Expedited Articles

(*Z*)- and (*E*)-2-((Hydroxymethyl)cyclopropylidene)methyladenine and -guanine. New Nucleoside Analogues with a Broad-Spectrum Antiviral Activity[1]

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New nucleoside analogues **14**−**17** based on a methylenecyclopropane structure were synthesized and evaluated for antiviral activity. Reaction of 2,3-dibromopropene (**19**) with adenine (**18**) led to bromoalkene **20**, which was benzoylated to give N^6,N^6-dibenzoyl derivative **23**. Attempts to convert **20** or **23** to bromocyclopropanes **21** and **22** by reaction with ethyl diazoacetate catalyzed by $Rh_2(OAc)_4$ were futile. By contrast, 2,3-dibromopropene (**19**) afforded smoothly (*E*)- and (*Z*)-dibromocyclopropane carboxylic esters **24** + **25**. Alkylation of adenine (**18**) with **24** + **25** gave (*E*)- and (*Z*)-bromo derivatives **21** + **22**. Base-catalyzed elimination of HBr resulted in the formation of (*Z*)- and (*E*)-methylenecyclopropanecarboxylic esters **26** + **27**. More convenient one-pot alkylation−elimination of adenine (**18**) or 2-amino-6-chloropurine (**30**) with **24** + **25** afforded (*Z*)- and (*E*)-methylenecyclopropane derivatives **26** + **27** and **31** + **32**. The *Z*-isomers were always predominant in these mixtures ($Z/E \sim 2/1$). Reduction of **26** + **27** and **31** + **32** with DIBALH afforded (*Z*)- and (*E*)-methylenecyclopropane alcohols **14** + **16** and **33** + **34**. The latter were resolved directly by chromatography. Compounds **14** + **16** were converted to N^6-(dimethylamino)methylene derivatives **28** and **29** which were separated and deprotected to give **14** and **16**. Reaction of **33** and **34** with HCO_2H led to guanine analogues **15** and **17**. The ^{1}H NMR spectra of the *Z*-analogues **14** and **15** are consistent with an *anti*-like conformation of the nucleobases. By contrast, ^{1}H NMR and IR spectra of bromo ester **21** are indicative of *syn*-conformation of adenine. Several *Z*-(hydroxymethyl)methylenecyclopropanes exhibited in vitro antiviral activity in micromolar or submicromolar range against human and murine cytomegalovirus (HCMV and MCMV), Epstein−Barr virus (EBV), human herpes virus 6 (HHV-6), varicella zoster virus (VZV), and hepatitis B virus (HBV). Analogues **14**, **15**, and **33** were the most effective agents against HCMV (IC_{50} 1−2.1, 0.04−2.1, and 0.8−5.6 μM), MCMV (IC_{50} 2.1, 0.3, and 0.3 μM) and EBV in H-1 (IC_{50} 0.2, 0.3, and 0.7 μM) and Daudi cells (IC_{50} 3.2, 5.6, and 1.2 μM). Adenine analogue **14** was active against HBV (IC_{50} 2 μM), VZV (IC_{50} 2.5 μM), and HHV-6 (IC_{50} 14 μM). Synadenol (**14**) and the *E*-isomer (**16**) were substrates of moderate efficiency for adenosine deaminase from calf intestine. The *E*-isomer **16** was more reactive than *Z*-isomer **14**. The deamination of **14** effectively stopped at 50% conversion. Synadenol (**14**) was also deaminated by AMP deaminase from *aspergillus* sp.

The synthesis and biological evaluation of carbocyclic nucleoside analogues are of much current interest.[2−5] Studies of cyclopentane derivatives **1** (Chart 1) initiated more than three decades ago[6,7] were more recently complemented by investigations of ring-contracted analogues comprising a four- or three-membered ring. The rationale for synthesis of cyclobutane analogues was largely based on oxetane structure of antibiotic oxetanocin A (**2**) which is an effective antiviral agent.[8,9] This approach led to a discovery of potent antivirals *carba*-oxetanocin A and G (**3** and **4**).[5] By contrast, design and synthesis of analogues comprising a cyclopropane moiety have not produced compounds with antiviral activity despite a significant recent effort.[5,10−15] Guanine analogue **5** of the *Z*-configuration is a notable exception.[16] The latter compound was designed as a conformationally constrained analogue of acyclovir. It was the only analogue with an antiherpetic potency (HSV-1 and

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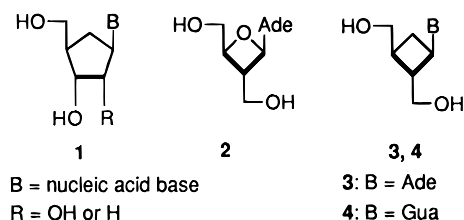
Chart 1



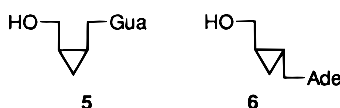
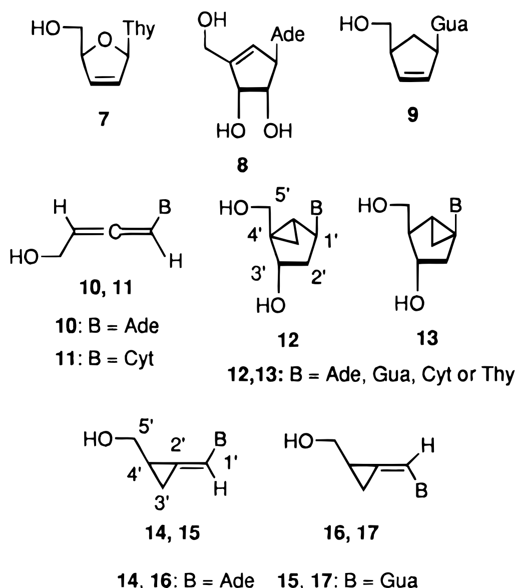


Chart 2



HSV-2) comparable to that of acyclovir in a series of cyclopropane derivatives comprising adenine, guanine, and thymine nucleobases.[16] A moderate antiviral effect of the *E*-analogue **6** was also noted. Nevertheless, compound **5** was inactive in vivo against HSV-1 in a murine encephalitis model, and activity against viruses other than HSV-1 or HSV-2 was not reported.

There is considerable evidence that introduction of a rigid structural element into nucleoside or carbocyclic nucleoside structure can lead to effective antiviral analogues. Thus, the presence of a double bond in cyclic nucleoside analogues such as anti-AIDS drug stavudine[17] (**7**, d4T, Zerit, Chart 2) or carba analogues neplanocin[5] A (**8**) and carbovir[5] (**9**) is a structural feature important for their potent antiviral and (compound **8**) antitumor effects. In the acyclic analogue series, introduction of a very rigid allenic moiety as a linker between the heterocyclic base and hydroxymethyl group led to compounds effectively inhibiting the replication of HIV, such as adenallene (**10**) and cytallene[18,19] (**11**). Severe conformational constraints imposed by annealing a cyclopropane ring with the cyclopentane moiety of carbocyclic nucleosides in compounds **12** and **13** are also compatible with the potent antiviral effects of these analogues.[20]

For these reasons, we became interested in synthesis and biological evaluation of nucleoside analogues where the ribofuranose portion is replaced by the methylenecyclopropane moiety. Compounds **14**−**17** are of particular interest because, on one hand, these structures relate to saturated compounds **5** and **6**, but they are considerably more rigid. On the other hand, compounds **14**−**17** can also be regarded as analogues of adenallene (**10**) where a single double bond distal from the nucleobase was replaced with a cyclopropane ring. Such a structural change would not impose serious steric limitations on the molecule which could otherwise interfere with the necessary receptor or enzyme binding. Also, there is a distinct stereoelectronic similarity between the double bond and cyclopropane ring.[21] Importantly, the presence of the 4′-hydroxymethyl group juxtaposed to a heterocyclic ring of **14** and **15** can lead to some restriction of rotation of both functions. This situation is different from that found in allene analogues **10** and **11** but similar to ordinary nucleosides.[22] Last but not least, allenic analogues such as adenallene (**10**) comprise a system with axial chirality whereas methylenecyclopropanes **14**−**17** are centrochiral with a single center of asymmetry.

