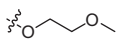
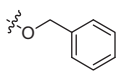


#	R	50% inhibitory concentration[a] (μM)												
		IC_{50}				CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}
		HSV-1 (CPE)	HSV-2 (CPE)	VZV (CPE)	HCMV (CPE)	HFF	EBV (hybrid)	Akata	HHV-6A (hybrid)	HSB-2	HHV-6B (hybrid)	MOLT-3	HHV-8 (QPCR)	BCBL-1
Cyclopropavir (CPV)		>100	>100	>100	1.4	>300	45	>20	1.3	>20	8.0	>20	3.0	>20
DAP[b]	NH_2	>300	>300	>300	>300	>300	11	>20	>20	>20	16	>20	30	67
5a		>300	>300	>300	>300	265	>24	>24	>20	>20	>20	>20	62	67
5b		>300	>300	>300	>300	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
5c		>300	>300	>300	160	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
5d		>60	>60	>60	24	>300	>20	>20	>20	>20	>20	>20	71	71.5
5e		>300	>300	>300	>60	>300	>24	>24	>20	>20	>20	>20	>20	>20
5f		>300	>300	>300	>300	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
5g		>60	>60	>60	>60	>300	>20	>20	>20	>20	>20	>20	63	71.7
5h		>300	>300	>300	>300	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
5i		>300	>300	>60	>60	>300	>24	>24	>20	>20	>20	>20	n.d.[c]	19.9
5j		>300	>300	180	>300	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
5k		>300	>300	>300	>300	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
5l		>300	>300	>300	>300	>300	>24	>24	>20	>20	n.d.[c]	n.d.[c]	>20	>20
Acyclovir (ACV)		2.9	2.8	1.9		>300								
Ganciclovir (GCV)					2.1	>300								

[a] Antiviral and cytotoxicity assays were performed in duplicate wells as described in the text.
[b] DAP is 2,6-diaminopurine.
[c] n.d. means value not determined.

Table 2
Antiviral activity and cytotoxicity for 6-alkoxy-substituted MCPN analogs



#	R	50% inhibitory concentration[a] (μM)												
		IC_{50}				CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}
		HSV-1 (CPE)	HSV-2 CPE)	VZV (CPE)	HCMV (plaque)	HFF	EBV (hybrid)	Akata	HHV-6A (hybrid)	HSB-2	HHV-6B (hybrid)	MOLT-3	HHV-8 (QPCR)	BCBL-1
Cyclopropavir (CPV)		>100	>100	>100	1.4	>300	45	>20	1.3	>20	8.0	>20	3.0	>20
AMP[b]		>300	>300	7.4	2.8	171	1	>20	>20	>20	>20	>20	13	51
6a		>300	>300	>100	1.6	>300	11.9	>20	>20	>20	11.2	>20	>20	>20
6b		>300	>300	2.9	1.1	>300	8.9	>20	18	>20	6.6	>20	16	>20
6c		>300	>300	>300	1.1	>300	11.0	>20	>20	>20	11.4	>20	17	>20
6d		>300	>300	>100	1.6	>300	1.6	>20	>20	>20	3.2	>20	>20	>20
6e		>300	>300	>60	0.5	259	0.5	>20	>20	>20	>20	>20	>20	>20
6f		>300	>300	n.d.[c]	3.9	>200	7.3	>20	>20	>20	9.5	>20	>20	>20
6g		>300	>300	>100	29	>300	13	>20	>20	>20	11.5	>20	>20	>20
6h		>300	>300	>300	5.6	>300	1.4	>20	>20	>20	8.9	>20	19	>20
6i		>300	>300	13.9	17	>300	20	>20	>20	>20	>20	>20	>20	>20
6j		>300	>300	53.0	45	>300	>20	>20	>20	>20	>20	>20	>20	>20
Acyclovir (ACV)		2.9	2.8	1.9		>300								
Ganciclovir (GCV)					2.1	>300								

[a] Antiviral and cytotoxicity assays were performed in duplicate wells as described in the text.
[b] AMP is 2-amino-6-methoxypurine.
[c] n.d. means value not determined.

2-amino-6-alkoxypurine analog series **6a–j** demonstrated broad antiviral activity, especially against EBV, HCMV, and HHV-6b, with some analogs possessing activity moderate against VZV and HHV-8 as well; the antiviral activity of these compounds was similar to that of the corresponding 1st generation analogs. Antiviral activity generally varied with the size of the ether carbon chain; with the optimal size for broad activity being about 4 or 5 carbons in length (**6d and 6e,** respectively), although any analog with less than 6-carbon ether functionalities (e.g., **6a**, **6b**, **6c**, etc.) also had good activity. Inclusion of a heteroatom in the ether unit (i.e., **6i**) decreased the activity relative to the corresponding hydrocarbon (**6d**), but the incorporation of halogens (i.e., **6h**) was largely tolerated. The benzyl ether **6j**, however, was much less active than the other analogs.

The thioether series (**7a–i**) was somewhat less potent in the antiviral assays than the corresponding ethers, but several compounds did possess notable antiviral potency, especially against EBV. The most potent compound among the thioether analogs was the isopropylthio analog **7c**, which had the best activity against HCMV, and the cyclopentylthio analog **7f**, which had the highest activity against EBV. Interestingly, both of these compounds are synthesized from secondary thiols, whereas the compounds synthesized from primary thiols (e.g., **7b** and **7d**) had somewhat lower activity against HCMV. Because of the more limited commercial availability of secondary thiols, however, we were unable to determine whether this was a general phenomenon, or merely a coincidence. Compared to the 1st generation compounds, the 6-alkylthio analogs in the current study were somewhat less potent against HCMV and HHV-6, but much more potent against EBV

3. Conclusion

In conclusion, we have synthesized a range of second-generation methylenecyclopropane nucleosides designed to test the antiviral activity and antiviral spectrum for the series. The compounds were synthesized by nucleophilic displacement of a versatile 2-amino-6-chloropurine intermediate that could be further elaborated into the desired target compounds. The 2-amino-6-alkylamino analogs were generally much less active then their corresponding 1st generation counterparts. The 2-amino-6-alkylthio analogs generally had potent anti-EBV activity and moderate activity against HHV-6A and HHV-6B. The most potent compounds were the 2-amino-6-alkoxypurine analogs; these compounds demonstrated potent and selective antiviral activity against several different human herpesviruses, including HCMV, EBV, and HHV-6B. Additional studies evaluating the suitability of the ether series for additional development are underway, and will be reported in the future.