As expected, the distance between the heterocyclic base and hydroxymethyl group (N^9−$C^{5'}$) in the *Z*-analogue **14** (4.10 Å) is shorter than in the *E*-isomer **16** (4.71 Å). A similar distance (N^9−$C^{4'}$) in adenallene (**10**, 4.46 Å) lies between both values. This criterion alone cannot be a sole determinant of the biological activity, but there are indications[16,23] that the *Z*-series may have a greater potential for an antiviral effect.

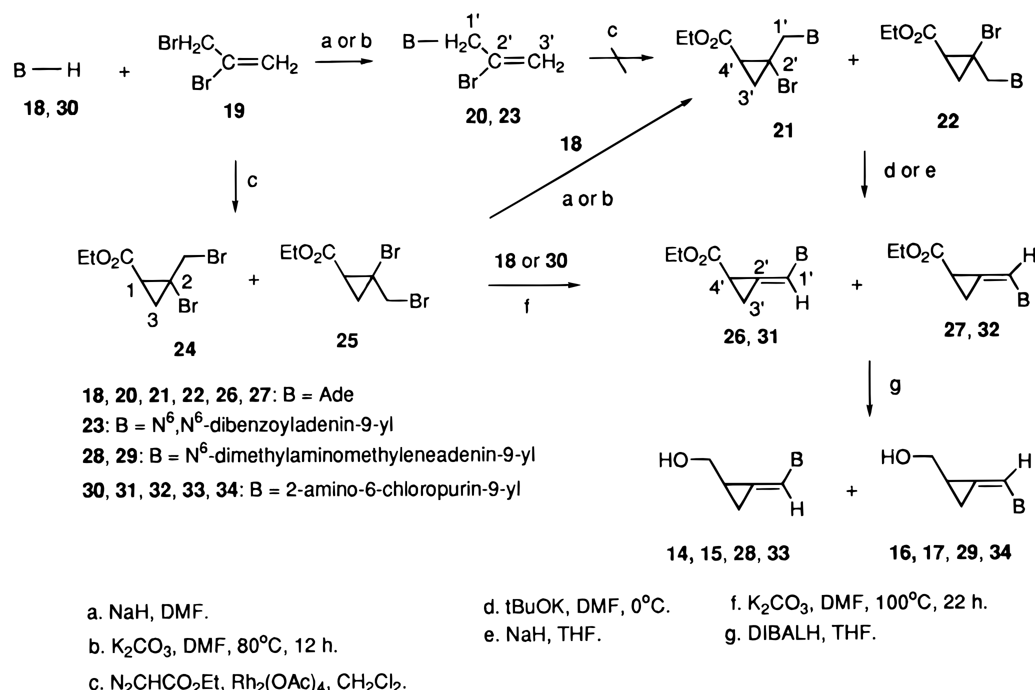
Synthesis

Initial experiments were aimed at introducing the cyclopropane moiety into an unsaturated precursor containing a nucleobase (Scheme 1). Thus, alkylation of adenine (**18**) with 2,3-dibromopropene (**19**) using NaH or K_2CO_3 in DMF gave 9-(2-bromopropen-1-yl)adenine (**20**) in 52−59% yield. Nevertheless, all attempts at cyclopropanation of **20** using ethyl diazoacetate and $Rh_2(OAc)_4$ catalyst[24] were fruitless, and no cyclopropane esters **21** or **22** were obtained. Benzoylation of **20** gave the N^6,N^6-dibenzoyl derivative **23**, but again, reaction with ethyl diazoacetate failed to give any desired products. We assume that adverse complexation of the heterocyclic base with the catalyst could be responsible for this result. Therefore, we have chosen to prepare a suitable alkylating agent with a preformed cyclopropane moiety with the aim to alkylate directly the appropriate nucleobase.

Such an agent was found in ethyl (*E*)- and (*Z*)-2-bromo-2-(bromomethyl)cyclopropane-1-carboxylates (**24** and **25**). The corresponding dichloro derivatives (**24** and **25, Br = Cl**) were reported in the literature[25] by addition of ethyl diazoacetate to 2,3-dichloropropene catalyzed by $CuSO_4$. However, a similar reaction with 2,3-dibromopropene (**19**) failed to give carboxylates[26] **24** or **25**. The latter were considered preferable for the intended alkylation of nucleobases, and therefore, more successful variants of this reaction were sought. Addition of ethyl diazoacetate to **19** catalyzed[24] by $Rh_2(OAc)_4$ in CH_2Cl_2 proceeded smoothly to give a mixture of *E*- and *Z*-isomers **24** and **25** in 91% yield (the isomeric assignment is tentative). The *E*/*Z* ratio was 1.5:1 as determined by ^{1}H NMR spectra.

Alkylation of sodium salt of adenine (**18**) with a mixture of **24** and **25** in DMF at 80 °C for 12 h afforded

Scheme 1

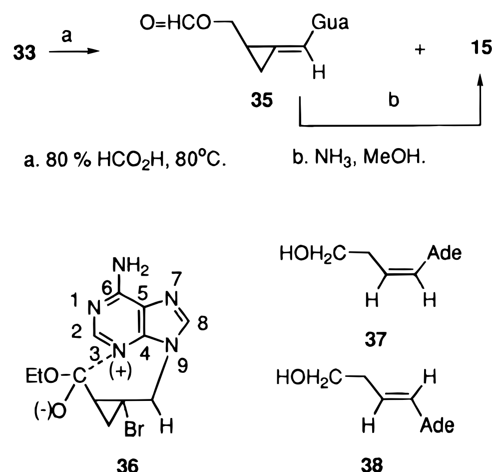


E-isomer **21** in 30% yield and the corresponding *Z*-isomer **22** (17.6%), which were separated by chromatography on silica gel. Elimination of HBr from an unseparated mixture of isomers **21** and **22** was effected smoothly with tBuOK in DMF at 0 °C to give (*Z*)- and (*E*)-methylenecyclopropanecarboxylic esters[27] **26** and **27** (70% yield) which could not be separated by chromatography. The *Z*/*E* ratio (1.5:1) was the same as the *E*/*Z* ratio of dibromo derivatives **21** and **22**. When eliminations were performed separately with pure ester **21** or **22**, the *Z*/*E* ratios were 1.5:1 and 1:1.5, respectively. When tBuOK was replaced with K_2CO_3 (3−10 h at 100 °C), the *Z*/*E* ratios were 2.5:1 and 2:1. Thus, configuration of the starting bromo ester does not have a profound influence on isomeric composition of the resultant methylenecyclopropane derivatives. Elimination of HBr from the 1:1 mixture of **21** and **22** using NaH in THF gave a low (23%) yield of **26** and **27** in the same ratio. It is noteworthy that elimination of HBr from *cis*- and *trans*-1-carbethoxy-2-bromo-2-ethylcyclopropanes using NaH in EtOH/ether gave a mixture in which the *anti*-2-ethylene-1-carbethoxycyclopropane[28] was preponderant (*syn*/*anti* ratio was 1:4).