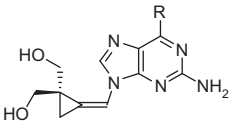
4. Experimental section

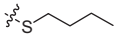
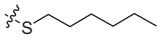
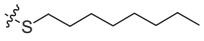
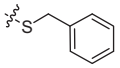
4.1. Biology

4.1.1. Cells and viruses

Human foreskin fibroblast (HFF) cells were prepared from human foreskin tissue obtained from the University of Alabama at Birmingham tissue procurement facility with approval from its IRB. The E-377 strain of HSV-1 and the MS strain of HSV-2 were a gift of Jack Hill (Burroughs Wellcome). The AD169 strain of HCMV and the Ellen strain of VZV were obtained from the American Type

Table 3
Antiviral activity and cytotoxicity for 6-alkylthio-substituted MCPN analogs



#	R	50% inhibitory concentration[a] (μM)													
		IC_{50}				CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}	
		HSV-1 (CPE)	HSV-2 (CPE)	VZV (CPE)	HCMV (plaque)	HFF	EBV (hybrid)	Akata	HHV-6A (hybrid)	HSB-2	HHV-6B (hybrid)	MOLT-3	HHV-8 (QPCR)	BCBL-1	
Cyclopropavir (CPV)			>100	>100	>100	1.4	>300	45	>20	1.3	>20	8.0	>20	3.0	>20
7a		>300	>300	>60	>50	>300	11.4	>20	>100	>100	11.6	>20	>20	>20	
7b		>300	>300	>300	42	>300	>20	>20	63	98	14.3	>20	>20	>20	
7c		>300	>300	>60	4.0	>300	3.1	>20	70	94	7.5	>20	>20	>20	
7d		>300	>300	>300	>50	>300	2.5	>20	75	89	7.0	>20	>20	>20	
7e		>300	>300	>50	31	>300	2.3	>20	>20	60	n.d.[b]	n.d.[b]	>20	>20	
7f		>300	>300	>300	>50	>300	0.9	>20	35	91	9.9	>20	>20	>20	
7g		>300	>300	>300	>50	>300	7.1	>20	>20	60	5.4	>20	>20	>20	
7h		>300	>300	>60	20	>300	>20	>20	>20	60	11.0	>20	>20	>20	
7i		>300	>300	>60	>50	>300	3.1	>20	88	>100	>20	>20	>20	>20	
Acyclovir (ACV)		2.9	2.8	1.9		>300									
Ganciclovir (GCV)					2.1	>300									

[a] Antiviral and cytotoxicity assays were performed in duplicate wells as described in the text.
[b] n.d. means value not determined.

Culture Collection (ATCC) (Manassas, VA). The phenotype of these isolates as well as the genotype of relevant genes was reported previously.[19] Epstein-Barr virus (EBV)-infected Akata cells were a gift from John Sixbey (Louisiana State University, Baton Rouge). HSB-2 cells and the GS strain of human herpesvirus 6 variant A (HHV-6A), as well as BCBL1 cells infected with HHV-8, were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. The Z29 strain of the B variant of human herpesvirus 6 (HHV-6B) and MOLT-3 cells were obtained from Scott Schmid at the Centers for Disease Control and Prevention, Atlanta, GA.

4.1.2. Evaluation of antiviral activity against HSV-1, HSV-2, HCMV, and VZV

Antiviral activity was evaluated in primary CPE reduction assays. Briefly, compounds were diluted in 96-well plates containing monolayers of HFF cells; the cells were then infected at a multiplicity of infection of approximately 0.01 PFU/cell for HSV-1 or HSV-2. Approximately 100 infected cells were used to initiate infection with VZV. Infected monolayers were incubated in a humidified 5% CO_2 incubator for 3 days for HSV-1 and HSV-2, 7 days for VZV, and 14 days for CMV. After the incubation period, the media was aspirated and the cells stained with a 0.1% crystal violet in a formalin solution for 4 h. The stain was removed and the monolayers were rinsed with water until all excess stain was removed. The plates were allowed to dry for 24 h and the absorbance at 595 nm was used to calculate compound concentrations that reduced viral CPE by 50% (EC_{50}). The same drug concentrations used to determine efficacy were also used on uninfected cells in each assay to determine the cytotoxicity of each experimental compound. The drug concentration sufficient to reduce cell number by 50% as estimated by crystal violet staining was used to determine cytotoxicity (CC_{50}).

Antiviral activity against HCMV was determined in plaque reduction assays by methods reported previously.[20] Briefly, monolayers of HFF cells in 6-well plates were prepared 2 days prior to infection which were then infected with virus suspensions sufficient to yield 30 plaques per well. Compound dilutions were then prepared in MEM with 2% FBS and were supplemented with standard concentrations of L-glutamine, penicillin, gentamycin, and pooled human gamma globulin. After a 1 h adsorption period, compound solutions were added and the infected monolayers were incubated for 8 days. Cell monolayers were then stained with neutral red and plaques were enumerated using a stereomicroscope. The experimental data were used to interpolate EC_{50} values by standard methods.

4.1.3. Evaluation of activity against EBV

Determination of antiviral activity against the Akata strain of EBV was described previously.[21] Briefly, Akata cells were passaged routinely in RPMI 1640 supplemented with 10% FBS and standard concentrations of L-glutamine, penicillin, and gentamicin. Compounds were diluted directly in growth media in 96-well round bottom assay plates. Wells containing compound dilutions were then seeded with latently infected Akata cells that were induced to undergo a productive infection by the addition of a goat anti-human immunoglobulin G antibody. After 72 h, the DNA in the cultures was denatured and transferred onto a positively charged nylon membrane and viral DNA was quantified using a digoxigenin-labeled probe and a monoclonal antibody specific for the hapten. Compound concentrations sufficient to reduce viral DNA by 50% were used to calculate EC_{50} values. Assays for cytotoxicity were prepared in the same manner as those for the antiviral assay but with uninfected cells. Cells were incubated with drug for seven days using the same conditions as the antiviral assay and 50 μL of

CellTiter-Glo was added to each well. Luminescence was quantified on a luminometer and concentrations of compounds sufficient to reduce cell numbers by 50% (CC_{50}) were calculated from the data.