From a preparative viewpoint, it is more convenient to perform alkylation and elimination of HBr in one pot. Thus, reaction of dibromo esters **24** + **25** with adenine (**18**) using K_2CO_3 in DMF at 100 °C for 22 h gave a mixture of **26** and **27** in 39% yield and with a somewhat improved *Z*/*E* ratio (2:1).

Reduction of esters **26** + **27** using DIBALH[29] in THF afforded a mixture of synadenol[30] (**14**) and *E*-isomer **16** in 75% yield; the compounds were not separable by chromatography. The latter were converted to the respective N^6-(dimethylamino)methylene derivatives **28** and **29** by reaction with *N*,*N*-dimethylformamide dimethyl acetal in DMF[31] that were amenable to chromatographic separation in 38 and 37% yield, respectively. Deprotection with NH_3 in MeOH then afforded pure *Z*- and *E*-isomers **14** and **16** (85 and 86%).

Scheme 2



Synguanol[30] (**15**) and the respective *E*-isomer **17** were obtained as follows. 2-Amino-6-chloropurine (**30**) was alkylated with a mixture of dibromo esters **24** + **25** according to the one-pot procedure described above for **26** and **27**. The *Z*- and *E*-esters **31** and **32** were obtained in 56% yield and in the ratio of 2:1. Again, it was not possible to resolve the isomers by chromatography, and therefore, reduction with DIBALH in THF was performed with this mixture. In this case, however, a larger excess of DIBALH added portionwise was mandatory for success. Products **33** and **34** were separated by chromatography in 41.8 and 23.3% yield, respectively. Compound **33** was readily converted to synguanol (**15**) by hydrolysis[32] with 80% HCO_2H at 80 °C (Scheme 2). Some 5′-O-formyl derivative **35** was formed during the procedure, but after treatment with NH_3 in MeOH, synguanol (**15**) was obtained in 85% yield. The *E*-isomer **17** was obtained from **34** in a similar fashion (83% yield).

It is of interest to note that polarity (lipophilicity) plays a crucial role in separation of (*Z*)- and (*E*)-

Table 1. Chemical Shifts (∂) of the Heterocyclic Protons of 9-((2-(Hydroxymethyl)cyclopropylidene)methyl)purines and Related Compounds[a]

compd	H_8	H_2	compd	H_8	H_2
5[b]	7.78		**16**	8.48	8.17
5 (Gua = Ade)[b]	8.25	8.16	**17**	8.04	
6[b]	8.23	8.14	**28**	8.86	8.42
6 (Ade = Gua)[b]	7.77		**29**	8.56	8.43
10[c]	8.17		**33**	8.70	
14	8.74	8.17	**34**	8.48	
15	8.31				

[a] CD_3SOCD_3. [b] Reference 16. [c] Reference 33.

Table 2. Chemical Shifts (∂) of the Heterocyclic Protons of 9-((2-Carbethoxycyclopropylidene)methyl)purines[a]

compd	H_8	H_2
21	8.10	
22	8.17, 8.13	
26[b]	8.23	8.15
27[b]	8.52	8.18
31[b]	8.19	
32[b]	8.46	

[a] CD_3SOCD_3. [b] Determined with a mixture of isomers.

cyclopropylidenemethyl derivatives by chromatography. Thus, a mixture of isomers **33** + **34** comprising the less polar 2-amino-6-chloropurine are separable whereas in case of the more polar adenine derivatives **14** + **16** a lipophilic "handle" (see compounds **28** and **29**) is essential for separation. By contrast, unsaturated carboxylic ester intermediates **26** + **27** and **31** + **32** are poorly separable by column chromatography.

NMR Spectra and Assignment of *Z*- and *E*-Isomers. The NMR spectra were indispensable for confirmation of the *Z*- and *E*-isomeric structures **14, 15** and **16, 17**. The H_8 and H_2 signals of the *Z*-isomer **14** and *E*-isomer **16** were unambiguously assigned by $^1H-^{13}C$ 2D NMR spectroscopy. Chemical shifts of the H_8 protons in (*Z*)-methylenecyclopropanes **14**, **15**, **28**, and **33** are located significantly downfield from those of the *E*-isomers **16**, **17**, **29**, and **34** (Table 1) whereas the H_2 chemical shifts are remarkably constant. This situation contrasts with that found in adenallene[33] (**10**) and saturated *Z*- and *E*-analogues[16] **5 (Gua = Ade)** and **6** where $\Delta\partial(H_8,H_2)$ are very small. Also, almost identical chemical shifts of the H_8 protons were noted in guanine analogues **5** and **6 (Ade = Gua)**.

These differences can be explained by a deshielding of the H_8 by 4′-hydroxymethyl group ($O_{5'}$) in an *anti*-like conformation of the purine base of methylenecyclopropane analogues **14** and **15**. It is to be expected that such effect will decrease in compounds with a freer rotation of the purine base. A similar deshielding was observed in adenine[34] nucleosides of an anti conformation, and it was suggested that $\Delta\partial(H_8,H_2)$ can serve as an indicator of ribose–base interactions. On the basis of these findings, a tentative assignment of *E*/*Z* stereochemistry to analogues **14**–**17** is possible. It is then also likely that in solution (DMSO) the *Z*-analogues **14** and **15** have their nucleobases in an *anti*-like conformation.

A different situation was encountered in the group of esters (Table 2). Saturated adenine analogues **21** and **22** exhibited a small $\Delta\partial(H_8,H_2)$ comparable with adenallene (**10**) or saturated hydroxymethyl derivatives **5** (Gua = Ade) and **6** (Table 1). The $\Delta\partial H_8$ of **21** and **22**

Table 3. NOE Enhancements of Relevant Protons in (*Z*)- and (*E*)-9-((2-(Hydroxymethyl)cyclopropylidene)methyl)adenine (**14** and **16**)

compd	H_{iir}	H_{obs}	% NOE
14	$H_{4'}$	H_8	4.2
	H_8	$H_{4'}$	2.8
	$H_{5'}$	H_8	0.9
	H_8	$H_{5'}$	0.9
	OH	H_8	3.8
	H_8	OH	1.9
	$H_{3'-cis}$[a]	$H_{4'}$	8.8
	$H_{3'-trans}$[a]	$H_{4'}$	0.8
16	$H_{3'-cis}$[a]	H_8	3.6
	$H_{3'-trans}$[a]	H_8	2.6
	H_8	$H_{3'-cis}$[a]	1.2
	H_8	$H_{3'-trans}$[a]	1.0
	$H_{3'-cis}$[a]	$H_{4'}$	9.3
	$H_{3'-trans}$[a]	$H_{4'}$	1.3

[a] Relative to $H_{4'}$.

was also small. Of interest is a significant shift of carbonyl frequency of the *E*-ester **21** (1725 cm^{-1}) relative to the corresponding *Z*-isomer **22** (1745 cm^{-1}). This may indicate a *syn*-like conformation of adenine in **21** (formula **36**), similar to that proposed for ethyl 2′,3′-O-isopropylideneadenosine-5′-carboxylate[35] (ν_{CO} 1726 cm^{-1}). The latter has a *cis* configuration of adenine and carboxylate functions as found in *E*-ester **21**.