4.1.4. Assessment of activity against HHV-6

Compounds were evaluated by methods reported previously.[20] Briefly, stock solutions were serially diluted in cell culture media in 96-well plates. Uninfected HSB-2 or Molt-3 cells were then seeded into 96 well plates and the infection was initiated with HHV-6A infected HSB-2 cells, or HHV-6B infected Molt-3 cells. Cultures were incubated for 7 days at 37 °C. Infected cells were then lysed in denaturation buffer (4.5 M NaCl, 1.2 M NaOH) and the lysate was applied to a positively charged nylon membrane to immobilize the DNA. A digoxygenin-labeled probe specific for viral DNA was used in a hybridization assay to quantify the accumulation of viral DNA and EC_{50} values were calculated from the experimental data. Cytotoxicity was determined in uninfected cells by the same methods used for Akata cells.

4.1.5. Activity against HHV-8

Compounds were evaluated by methods reported previously.[20] Briefly, BCBL1 cells were maintained in growth medium consisting of RPMI 1640 supplemented with 10% FBS, penicillin, gentamycin, and L-glutamine. Compounds were diluted in triplicate wells of a 96-well plate and cells that had been induced to undergo a lytic infection with phorbol 12-myristate 13-acetate were added to the assay plates. Cultures were incubated for 7 days at 37 °C in a humidified CO_2 incubator. Total DNA was prepared with a Wizard SV 96-well purification kit (Promega), and viral DNA was quantified by quantitative PCR using forward primer 5′-TTC CCC AGA TAC ACG ACA GAA TC-3′, reverse primer 5′-CGG AGC GCA GGC TAC CT-3′, and probe 5′-(6-carboxyfluorescein)-CCT ACG TGT TCG TCG AC-(6-carboxytetramethylrhodamine)-3′. Compound concentrations sufficient to reduce viral DNA accumulation by 50% (EC_{50}s) were calculated by standard methods. Cytotoxicity was determined with CellTiter-Glo by methods used for Akata cells.

4.2. Chemistry

4.2.1. General procedures

1,1-Bis(acetoxymethyl)-2-bromo-2-(bromomethyl)cyclopropane (**1**, Scheme 1) was obtained according to literature procedures;[12] all other reagents and solvents were obtained from commercial sources and used without additional purification. Synthesis of (*Z*)-2-amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}purine and (*E*)-2-amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}purine (**3a/b**). and (Z)-2-amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (**4a**) were carried out as large-scale modifications of the literature procedure[12] as described below. Evaporation of solvents was accomplished under reduced pressure (40–60 mmHg), at less than 40 °C, unless otherwise noted. Chromatography solvent systems are expressed in v/v ratios or as % vol. Melting points were taken on EZ-Melt automated melting point apparatus (Stanford Research Systems, Inc.) in manual mode, and are uncorrected. Thin-layer chromatography was performed on silica gel GHLF plates from Analtech (Newark, DE). Chromatograms were visualized under UV light at 254 nm. ^{1}H NMR spectra were obtained at 300 MHz on a Bruker DPX300 spectrometer. Chemical shift values for ^{1}H were determined relative to an internal tetramethylsilane standard (0.00 ppm). High-resolution mass spectrometry (HRMS) was performed at the Scripps Institute mass spectrometry facility (La Jolla, CA). Analytical HPLC was performed at CreaGen Biosciences (Woburn, MA). All compounds were found to be ⩾95% pure by analytical HPLC, except for **5a** (90%), **5b** (90%) and **5g** (89%).

4.2.2. (*Z*)-2-Amino-6-chloro-9-{[2,2-bis(acetoxymethyl)-cyclopropylidene]methyl}purine (3a) and (*E*)-2-Amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}-purine (3b)

To a stirred solution of 1,1-bis(acetoxymethyl)-2-bromo-2-(bromomethyl)cyclopropane (**1**, 329 g, 0.92 mol) and 2-amino-6-chloropurine (**2**, 136 g, 0.80 mol) in anhydrous DMF (2.0 L) cooled to 0 °C was added DBU (139 mL, 0.92 mol). The solution was allowed to warm to room temperature over 3 days with moderate stirring. Additional DBU (146 mL, 0.92 mol) was then added, and the solution was heated to 75 °C, and stirred at this temperature for 16 h. The reaction was poured into a mixture of H_2O (5.0 L), ice (1 kg), 85% phosphoric acid (280 mL), and EtOAc (6.0 L). The mixture was agitated, then separated. The organic layer was washed with 5% aqueous NaCl (5.0 L), saturated aqueous NaCl (2.5 L), then evaporated to a volume of 1.0 L and refrigerated for 24 h. The resulting precipitate was filtered, washed with 1:1 hex/EtOAc (250 mL), and dried to yield 189 g (65%) of **3a** and **3b** (∼1:1 mixture) as light-colored powder: ^{1}H NMR ($CDCl_3$) δ 8.43 (s, 1H), 8.32 (s, 1H), 7.40 (s, 1H), 7.30 (s, 1H), 7.06 (s, 2H), 7.02 (s, 2H), 4.28 (d, 4H), 4.06–4.15 (m, 4H), 2.04 (s, 6H), 1.94 (s, 6H), 1.89 (d, 2H), 1.64 (s, 2H).