The chemical shifts of the H_8 in unsaturated purine esters **26**, **27** and **31**, **32** exhibited a trend opposite to that found in hydroxymethyl derivatives **14**–**17**. Thus, the H_8 in *E*-esters **27** and **32** are more deshielded than in the *Z*-isomers **26** and **31**. It is possible that in the *Z*-esters **26** and **31**, a *syn*-like conformation of the base, similar to that shown in formula **36**, is at least partly responsible for a lack of deshielding effect. It should be also emphasized that the H_2 signals in both series of compounds (esters and hydroxymethyl derivatives) differ only to a negligible extent.

A final confirmation of assignment of *Z*- and *E*-isomers **14**–**17** came from the determination of NOE enhancements in a pair of *Z*- and *E*-isomers **14** and **16**. Thus, substantial NOE's observed in the *Z*-isomer **14** between the H_8 and OH as well as H_8 and $H_{4'}$ (Table 3) are in accord with the proximity of both protons and *anti*-like conformation of adenine base. A weaker effect was noted between the H_8 and $H_{5'}$. These interactions were absent in the *E*-isomer **16**. By contrast, the NOE data showed that the H_8 and cyclopropane protons $H_{3'}$ were close to each other in **16** whereas no such effect was seen in the *Z*-isomer **14**. These data are fully compatible with formulation of **14** and **16** as the *Z*- and *E*-isomers, respectively. Because the *Z*-isomers **14** and **33** were major products of reduction of the respective carboxylic ester precursors, it was then possible to assign the *Z*-configuration to esters more abundant in isomeric mixtures.

Other general conclusions regarding the *Z*- and *E*-isomerism are also possible. Thus, in the *E*-isomers the protons of hydroxymethyl group ($H_{5'}$) are either magnetically equivalent or the differences of chemical shifts are small. By contrast, similar protons in all *Z*-isomers are nonequivalent, and the signals are well separated. This is presently the most straightforward method for distinguishing the *Z*- and *E*-analogues **14**–**17**. By contrast, these protons are nonequivalent in both *Z*- and *E*-series[16] of analogues **5** and **6**. In all hydroxymethyl

Table 4. Inhibition of Human and Murine Cytomegalovirus (HCMV and MCMV) Replication by Methylenecyclopropane Analogues

compd	50 or 90% inhibitory concentration (IC_{50} or IC_{90}, μM): antiviral activity[a] HCMV plaque	HCMV yield	HCMV CPE	MCMV plaque	cytotoxicity in following cells[b] HFF	MEF	KB
14	2.1[c]	1.1[c]	1.0	2.1	>100[c]	>92	78[c]
15	2.1[c]	1.8	0.04	0.3	>100[c]	>429	>100
16	>100[c]	>100[c]	395	NT	>100[c]	NT	>100[c]
17	>100[c]	NT	>413	NT	>100[c]	NT	>100
33	5.0	0.8	5.6	0.3	>100	270	>100[c]
34	>100	11	>79.5	NT	NT	NT	>100
ganciclovir	7.4 ± 6.5[d]	1.6 ± 2.1[d]	2.6 ± 3.0	3.4 ± 1.7	NT	NT	NT

[a] For details, see the Experimental Section. Plaque reduction, yield reduction, and cytopathic effect inhibition (CPE) assays were performed in duplicate as described in the text. Results from plaque and CPE assays are reported as IC_{50}'s; those for yield reduction experiments as IC_{90}'s. NT = not tested. [b] Visual cytotoxicity was scored on stationary HFF and MEF cells at time of HCMV and MCMV plaque enumeration in cells not affected by the virus. Cytotoxicities of IC_{50} >100 μM were also found by neutral red uptake and in rapidly proliferating HFF cells. Inhibition of KB cell growth was determined as described in the text in quadruplicate assays. [c] Average of duplicate or triplicate experiments. [d] Averages ± standard deviation derived from 108 and 33 experiments, respectively.

analogues of the *Z*-configuration the chemical shift of hydroxy proton is located downfield from that in the *E*-isomers. An opposite trend was found in the ∂ values of $H_{1'}$ with the exception of compounds **28** and **29**.

The differences found among cyclopropane protons are also of significance for the *Z*- and *E*-isomer assignment especially for compounds lacking the hydroxymethyl group (carboxylic esters). The methylene group protons ($H_{3'}$) are nonequivalent in both isomeric series. The signals of $H_{3'}$ and $H_{4'}$ of the *Z*-isomers are more apart than those of the *E*-isomers. A similar pattern of the $C_{3'}$ and $C_{4'}$ signals was found in the ^{13}C NMR spectra. The *cis* hydrogen ($H_{3'}$) is always downfield from that of trans hydrogen. The coupling constants then follow the trend $^2J \geq {}^3J_{cis} > {}^3J_{trans}$. In addition, the assignments of *cis* and *trans* relationships within the cyclopropane moiety were confirmed by NOE enhancements (Table 3). As expected, the NOE is always significantly greater in *cis*- than in *trans*-orientated protons. The vicinal coupling constants $^3J_{cis}$ and $^3J_{trans}$ correspond well to similar values of cyclopropanes[36] but the geminal ^2J's (~8−10 Hz) are larger. This may be caused by the neighboring π-electron system.[36]

Biological Activity. A. Antiviral Effects. The efficacy of analogues **14**−**17** and **33**, **34** as antiviral agents was tested in cultures infected with the following viruses: human and murine cytomegalovirus (HCMV and MCMV), herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Epstein−Barr virus (EBV), varicella zoster virus (VZV), human herpes virus 6 (HHV-6), and hepatitis B virus (HBV). Assays with HIV-1-infected CEM-SS cells were also included. The results are summarized in Tables 4 − 8. It is evident that analogues of the *Z*-configuration are very effective inhibitors of a variety of herpetic viruses whereas the respective *E*-isomers are much less potent or inactive. Thus, synadenol (**14**) strongly interfered with the replication of HCMV in human foreskin fibroblast (HFF) cells as determined by plaque reduction and cytopathic effect inhibition assays (IC_{50} 2.1 and 1 μM, respectively) as well as yield reduction assay (IC_{90} 1.1 μM, Table 4). Synguanol (**15**) was even more effective with IC_{50} in plaque reduction and cytopathic effect inhibition assay 2.1 and 0.04 μM, respectively. The yield reduction assay gave an IC_{90} value of 1.8 μM. The efficacy of both analogues is comparable with that of ganciclovir, a drug which is licensed for treatment of HCMV infections. Somewhat surprisingly, the 2-amino-6-chloro analogue **33** was also effective, exhibiting IC_{50} values of 5 μM in plaque reduction, 5.6 μM in cytopathic effect inhibition and IC_{90} of 0.8 μM in yield reduction assay against HCMV.

Because analogues **14** and **15** are considered good candidates for studies of their effects on CMV infection in mice in vitro assays also included MCMV in mouse embryonic fibroblast (MEF) cell culture. Compounds **14**, **15**, and **33** proved as effective as in HCMV assays (IC_{50} 0.3−2.1 μM).

The results obtained with the (*Z*)-methylenecyclopropane analogues in CMV assays encouraged us to study the efficacy of these analogues against a broader range of herpesviruses. Activity against HSV-1 and -2 was of particular interest (Table 5). Although the efficacy of synadenol **14** in cytopathic effect inhibition assay (IC_{50} 10.6 μM) against HSV-2 was comparable to that of acyclovir (7.7 μM) and it exhibited IC_{50} of <0.14 μM against HSV-1, other assays indicated a much lower level of activity. It should be noted that in the corresponding saturated series of analogues,[16] compound **5** was strongly effective only against HSV-1 and less against HSV-2. Also, analogue **5** is a substrate for HSV-1 thymidine kinase (TK). A lack of activity of synguanol (**15**) against HSV-1 and HSV-2 may then be related to a poor substrate efficiency toward the latter enzyme. It is also noteworthy that analogues[37] **37** and **38** with a flexible methylene link replacing the cyclopropyl moiety of **14** and **16** were totally devoid of antiviral effects.

Synadenol (**14**) and synguanol (**15**) were also effective against EBV in Daudi cells with IC_{50} 3.2 and 5.6 μM, respectively (Table 6). The 2-amino-6-chloropurine derivative **33** was more potent (IC_{50} 1.2 μM) than acyclovir (IC_{50} 5.3 μM), whereas potency of **14** and **15** is comparable with that of acyclovir. Synadenol (**14**), synguanol (**15**), and 2-amino-6-chloropurine derivative **33** were the most effective analogues in H-1 cells infected with EBV, exhibiting IC_{50} values between 0.2 and 0.7 μM, respectively. Potency of all three analogues surpassed that of ganciclovir control (IC_{50} 5 μM). Synadenol (**14**) was as effective against VZV (IC_{50} 2.5 μM) as acyclovir (IC_{50} 3.5 μM), but synguanol (**15**) exhibited only a moderate effect (IC_{50} 61.3 μM). Last but not least, the efficacy of synadenol (**14**) and synguanol (**15**)

Table 5. Inhibition of Herpes Simplex Virus Type 1 and 2 (HSV-1 and −2) Replication by Methylenecyclopropane Analogues

	50% inhibitory concentration (IC_{50}, μM)					
	antiviral activity					
	HSV-1			HSV-2		
compd	ELISA[a,b]	CPE[b]	Vero[c]	CPE[b]	Vero[c]	cytotoxicity[b] CEM
14	33[d]	<0.14[e]	28	10.6[f]	59	>100
15	100	61.7	85	>413	>100	>100
16	>100[d]	36[g]	>100	395	>100	>100
17	100	>413	>100	>413	>100	>100
33	31[d]	>79.5	52	>79.5	55	80
34	>100	>79.5	95	>79.5	>100	60
control	3.5 ± 2.1[h]	2.6 ± 2.0[i]	9[i]	7.7 ± 8.6[i]	25[i]	>20[i]

[a] The plaque reduction assay was used to determine the activity of ganciclovir against HSV-1; all other compounds were assayed by ELISA in quadruplicate wells. [b] For cytotoxicity in HFF cells, see Table 4. [c] Cytotoxicity was determined in CEM cells. [d] Average of duplicate or triplicate experiments. [e] Plaque reduction 140 μM. [f] Plaque reduction 48 μM. [g] Plaque reduction >460 μM. [h] Ganciclovir. [i] Acyclovir.

Table 6. Inhibition of Epstein−Barr Virus (EBV), Human Herpes Virus 6 (HHV-6), and Varicella Zoster Virus (VZV) Replication by Methylenecyclopropane Analogues

	50% inhibitory concentration (IC_{50}, μM)					
	antiviral activity[a]					
	EBV				cytotoxicity	
compd	H-1	Daudi	HHV-6 ELISA	VZV Plaque[b]	H-1	Daudi
14	0.2	3.2	14[c]	2.5	>50	368
15	0.3	5.6	42.5[c]	61.3	>50	>214[d]
16	3	71.4	>100[c]	90.2	>50	>230
17	>50	>206	>100	263	>50	>206
33	0.7	1.2	50	>518	>50	>199
34	>10	143	NT	NT	<50	>199
control	5[e]	5.3 ± 1.9[f]	31 ± 14[g]	3.5 ± 2.0[f]	75[e]	>222

[a] For description of assays, see the Experimental Section. NT = not tested. [b] Plaque reduction assay in HFF cells. For cytotoxicity, see Table 4. [c] Average of duplicate or triplicate experiments. [d] IC_{50} 12.9 μM in rapidly proliferating Daudi cells. [e] Ganciclovir. [f] Acyclovir. [g] Foscarnet.

Table 7. Inhibition of Hepatitis B Virus (HBV) Replication in 2.2.15 Cells by Methylenecyclopropane Analogues[a]

	50% inhibitory concentration (IC_{50}, μM)	
compd	antiviral activity HBV	cytotoxicity[b] mtDNA synthesis[c]
14	2	>50
15	10	>100
16	>10	>50
17	>10	>100
33	>10	>50
34	>10	NT
ddC	1.4	0.07

[a] For details of the assays, see the Experimental Section, NT = not tested. [b] Cytotoxicity was determined in CEM cells (Table 5) and by effect of analogues on mitochondrial DNA (mtDNA) synthesis. The IC_{50} of ddC was 10 μM in CEM cells.

Table 8. Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Replication by Methylenecyclopropane Analogues

	50% inhibitory concentration (IC_{50}, μM)		
	antiviral activity[a] HIV_{IIIB} RT[a]		
compd	low moi	high moi	cytotoxicity CEM-SS[b]
14	0.8	20	>100
15	1.1	>30	>100
16	14	>100	>100
17	1.9	>30	>100
33	>100	NT	>100
34	>100	NT	>100
AZT	0.003	0.017	NT

[a] Activity of compounds against HIV strain III_B was determined in triplicate assays at low (~0.1 pfu/cell) and high (~1 pfu/cell) multiplicity of infection (moi) by supernatant reverse transcriptase (RT) activity as detailed in the Experimental Section. NT = not tested. [b] Visual cytotoxicity was determined by microscopic examination.

against HHV-6 (IC_{50} 14 and 42.5 μM, respectively) roughly corresponded to that of drug foscarnet (IC_{50} 31 μM).

In the area of nonherpetic viruses, synadenol (**14**) was the most effective agent against HBV in 2.2.15 cell culture (IC_{50} 2 μM) whereas synguanol (**15**) was less active with IC_{50} 10 μM (Table 7). Unlike ddC control, both analogues were devoid of cytotoxicity and long-term (mitochondrial) toxicity in the concentration range tested. The IC_{50} values of 2-amino-6-chloropurine *Z*-analogue **33** and *E*-isomers **16**, **17**, and **34** were above 10 μM.

The efficacy of all these analogues against HIV-1 in CEM-SS cells was of significantly lower magnitude than against most herpesviruses (Table 8). Thus, the antiretroviral effect of analogues **14** and **15** in micromolar range (IC_{50} 0.8−1.1 μM) was seen only at a lower virus titer. At higher concentrations of HIV-1, some effect (IC_{50} 20 μM) was noted only with synadenol (**14**).