4.2.3. (Z)-2-Amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (4a)

To a stirred solution of (*Z*)-2-amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}purine and (*E*)-2-amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}purine (1:1 **3a**:**3b**, 193 g, 0.53 mol) in MeOH (6.5 L) and H_2O (720 mL) was added potassium carbonate (75.5 g, 0.54 mol). The suspension was stirred at room temperature for 30 min, then AcOH (62 mL, 1.1 mol) was added in one portion, and the mixture stirred at room temperature for 16 h. The resulting precipitate was filtered and rinsed with MeOH (500 mL) to provide the undesired *E* isomer **4b**. The filtrate was evaporated, and EtOH (2.5 L) was added to the residue. The EtOH was then evaporated and the residue triturated in $CHCl_3$ (2.2 L) and MeOH (250 mL). The mixture was stirred at room temperature for 16 h, and the resulting solids filtered, rinsed with 10% MeOH/$CHCl_3$, and dried to yield 62.9 g (42%) of **4a** (containing ∼8% of **4b**) as a pale tan powder. An additional 1.1 g of pure **4a** was obtained upon concentration of the filtrate: ^{1}H NMR (DMSO-d_6) δ 1.34 (s, 2H), 3.47–3.66 (m, 4H), 5.04 (t, 2H), 7.03 (s, 2H), 7.18 (s, 1H), 8.81 (s, 1H).

4.2.4. General method A; 2-amino-6-alkylaminopurine analogs

To a solution of (*Z*)-2-amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (**4a**; 100 mg, 0.35 mmol) in absolute EtOH (3 mL) was added the respective amine (0.5 mL, excess). The mixture was heated to 85 °C for 2–48 h, then cooled and evaporated under vacuum. The crude mixture was then purified by flash chromatography on silica gel with 0–8% MeOH/$CHCl_3$. Product-containing fractions were pooled and evaporated, and the resulting solids recrystallized from hot CH_3CN to provide the desired product.

4.2.4.1. (*Z*)-2-Amino-6-methylamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5a). Intermediate **4a** was treated with a solution of methylamine in THF according to method A for 16 h to provide 66 mg (68%) of **5a** as a fluffy white powder: mp 231–233 °C; R_f 0.09 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H), 7.3–7.1 (br s, 1H), 7.15 (s, 2H), 5.89 (s, 2H), 4.98 (t, 2H), 3.55 (ddd, 4H), 2.88 (br s, 3H), 1.29 (d, 2H); HRMS (ESI) *m*/*z* calcd for $C_{12}H_{17}N_6O_2$ $[M+H]^+$ 277.1407, found 277.1405.

4.2.4.2. (*Z*)-2-Amino-6-(1-propylamino)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5b). Intermediate **4a** was treated with 1-aminopropane according to method A for 48 h to provide 80 mg (75%) of **5b** as a fluffy white powder: mp 190–192 °C; R_f 0.18 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H), 7.16 (s, 2H), 5.86 (s, 2H), 5.00 (t, 2H), 3.66 (dd, 2H), 3.62 (dd, 2H), 3.33 (m, 2H under H_2O), 1.57 (q, 2H), 1.30 (s, 2H), 0.88 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 160.52, 155.00, 149.51, 134.15, 116.00, 112.91, 111.49/109.65, 65–60 (indistinct multiplet), 30.62, 25–20 (indistinct multiplet), 13–8 (indistinct multiplet); HRMS (ESI) *m*/*z* calcd for $C_{14}H_{21}N_6O_2$ $[M+H]^+$ 305.1720, found 305.1720.

4.2.4.3. (*Z*)-2-Amino-6-(2-propylamino)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5c). Intermediate **4a** was treated with 2-aminopropane according to method A for 16 h to provide 66 mg (61%) of **5c** as a white powder: mp 163–165 °C; R_f 0.36 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 7.16 (s, 1H), 6.95 (d, 1H), 5.86 (s, 2H), 5.00 (t, 2H), 4.35 (br s, 1H), 3.64 (dd, 2H), 3.52 (dd, 2H), 1.30 (s, 2H), 1.17 (d, 6H); HRMS (ESI) *m*/*z* calcd for $C_{14}H_{21}N_6O_2$ $[M+H]^+$ 305.1720, found 305.1722.

4.2.4.4. (*Z*)-2-Amino-6-cyclopropylamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5d). Intermediate **4a** was treated with cyclopropylamine according to method A for 16 h to provide 80 mg (76%) of **5d** as a white powder: mp 172–175 °C; R_f 0.21 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H), 7.36 (s, 1H), 7.15 (s, 1H), 5.91 (s, 2H), 4.98 (s, 2H), 3.61 (d, 2H), 3.50 (d, 2H), 3.02 (s, 1H), 1.4–1.2 m, 2H), 0.7–0.5 (m, 4H); HRMS (ESI) *m*/*z* calcd for $C_{14}H_{19}N_6O_2$ $[M+H]^+$ 303.1564, found 303.1566.

4.2.4.5. (*Z*)-2-Amino-6-(1-butylamino)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5e). Intermediate **4a** was treated with 1-aminobutane according to method A for 48 h to provide 62 mg (55%) of **5e** as a fluffy white powder: mp 206–209 °C; R_f 0.19 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H), 7.16 (br s, 2H), 5.86 (br s, 2H), 5.00 (t, 2H), 3.64 (dd, 2H), 3.52 (dd, 2H), 3.41 (br s, 2H), 1.57–1.50 (m, 2H), 1.36–1.30 (m, 4H), 0.90 (t, 3H); HRMS (ESI) *m*/*z* calcd for $C_{15}H_{23}N_6O_2$ $[M+H]^+$ 319.1877, found 319.1874.

4.2.4.6. (*Z*)-2-Amino-6-isobutylamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5f). Intermediate **4a** was treated with isobutylamine according to method A for 48 h to provide 62 mg (55%) of **5f** as a fluffy white powder: mp 190–192 °C; R_f 0.18 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 7.22 (br s, 1H), 7.17 (s, 1H), 5.85 (s, 2H), 5.00 (t, 2H), 3.67 (dd, 2H), 3.52 (dd, 2H), 3.4–3.1 (br s, 2H), 1.93 (quint, 1H), 1.30 (s, 2H), 0.88 (d, 6H); HRMS (ESI) *m*/*z* calcd for $C_{15}H_{23}N_6O_2$ $[M+H]^+$ 319.1877, found 319.1877.