As already mentioned, all *E*-isomers were devoid of significant antiviral activity. Adenine analogue **16** is the sole exception with an IC_{50} of 3 μM against EBV in H-1 cell culture, but this activity is one order of magnitude lower than that of synadenol (**14**). Also, compound **16** had only a moderate effect against EBV in Daudi cells (IC_{50} 71.4 μM).

All effective analogues displayed antiviral activity in nontoxic concentration ranges. The IC_{50} values determined in stationary HFF, MEF, CEM, Daudi, and KB cells were all above 50 μM and in most cases higher than 100 μM. In rapidly proliferating HFF and Daudi cells,

synadenol (**14**) was noncytotoxic (IC_{50} >100 μM) but synguanol (**15**) was moderately cytotoxic in Daudi cells (IC_{50} 13 μM). The cytotoxicity of analogues tested in uninfected H-1 cells showed IC_{50}'s above 50−100 μM. The IC_{50} values for inhibition of mitochondrial DNA synthesis in CEM cells, a measure of long-term cytotoxicity,[38] were in the same range.

B. Antitumor Activity. The antitumor activity of synadenol (**14**) and synguanol (**15**) was at best moderate. In murine leukemia L1210 clonogenic assay,[39] the IC_{50} values were 50 (**14**) and 40 μM (**15**), respectively. The *E*-isomers **16** and **17** exhibited IC_{50} >100 and 55 μM. Analogues **15**−**17** were also inactive in a zone assay[39] against mouse solid tumors C-38 and M-17/Adr as well as against human tumor cell line H-116. With synadenol (**14**) only a moderate antitumor effect was observed at 500 μg/disk.

C. Adenosine Deaminase (ADA) and AMP Deaminase (AMPDA). Synadenol (**14**) and *E*-isomer **16** are substrates for ADA from calf intestine. The latter analogue is a better substrate than the *Z*-isomer **14**, in agreement with a pattern observed previously.[23] Thus, compound **16** was deaminated in less than 24 h whereas the reaction of **14** was significantly slower. The reaction proceeded to about 45% conversion after 48 h, which did not appreciably change after a total of 120 h (50% conversion). This indicated that deamination of synadenol (**14**) may exhibit a significant degree of enantioselectivity.[40] A further exploration of this important facet is in progress. Synadenol (**14**) is a good substrate for AMPDA from *aspergillus* sp.[41] (95% deamination after 4.5 h).

Additional in vitro and in vivo tests as well as studies of structure−activity relationships and mechanism of action of the most potent analogues are being continued and they will be reported at a later date.

Experimental Section

General Methods. See reference 40. The NMR spectra were determined in CD_3SOCD_3 at 300 or 500 MHz (^{1}H) unless stated otherwise. For IR spectra, KBr pellets were used and UV spectra were measured in ethanol. The FAB mass spectra were recorded using a thioglycerol matrix. Adenosine deaminase from calf intestine (Type II) and AMP deaminase from *aspergillus* sp. were products of Sigma Chemical Co., St. Louis, MO.

9-(2-Bromo-2-propen-1-yl)adenine (20). A. Using Sodium Salt of Adenine (18). 2,3-Dibromopropene (**19**, 80%, 0.90 mL, 7.0 mmol) was added into a suspension of sodium salt of adenine made from adenine (**18**, 946 mg, 7.0 mmol) and NaH (60% dispersion in mineral oil, 310 mg, 7.7 mmol) in DMF (30 mL) at room temperature with stirring under N_2 over 5 min. The stirring was continued for another hour, and DMF was evaporated in vacuo (oil pump). The residue was triturated with CH_2Cl_2−MeOH (85:15, 60 mL). The insoluble portion was filtered off using a Celite bed, and it was washed with the same solvent. The combined washings were evaporated to give a syrup which was chromatographed on a silica gel column using gradient 94:6 → 9:1 CH_2Cl_2−MeOH to give compound **20** (1.05 g, 59%): mp 183−185 °C after recrystallization from CH_2Cl_2−MeOH (99:1); UV λ_{max} 260 nm (ϵ 15 000), 209 (ϵ 20 300); IR 3305 and 3150 (s, NH_2), 1665, 1600, and 1582 cm^{-1} (s, olefin and adenine); ^{1}H NMR ∂ 5.06 (s, 2H, $H_{1'}$), 5.67 (d, 1H, ^2J = 2.4 Hz) and 5.81 (dd, 1H, ^2J = 2.4 Hz, ^4J = 1.2 Hz, $H_{3'}$), 7.28 (s, 2H, NH_2), 8.124 (s, 1H) and 8.117 (s, 1H, H_2 and H_8); ^{13}C NMR 50.51 ($C_{1'}$), 120.81 ($C_{3'}$), 127.63 ($C_{2'}$), 118.79 (C_5), 141.27 (C_8), 149.84 (C_4), 153.20 (C_2), and 156.29 (C_6); EI-MS 253 and 255 (M, 22.4, 21.9), 174 (M − Br, 100.0), 147 (M − BrC=CH$_2$ − H, 23.7), 135 (adenine, 7.5); HRMS calcd for $C_8H_8{}^{79}BrN_5$ M 252.9963, found M 252.9953. Anal. ($C_8H_8BrN_5$) C, H, Br, N.

B. Using K_2CO_3. The suspension of adenine (**18**, 946 mg, 7.0 mmol), K_2CO_3 (967 mg, 7.0 mmol), and 2,3-dibromopropene (**19**, 80%, 0.90 mL, 7.0 mmol) in DMF (25 mL) was stirred at room temperature under N_2 for 45 h. The workup followed method A to give compound **20** (925 mg, 52%), which was identical with the product described above.

***N*^{6},*N*^{6}-Dibenzoyl-9-(2-bromo-2-propen-1-yl)adenine (23).** Benzoyl chloride (0.79 mL, 6.80 mmol) was added dropwise with stirring into a suspension of compound **20** (430 mg, 1.69 mmol) in pyridine (25 mL) at 0 °C. The stirring was then continued at room temperature for 16 h. Pyridine was evaporated in vacuo, and the residue was partitioned between CH_2Cl_2 (100 mL) and saturated aqueous $NaHCO_3$ (30 mL). The organic phase was washed with water and saturated NaCl (30 mL each), and it was dried (Na_2SO_4). Evaporation gave a brown solid which was recrystallized from benzene−cyclohexane (1:1) to furnish compound **23** (580 mg, 74%), mp 196−198 °C. The mother liquor was evaporated, and the residue was chromatographed on a silica gel column to give another portion (125 mg, 16%) of product **23**: UV λ_{max} 271 nm (shoulder, ϵ 18 100), 250 (ϵ 23 200), 208 (ϵ 34 700); IR 1715 (C=O, amide), 1650 (w, olefin), 1605 and 1582 cm^{-1} (purine ring); ^{1}H NMR ($CDCl_3$) ∂ 5.09 (s, 2H, $H_{1'}$), 5.73 (d, 1H, ^2J = 2.1 Hz) and 5.88 (d, 1H, ^2J = 2.1 Hz, $H_{3'}$), 8.14 (s, 1H) and 8.68 (s, 1H, H_2 and H_8), 7.35 (t, 4H, H_{meta}, ^3J = 7.7 Hz), 7.48 (t, 2H, H_{para}, ^3J = 7.4 Hz) and 7.85 (d, 4H, H_{ortho}, ^3J = 7.5 Hz, C_6H_5CO); ^{13}C NMR (CD_3SOCD_3) 51.38 ($C_{1'}$), 125.37 ($C_{3'}$), 127.12 ($C_{2'}$), 172.28 (C=O), 121.43 (C_5), 144.62 (C_8), 151.98 (C_4), 152.51 (C_2), and 153.05 (C_6); EI-MS 460 and 462 (M − H, 3.5, 3.3), 459 and 461 (M − 2H, 2.6, 3.2), 432 and 434 (M − CO − H, 7.8, 7.6), 431 and 433 (M − CO − 2H, 2.3, 4.1), 404 and 406 (M − 2 × CO, 2.6, 2.4), 355 and 357 (M − COPh − H, 24.3, 24.3), 339 and 341 (7.0, 7.3), 327 and 329 (2.5, 2.7), 104 (PhCO, 100.0), 77 (Ph, 50.1); HRMS calcd for $C_{22}H_{16}{}^{79}BrN_5O_2$ M 461.0487, found M 461.0495.