4.2.4.7. (*Z*)-2-Amino-6-(cyclopentylamino)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5g). Intermediate **4a** was treated with cyclopentylamine according to method A for 16 h to provide 101 mg (88%) of **5g** as an off-white solid: mp 110–113 °C; R_f 0.35 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H), 7.15 (t, 1H), 7.08 (d, 1H), 5.84 (s, 2H), 4.98 (t, 2H), 4.6–4.4 (s, 1H), 3.62 (dd, 2H), 3.50 (dd, 2H), 2.0–1.8 (m, 2H), 1.8–1.6 (m, 2H), 1.6–1.4 (m, 4H), 1.28 (d, 2H); HRMS (ESI) *m*/*z* calcd for $C_{16}H_{23}N_6O_2$ $[M+H]^+$ 331.1877, found 331.1877.

4.2.4.8. (*Z*)-2-Amino-6-(cyclohexylamino)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5h). Intermediate **4a** was treated with cyclohexylamine according to method A for 16 h to provide 83 mg (68%) of **5h** as a pale-yellow chunky crystalline solid: mp 183–184 °C; R_f 0.50 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 7.16 (s, 1H), 6.92 (d, 1H), 5.85 (s, 2H), 5.00 (t, 2H), 4.07 (br s, 1H), 3.64 (dd, 2H), 3.51 (dd, 2H), 1.83–1.72 (dd, 4H), 1.60 (d, 1H), 1.36–1.27 (m, 7H); HRMS (ESI) *m*/*z* calcd for $C_{17}H_{25}N_6O_2$ $[M+H]^+$ 345.2033, found 345.2031.

4.2.4.9. (*Z*)-2-Amino-6-(benzylamino)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5i). Intermediate **4a** was treated with benzylamine according to method A for 16 h to provide 58 mg (46%) of **5i** as a white powder: mp 175–176 °C; R_f 0.30 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 7.9–7.7 (br s, 1H), 7.33–7.15 (m, 6H), 5.7–5.8 (br s, 2H), 4.97 (t, 2H), 4.8–4.5 (br s, 2H), 3.62 (dd, 2H), 3.50 (dd, 2H), 1.28 (d, 2H); HRMS (ESI) *m*/*z* calcd for $C_{18}H_{21}N_6O_2$ $[M+H]^+$ 353.1720, found 353.1719.

4.2.4.10. (*Z*)-2-Amino-6-dimethylamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5j). Intermediate **4a** was treated with dimethylamine according to method A for 48 h to provide 82 mg (80%) of **5j** as a white powder: mp 179–181 °C; R_f 0.32 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H), 7.19 (s, 1H), 5.90 (s, 2H), 4.99 (t, 2H), 3.65 (dd, 2H), 3.50 (dd, 2H), 3.35 (s, 6H, overlapping H_2O peak), 1.30 (t, 2H); HRMS (ESI) *m*/*z* calcd for $C_{13}H_{19}N_6O_2$ $[M+H]^+$ 291.1564, found 291.1563.

4.2.4.11. (*Z*)-2-Amino-6-(pyrrolidin-1-yl)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5k). Intermediate **4a** was treated with pyrrolidine according to method A for 16 h to provide 60 mg (54%) of **5k** as a white powder: mp 217–218 °C; R_f 0.34 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H), 7.18 (s, 1H), 5.88 (s, 2H), 5.00 (t, 2H), 3.95 (br s, 2H), 3.67–3.63 (m, 3H), 3.55–3.49 (m, 3H), 1.90 (br s, 4H), 1.30 (s, 2H); HRMS (ESI) *m*/*z* calcd for $C_{15}H_{21}N_6O_2$ $[M+H]^+$ 317.1720, found 317.1719.

4.2.4.12. (*Z*)-2-Amino-6-(morpholin-4-yl)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5l). Intermediate **4a** was treated with morpholine according to method A for 16 h to provide 60 mg (51%) **5l** as light brown micro-crystalline solid: mp 214–215 °C; R_f 0.38 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 7.19 (s, 1H), 6.01 (s, 2H), 4.98 (t, 2H), 4.09 (s, 4H), 3.69–3.62 (m, 6H), 3.54–3.48 (m, 2H), 1.30 (s, 2H); HRMS (ESI) *m*/*z* calcd for $C_{15}H_{21}N_6O_3$ $[M+H]^+$ 333.1670, found 333.1672.

4.2.5. General method B; 2-amino-6-alkoxypurine analogs

A solution of the respective alcohol (1 mL) in DMF (1 mL) was treated with sodium hydride (67 mg, 1.67 mmol, 4.2 eq) at room temperature and stirred for 30 minutes. (*Z*)-2-amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (**4a**; 100 mg, 0.35 mmol) was added in one portion, and the resulting mixture was heated to 70 °C for 4–48 h. The reaction was then cooled to room temperature, and glacial acetic acid (200 μL) was added. The crude mixture was adsorbed onto silica gel (12 g), and suction was applied for 16 h. The product was eluted with 20% MeOH/CH_2Cl_2, and evaporated to provide an oil that was further purified by flash chromatography on silica gel with 0–10% MeOH/$CHCl_3$. Product-containing fractions were pooled and evaporated, and the resulting solids recrystallized from hot CH_3CN to provide the desired product.

4.2.5.1. (*Z*)-2-Amino-6-ethoxy-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6a). Intermediate **4a** was treated with ethanol according to method B for 48 h to provide 45 mg (44%) of **6a** as an off-white powder: mp 182–187 °C; R_f 0.19 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.59 (s, 1H), 7.21 (s, 1H), 6.51 (s, 2H), 5.02 (t, 2H), 4.47 (q, 2H), 3.67 (dd, 2H), 3.52(dd, 2H), 1.4–1.33 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 160.35,

160.11, 152.51, 136.51, 116.99, 113.42, 110.26, 62.17, 61.67, 30.62, 14.58, 10.99; HRMS (ESI) *m/z* calcd for $C_{13}H_{18}N_5O_3$ $[M+H]^+$ 292.1404, found 292.1403.

4.2.5.2. (*Z*)-2-Amino-6-(1-propyloxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6b). Intermediate **4a** was treated with 1-propanol according to method B for 24 h to provide 55 mg (51%) of **6b** as a white powder: mp 195–202 °C; R_f 0.17 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.58 (s 1H), 7.2 (s, 1H), 6.48 (s, 2H), 5.00 (t, 2H), 4.37 (t, 2H), 3.66 (dd, 2H), 3.51 (dd, 2H), 1.77 (q, 2H), 1.33 (s, 2H), 0.97 (t, 3H); HRMS (ESI) *m/z* calcd for $C_{14}H_{20}N_5O_3$ $[M+H]^+$ 306.1561, found 306.1561.

4.2.5.3. (*Z*)-2-Amino-6-allyloxy-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6c). Intermediate **4a** was treated with allyl alcohol according to method B for 24 h to provide 60 mg (46%) of **6c** as a white powder: mp 203–206 °C; R_f 0.20 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1H), 7.21 (s, 1H), 6.53 (s, 2H), 6.17–6.06 (m, 1H), 5.42 (d, 1H), 5.28 (d, 1H), 5.02 (t, 2H), 4.97 (d, 2H), 3.67 (dd, 2H), 3.54 (dd, 2H), 1.34 (s, 2H); HRMS (ESI) *m/z* calcd for $C_{14}H_{18}N_5O_3$ $[M+H]^+$ 304.1404, found 304.1404.

4.2.5.4. (*Z*)-2-Amino-6-(1-butyloxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6d). Intermediate **4a** was treated with 1-butanol according to method B for 4 h to provide 66 mg (58%) of **6d** as an off-white microcrystalline solid: mp 210–212 °C; R_f 0.17 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1H), 7.20 (s, 1H), 6.48 (s, 2H), 5.01 (t, 2H), 4.42 (t, 2H), 3.66 (dd, 2H), 3.51 (dd, 2H), 1.73 (quint, 2H), 1.46–1.40 (m, 2H), 1.32 (s, 2H), 0.94 (t, 3H); HRMS (ESI) *m/z* calcd for $C_{15}H_{22}N_5O_3$ $[M+H]^+$ 320.1717, found 320.1717.

4.2.5.5. (Z)-2-Amino-6-(1-pentyloxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6e). Intermediate **4a** was treated with 1-pentanol according to method B for 30 min to provide 73 mg (62%) of **6e** as a white powder: mp 148–149 °C; R_f 0.32 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1H), 7.20 (s, 1H), 6.47 (s, 2H), 5.00 (t, 2H), 4.41 (t, 2H), 3.67 (dd, 2H), 3.51 (dd, 2H), 1.76 (t, 2H), 1.37–1.33 (m, 6H), 0.90 (t, 3H); HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_5O_3$ $[M+H]^+$ 334.1874, found 334.1876.

4.2.5.6. (*Z*)-2-Amino-6-(1-hexyloxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6f). Intermediate **4a** was treated with 1-hexanol according to method B for 4 h to provide 56 mg (45%) of **6f** as a white powder: mp 142–143 °C; R_f 0.20 (5% MeOH / $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1H), 7.20 (s, 1H), 6.48 (s, 2H), 5.00 (t, 2H), 4.41 (t, 2H), 3.66 (dd, 2H), 3.51 (dd, 2H), 1.75 (t, 2H), 1.5–1.3 (m, 8H), 0.88 (t, 3H); HRMS (ESI) *m/z* calcd for $C_{17}H_{26}N_5O_3$ $[M+H]^+$ 348.2030, found 348.2031.

4.2.5.7. (*Z*)-2-Amino-6-(1-octyloxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6g). Intermediate **4a** was treated with 1-octanol according to method B for 4 h to provide 67 mg (50%) of **6g** as a white powder: mp 148–150 °C; R_f 0.20 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1H), 7.20 (s, 1H), 6.48 (s, 2H), 5.00 (t, 2H), 4.40 (t, 2H), 3.66 (dd, 2H), 3.51 (dd, 2H), 1.75 (t, 2H), 1.5–1.3 (m, 12H), 0.86 (t, 3H); HRMS (ESI) *m/z* calcd for $C_{19}H_{30}N_5O_3$ $[M+H]^+$ 376.2343, found 376.2343.

4.2.5.8. (*Z*)-2-Amino-6-(2,2,2-trifluoroethoxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6h). Intermediate **4a** was treated with 2,2,2,-trifluoroethanol according to method B for 24 h to provide 50 mg (41%) of **6h** as a white powder: mp 182–192 °C; R_f 0.20 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.69 (s, 1H), 7.22 (s, 1H), 6.75 (s, 2H), 5.17 (q, 2H), 5.05 (s, 2H), 3.68 (d, 2H), 3.52 (d, 2H), 1.35 (s, 2H); HRMS (ESI) *m/z* calcd for $C_{13}H_{15}F_3N_5O_3$ $[M+H]^+$ 346.1121, found 346.1121.

4.2.5.9. (*Z*)-2-Amino-6-(2-methoxyethoxy)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6i). Intermediate **4a** was treated with 2-methoxyethanol according to method B for 24 h to provide 86 mg (75%) of **6i** as an off-white powder: mp 210–213 °C; R_f 0.20 (5% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz-DMSO-d_6) δ 8.59 (s, 1H), 7.20 (s, 1H), 6.53 (s, 2H), 5.01 (s, 2H), 4.54 (s, 2H), 3.8–3.6 (m, 4H), 3.6–3.4 (m, 2H), 3.31 (s, 3H, overlaps H_2O peak), 1.33 (s, 2H); HRMS (ESI) *m/z* calcd for $C_{14}H_{20}N_5O_4$ $[M+H]^+$ 322.1510, found 322.1508.

4.2.5.10. (Z)-2-Amino-6-benzyloxy-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6j). Intermediate **4a** was treated with benzyl alcohol according to method B for 24 h to provide 55 mg (44%) of **6j** as a white powder: mp 182–187 °C; R_f 0.19 (5% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1H), 7.52 (d, 2H), 7.44–7.36 (m, 3H), 7.22 (s, 1H), 6.60 (s, 2H), 5.52 (s, 2H), 5.02 (t, 2H), 3.67 (dd, 2H), 3.52 (dd, 2H), 1.34 (s, 2H); HRMS (ESI) *m/z* calcd for $C_{18}H_{20}N_5O_3$ $[M+H]^+$ 354.1561, found 354.1561.