Ethyl *(E)*- and *(Z)*-2-Bromo-2-(bromomethyl)cyclopropane-1-carboxylates (24 and 25). Ethyl diazoacetate (90% in CH_2Cl_2, 23.3 mL, 0.20 mol) was added into a solution of dirhodium tetraacetate (22.1 mg, 0.05 mmol) in 2,3-dibromopropene (**19**, 97%, 68.0 g, 0.34 mol) and CH_2Cl_2 (3 mL) with the aid of a syringe pump at a rate of 1.1 mL/hour with stirring at room temperature. The reaction mixture was distilled in vacuo, and the distillate was trapped at −78 °C to recover unreacted 2,3-dibromopropene (**19**, 20 g, 29%). Water (100 mL) was added to the oily residue followed by a portionwise addition of powdered $KMnO_4$ at 0 °C with stirring. A total of 30 g of permanganate was consumed. The excess of permanganate was removed by addition of solid $Na_2S_2O_3$. The mixture was filtered using a Celite pad, and the solids were washed with ether (4 × 80 mL) with the aid of a sonicator. The filtrate was extracted with ether (4 × 70 mL). The combined ether portions were washed with saturated aqueous $NaHCO_3$ (2 × 100 mL), water (2 × 250 mL), and saturated NaCl (2 × 100 mL). After drying (Na_2SO_4) and evaporation of ether, a mixture of products **25** and **26** was obtained as a yellow oil (51.9 g, 91% yield), $n_D{}^{25}$ = 1.5131. It was of sufficient purity to be used in the subsequent steps. The ^{1}H NMR spectrum indicated a mixture of *E*- and *Z*-isomers **24** and **25** in the ratio of 1.5:1. Distillation of a sample of this product (5.0 g) gave colorless liquid (4.5 g): bp 59−64 °C/0.25 mmHg; $n_D{}^{25}$ = 1.5139; IR (neat) 1733 (C=O, ester), 1036 and 868 cm^{-1} (cyclopropane ring); ^{1}H NMR ($CDCl_3$) ∂, *E*-isomer **24**, 1.30 (t, 3H, ^3J = 7.2 Hz, CH_3), 1.73 (t, 1H, ^2J = $^3J_{trans}$ = 6.8 Hz) and 1.85 (dd, 1H, ^2J = 6.3 Hz, $^3J_{cis}$ = 9.0 Hz, H_3), 2.47 (dd, 1H, $^3J_{cis}$ = 9.0 Hz, $^3J_{trans}$ = 7.1 Hz, H_1), 3.97 (d, 2H, J_{AB} = 11.4 Hz, CH_2Br), 4.19 (q, 2H, ^3J = 7.5 Hz, OCH_2); *Z*-isomer **25**, 1.29 (t, 3H, ^3J = 7.2 Hz, CH_3), 1.52 (dd, 1H, ^2J = 6.9 Hz, $^3J_{cis}$ = 9.3 Hz) and 1.89 (t, 1H, ^2J = $^3J_{trans}$ = 7.1 Hz, H_3), 2.10 (dd, 1H, $^3J_{cis}$ = 9.3 Hz, $^3J_{trans}$ = 7.1 Hz, H_1), 3.76 (AB, 2H, J_{AB} = 11.1 Hz, CH_2Br), 4.21 (q, 2H, ^3J = 7.2 Hz, OCH_2); ^{13}C NMR ($CDCl_3$), *E*-isomer **24**, 14.14 (CH_3), 26.43 (C_3), 31.44 (C_1), 37.21 (C_2),

38.91 (CH_2Br), 61.66 (OCH_2), 169.32 (C=O); *Z*-isomer **25**, 14.30 (CH_3), 22.34 (C_3), 29.01 (C_1), 35.86 (C_2), 42.02 (CH_2Br), 61.59 (OCH_2), 168.12 (C=O); EI-MS 288 (M, 0.3), 286 (M, 1.9) and 284 (M, 0.5), 256 (1.6), 258 (2.6) and 260 (1.2), 243 (5.0), 241 (10.2) and 239 (5.3), 211 (7.0), 213 (13.8) and 215 (7.4), 205 (52.2) and 207 (51.0), 177 (96.7) and 179 (94.4), 159 (7.1) and 161 (7.0), 131 (15.2) and 133 (16.8), 97 (30.4), 81 (15.3), 69 (16.3), 53 (70.8), 39 (15.9), 28 (CO, 100.0); HRMS calcd for $C_7H_{10}{}^{79}Br_2O_2$ M 283.90475, found M 283.9048. Anal. ($C_7H_{10}Br_2O_2$) C, H, Br.

***(E)*-9-((1-Bromo-2-carbethoxycyclopropyl)methyl)adenine (21) and *(Z)*-9-((1-Bromo-2-carbethoxycyclopropyl)methyl)adenine (22).** A mixture of dibromo esters **24** and **25** (858 mg, 3.0 mmol) was added into a suspension of sodium salt of adenine which was prepared from adenine (**18**, 405 mg, 3.0 mmol) and NaH (60% dispersion in mineral oil, 120 mg, 3.0 mmol) in DMF (25 mL) with stirring under N_2 at room temperature. The resultant mixture was heated at 80 °C (bath temperature) for 12 h. The solvent was evaporated in vacuo, and the residue was adsorbed on silica gel (3 g) which was then put on the top of a silica gel (70 g) column. The elution was performed with CH_2Cl_2−MeOH (96:4 and 94:6) to give the *E*-isomer **21** (305 mg, 29.9% yield) and *Z*-isomer **22** (180 mg, 17.6% yield). Analytical samples were obtained by recrystallization from benzene−ethyl acetate (*E*-isomer **21**) and benzene (*Z*-isomer **22**).