4.2.6. General method C; 2-amino-6-thioalkylpurine analogs

To a solution of (*Z*)-2-amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (**4a**; 100 mg, 0.35 mmol) and the respective thiol in DMF (1 mL) was added 1.0 M aq. NaOH, (1.0 mL, 1.0 mmol). The reaction mixture was stirred at room temperature for 0.5–24 h, then glacial AcOH (200 μL) was added in one portion. The crude mixture was adsorbed onto silica gel (12 g), and suction was applied for 16 h. The product was eluted with 20% MeOH/CH_2Cl_2, and evaporated to provide an oil that was further purified by flash chromatography on silica gel with 0–10% MeOH/$CHCl_3$. Product-containing fractions were pooled and evaporated, and the resulting solids recrystallized from hot CH_3CN/H_2O to provide the desired product.

4.2.6.1. (*Z*)-2-Amino-6-methylthio-9-{[2,2-bis (hydroxymethyl)cyclopropylidene]methyl}purine (7a). Intermediate **4a** was treated with sodium thiomethoxide (145 mg, 2.1 mmol) according to a modification of method C in which no sodium hydroxide was added. The reaction proceeded for 30 min to provide 66 mg (58%) of **7a** as an off-white powder: mp 146–147 °C; R_f 0.28 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.20 (s, 1H), 6.59 (s, 2H), 4.99 (t, 2H), 3.65 (dd, 2H), 3.51 (dd, 2H), 2.58 (s, 3H), 1.34 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 160.16, 159.85, 149.11, 138.81, 123.843, 117.56, 111.26, 62.12, 30.69, 11.79, 9.91; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}N_5O_2S$ $[M+H]^+$ 294.1019, found 294.1018.

4.2.6.2. (*Z*)-2-Amino-6-(1-propylthio)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7b). Intermediate **4a** was treated with 1-propanethiol (0.13 mL, 1.4 mmol) according to method C for 30 min to provide 66 mg (58%) of **7b** as an off-white powder: mp 212–214 °C; R_f 0.25 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.20 (s, 1H), 6.57 (s, 2H), 5.00 (t, 2H), 3.67 (dd, 2H), 3.51 (dd, 2H), 3.28 (t, 2H), 1.69 (q, 2H), 1.34 (s, 2H), 1.00 (t, 3H); HRMS (ESI) *m/z* calcd for $C_{14}H_{20}N_5O_2S$ $[M+H]^+$ 322.1332, found 322.1331.

4.2.6.3. (*Z*)-2-Amino-6-(2-propylthio)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7c). Intermediate **4a** was treated 2-butanethiol (0.13 mL, 1.4 mmol) according to method C for 30 min to provide 60 mg (65%) of **7c** as an off-white

powder: mp 163–183 °C; R_f 0.25 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.20 (s, 1H), 6.58 (s, 2H), 5.01 (t, 2H), 4.28 (quint, 1H), 3.67 (dd, 2H), 3.50 (dd, 2H), 1.39 (d, 6H), 1.33 (s, 2H); HRMS (ESI) m/z calcd for $C_{14}H_{20}N_5O_2S$ $[M+H]^+$ 322.1332, found 322.1330.

4.2.6.4. (*Z*)-2-Amino-6-(1-butylthio)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7d). Intermediate **4a** was treated with 1-butanethiol (0.15 mL, 1.4 mmol) according to method C for 30 min to provide 32 mg (13%) of **7d** as an off-white powder: mp 185–195 °C; R_f 0.23 (10%MeOH/$CHCl_3$); ^{1}H MR (DMSO-d_6) δ 8.64 (s, 1H), 7.20 (s, 1H), 6.55 (s, 2H), 4.99 (t, 2H), 3.67 (dd, 2H), 3.53 (dd, 2H), 3.27 (t, 2H, overlapping H_2O peak), 1.65 (quint, 2H), 1.42 (quint, 2H), 1.33 (s, 2H), 0.91 (t, 3H); HRMS (ESI) m/z calcd for $C_{15}H_{22}N_5O_2S$ $[M+H]^+$ 336.1489, found 336.1489.

4.2.6.5. (*Z*)-2-Amino-6-isobutylthio-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7e). Intermediate **4a** was treated with 2-methyl-1-propanethiol (0.15 mL, 1.4 mmol) according to method C for 30 min to provide 26 mg (11%) of **7e** as an off-white powder: mp 174–177 °C; R_f 0.28 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.64 (s, 1H), 7.19 (s, 1H), 6.54 (s, 2H), 4.99 (t, 2H), 4.18 (q, 1H), 3.67 (dd, 2H), 3.50 (dd, 2H), 1.69 (quint, 2H), 1.38–1.33 (m, 5H), 0.99 (t, 3H); HRMS (ESI) m/z calcd for $C_{15}H_{22}N_5O_2S$ $[M+H]^+$ 336.1489, found 336.1488.

4.2.6.6. (*Z*)-2-Amino-6-cyclopentylthio-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7f). Intermediate **4a** was treated with cyclopentanethiol (0.15 mL, 1.4 mmol) according to method C for 30 min to provide 80 mg (65%) of **7f** as a white powder: mp 188–190 °C; R_f 0.24 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.64 (s, 1H), 7.20 (s, 1H), 6.55 (s, 2H), 4.99 (t, 2H), 4.29 (t, 1H), 3.67 (dd, 2H), 3.50 (dd, 2H), 2.4–2.1 (m, 2H), 1.9–1.5 (m, 6H), 1.34 (s, 2H); HRMS (ESI) m/z calcd for $C_{16}H_{22}N_5O_2S$ $[M+H]^+$ 348.1489, found 348.1489.

4.2.6.7. (*Z*)-2-Amino-6-(1-hexylthio)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7g). Intermediate **4a** was treated with 1-hexanethiol (0.20 mL, 1.4 mmol) according to method C for 30 min to provide 48 mg (55%) of **7g** as an off-white powder: mp 148–150 °C; R_f 0.23 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.20 (s, 1H), 6.55 (s, 2H), 5.00 (t, 2H), 3.67 (dd, 2H); 3.51 (dd, 2H), 3.28 (t, 2H), 1.66 (quint, 2H), 1.5–1.2 (m, 8H), 0.87 (t, 3H); HRMS (ESI) m/z calcd for $C_{17}H_{26}N_5O_2S$ $[M+H]^+$ 364.1802, found 364.1800.

4.2.6.8. (*Z*)-2-Amino-6-(1-octylthio)-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7h). Intermediate **4a** was treated with 1-octanethiol (0.26 mL, 1.4 mmol) according to method C for 30 min to provide 89 mg (32%) of **7h** as a white powder: mp 136–137 °C; R_f 0.28 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.64 (s, 1H), 7.20 (s, 1H), 6.55 (s, 2H), 4.99 (t, 2H), 3.67 (dd, 2H), 3.50 (dd, 2H), 3.27 (t, 2H, overlapping H_2O peak), 1.66 (quint, 2H), 1.5–1.2 (m, 12H), 0.85 (t, 3H); HRMS (ESI) m/z calcd for $C_{19}H_{30}N_5O_2S$ $[M+H]^+$ 392.2115, found 392.2116.

4.2.6.9. (*Z*)-2-Amino-6-benzylthio-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (7i). Intermediate **4a** was treated with benzyl mercaptan (0.17 mL, 1.4 mmol) according to method C for 30 min to provide 74 mg (56%) of **7i** as a white powder: mp 202–204 °C; R_f 0.26 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.48 (d, 2H), 7.33–7.20 (m, 4H), 6.69 (s, 2H), 4.98 (t, 2H), 4.56 (s, 2H), 3.66 (dd, 2H), 3.50 (dd, 2H), 1.34 (s, 2H); HRMS (ESI) m/z calcd for $C_{18}H_{20}N_5O_2S$ $[M+H]^+$ 370.1332, found 370.1333.

Acknowledgments

This work was supported by National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID) contracts N01-AI-30049, and HHSN2722011000010C (M.N.P) and Grant 1R43AI082799.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.04.049.

References and notes

1. *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*; Arvin, A., Campadelli-Fiume, G., Mocarski, E., Moore, P. S., Roizman, B., Whitley, R., Yamanishi, K., Eds.; Cambridge University Press: Cambridge, 2007.
2. *The Human Herpesviruses*; Roizman, B., Whitley, R. J., Lopez, C., Eds.; Raven Press: New York, 1993.
3. Pellett, P. E.; Roizman, B. The Family Herpesviridae: A Brief Introduction. In *Fields Virology*; Knipe, D. M., Howley, P. M., Griffin, D. E., Lamb, R. A., Martin, M. A., Roizman, B., Straus, S. E., Eds., 5th ed.; Lippincott Williams & Wilkins: Philadelphia, 2007; pp 2479–2499.
4. O'Brien, J. J.; Campoli-Richards, D. M. *Drugs* **1989**, *37*, 233.
5. Beutner, K. R. *Antiviral Res.* **1995**, *28*, 281.
6. Faulds, D.; Heel, R. C. *Drugs* **1990**, *39*, 597.
7. Cvetkovic, R. S.; Wellington, K. *Drugs* **2005**, *65*, 859.
8. Plosker, G. L.; Noble, S. *Drugs* **1999**, *58*, 325.
9. Wagstaff, A. J.; Bryson, H. M. *Drugs* **1994**, *48*, 199.
10. Zemlicka, J. Methylenecyclopropane Analogues of Nucleosides as Anti-herpes Agents In *Advances in Antiviral Drug Design*; DeClercq, E., Ed.; Elsevier Science: New York, 2007; Vol. 5, pp 113–165.
11. Qiu, Y. L.; Ksebati, M. B.; Ptak, R. G.; Fan, B. Y.; Breitenbach, J. M.; Lin, J. S.; Cheng, Y. C.; Kern, E. R.; Drach, J. C.; Zemlicka, J. *J. Med. Chem.* **1998**, *41*, 10.
12. Zhou, S.; Breitenbach, J. M.; Borysko, K. Z.; Drach, J. C.; Kern, E. R.; Gullen, E.; Cheng, Y. C.; Zemlicka, J. *J. Med. Chem.* **2004**, *47*, 566–575.
13. Kern, E. R.; Kushner, N. L.; Hartline, C. B.; Williams-Aziz, S. L.; Harden, E. A.; Zhou, S.; Zemlicka, J.; Prichard, M. N. *Antimicrob. Agents Chemother.* **2005**, *49*, 1039.
14. Chen, X.; Kern, E. R.; Drach, J. C.; Gullen, E.; Cheng, Y. C.; Zemlicka, J. *J. Med. Chem.* **2003**, *46*, 1531.
15. Kushner, N. L.; Williams, S. L.; Hartline, C. B.; Harden, E. A.; Bidanset, D. J.; Chen, X.; Zemlicka, J.; Kern, E. R. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 2105.
16. Kern, E. R.; Bidanset, D. J.; Hartline, C. B.; Yan, Z.; Zemlicka, J.; Quenelle, D. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 4745.
17. Bowlin, T. L.; Brooks, J.; Zemlicka, J. *Antiviral Res.* **2009**, *82*, A46.
18. Zhou, S.; Drach, J. C.; Prichard, M. N.; Zemlicka, J. *J. Med. Chem.* **2009**, *52*, 3397.
19. Prichard, M. N.; Quenelle, D. C.; Hartline, C. B.; Harden, E. A.; Jefferson, G.; Frederick, S. L.; Daily, S. L.; Whitley, R. J.; Tiwari, K. N.; Maddry, J. A.; Secrist, J. A., 3rd; Kern, E. R. *Antimicrob. Agents Chemother.* **2009**, *53*, 5251.
20. Williams-Aziz, S. L.; Hartline, C. B.; Harden, E. A.; Daily, S. L.; Prichard, M. N.; Kushner, N. L.; Beadle, J. R.; Wan, W. B.; Hostetler, K. Y.; Kern, E. R. *Antimicrob. Agents Chemother.* **2005**, *49*, 3724.
21. Prichard, M. N.; Daily, S. L.; Jefferson, G. M.; Perry, A. L.; Kern, E. R. *J. Virol. Methods* **2007**, *144*, 86.