E-isomer **21**: mp 200−203 °C; UV λ_{max} 260 (ϵ 14 900), 209 nm (ϵ 19 500); IR 3340 and 3150 (NH_2), 1725 (C=O, ester), 1670, 1600, and 1580 (adenine ring), 1045, 1015, and 867 cm^{-1} (cyclopropane ring); ^1H NMR ∂ 1.23 (t, 3H, ^3J = 7.2 Hz, CH_3), 1.76 (dd, 1H, ^2J = 6.9 Hz, $^3J_{cis}$ = 9.0 Hz) and 2.00 (t, 1H, ^2J = $^3J_{trans}$ = 6.9 Hz, $H_{3'}$), 2.49 (dd, 1H, $^3J_{cis}$ = 9.3 Hz, $^3J_{trans}$ = 7.5 Hz, $H_{4'}$), 4.18 (q, 2H, ^3J = 7.2 Hz, OCH_2), 4.66 (s, 2H, $H_{1'}$), 7.24 (s, 2H, NH_2), 8.10 (s, 2H, H_2 and H_8); ^{13}C NMR 13.52 (CH_3), 22.25 ($C_{3'}$), 28.57 ($C_{4'}$), 36.28 ($C_{2'}$), 46.64 ($C_{1'}$), 60.75 (OCH_2), 168.82 (C=O), 118.10 (C_5), 140.46 (C_8), 149.20 (C_4), 152.03 (C_2), 155.49 (C_6); FAB-MS 341 and 343 (M + 2H, 20.3, 18.6), 340 and 342 (M + H, 98.4, 100.0), 262 (8.0), 214 (4.3), 188 (5.9), 136 (25.2). Anal. ($C_{12}H_{14}BrN_5O_2$) C, H, Br, N.

Z-isomer **22**: mp 186−189 °C; UV λ_{max} 260 (ϵ 14 700), 209 nm (ϵ 18 700); IR 3350 and 3160 (NH_2), 1745 (C=O, ester), 1655, 1605, and 1585 (adenine ring), 1045, 1015, and 865 cm^{-1} (cyclopropane ring); ^1H NMR ($CDCl_3$) ∂ 1.14 (t, 3H, ^3J = 7.2 Hz, CH_3), 1.57 (t, 1H, ^2J = $^3J_{trans}$ = 6.8 Hz) and 1.93 (dd, 1H, ^2J = 6.6 Hz, $^3J_{cis}$ = 9.0 Hz, $H_{3'}$), 2.64 (dd, 1H, $^3J_{cis}$ = 9.0 Hz, $^3J_{trans}$ = 7.2 Hz, $H_{4'}$), 4.07 (q, 2H, ^3J = 7.2 Hz, OCH_2), 4.52 (AB, 2H, J_{AB} = 14.7 Hz, $H_{1'}$), 7.27 (s, 2H, NH_2), 8.13 and 8.17 (2s, 2H, H_2 and H_8); ^{13}C NMR ($CDCl_3$) 13.70 (CH_3), 19.95 ($C_{3'}$), 26.05 ($C_{4'}$), 36.99 ($C_{2'}$), 51.93 ($C_{1'}$), 60.42 (OCH_2), 167.63 (C=O), 118.01 (C_5), 140.30 (C_8), 149.23 (C_4), 152.14 (C_2), 155.55 (C_6); FAB-MS 341 and 343 (M + 2H, 19.0, 18.2), 340 and 342 (M + H, 96.3, 100.0), 262 (32.1), 214 (12.1), 188 (12.5), 136 (78.3). Anal. ($C_{12}H_{14}BrN_5O_2$) C, H, Br, N.

***(Z)*-9-((2-Carbethoxycyclopropylidene)methyl)adenine (26) and *(E)*-9-((2-Carbethoxycyclopropylidene)methyl)adenine (27). A. Using a Mixture of Bromo Esters 21, 22, and Potassium *tert*-Butoxide.** Potassium *tert*-butoxide (505 mg, 4.5 mmol) was added into a solution of a mixture of bromo esters **21** and **22** (1.5:1, 1.02 g, 3 mmol) in DMF (35 mL) at 0 °C with stirring under N_2. The stirring was continued for 2 h. The reaction mixture was then added dropwise into saturated aqueous NH_4Cl (20 mL) at 0 °C with stirring. After 15 min, the solvents were removed by evaporation in vacuo. The crude product was chromatographed on a silica gel column using CH_2Cl_2−MeOH (9:1) as an eluent to give the mixture of compounds **26** and **27** (545 mg, 70%), mp 166−175 °C after recrystallization from benzene. The ^1H NMR indicated the presence of *Z*- and *E*-isomers **26** and **27** in the ratio of 1.5:1: UV max 276 λ_{nm} (shoulder, ϵ 7600), 256 (shoulder, ϵ 11 800), 228 (ϵ 22 500); IR 3350 and 3160 (NH_2), 1735 (C=O, ester), 1660, 1605, and 1585 cm^{-1} (adenine ring and olefin); *Z*-isomer **26**: ^1H NMR ∂ 1.13 (t, 3H, ^3J = 7.3 Hz, CH_3), 1.91 (ddd, 1H, ^2J = 8.5 Hz, $^3J_{trans}$ = 5.0 Hz, ^4J = 2.0 Hz) and 2.00 (td, 1H, ^2J = $^3J_{cis}$ = 8.5 Hz, ^4J = 2.0 Hz, $H_{3'}$), 2.89 (ddd, 1H, $^3J_{cis}$ = 8.3 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 2.0 Hz, $H_{4'}$), 4.02−4.13 (m, 2H, OCH_2), 7.40 (s, 2H, NH_2), 7.54 (q, 1H, ^4J = 2.0 Hz, $H_{1'}$), 8.15 (s, 1H) and 8.23 (s, 1H, H_2 and H_8); ^{13}C NMR 10.70 ($C_{3'}$), 14.52 (CH_3), 19.78 ($C_{4'}$), 61.10 (OCH_2), 111.71 ($C_{1'}$), 112.85 ($C_{2'}$), 170.71 (C=O), 118.90 (C_5), 137.75 (C_8), 148.76 (C_4), 153.64 (C_2), 156.56 (C_6). *E*-isomer **27**: ^1H NMR ∂ 1.19 (t, 3H, ^3J = 7.3 Hz, CH_3), 2.07 (ddd, 1H, ^2J = 9.5 Hz, $^3J_{trans}$ = 5.0 Hz, ^4J = 3.0 Hz) and 2.17 (td, 1H, ^2J = 9.0 Hz, $^3J_{cis}$ = 8.2 Hz, ^4J = 2.5 Hz, $H_{3'}$), 2.64 (ddd, 1H, $^3J_{cis}$ = 8.0 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 1.8 Hz, $H_{4'}$), 4.02−4.13 (m, 2H, OCH_2), 7.39 (s, 2H, NH_2), 7.59 (q, 1H, $H_{1'}$), 8.18 (s, 1H, H_2), 8.52 (s, 1H, H_8); ^{13}C NMR 13.30 ($C_{3'}$), 14.52 (CH_3), 17.52 ($C_{4'}$), 61.10 (OCH_2), 112.18 ($C_{1'}$), 113.16 ($C_{2'}$), 171.29 (C=O), 118.90 (C_5), 137.99 (C_8), 148.76 (C_4), 153.64 (C_2), 156.56 (C_6); EI-MS 259 (M, 54.2), 245 (6.1), 230 (24.3), 214 (28.4), 202 (9.9), 187 (100.0), 160 (19.6), 135 (22.4). Anal. ($C_{12}H_{13}N_5O_2$) C, H, N.

B. Using NaH. A mixture of NaH (60% in mineral oil, 120 mg, 3 mmol) and bromo derivatives **21** + **22** (1:1) in THF (8 mL) was stirred at room temperature under N_2 for 5 h. Acetic acid (0.2 mL) was then added, and solvent was evaporated. Chromatography on a silica gel column using CH_2Cl_2−MeOH (9:1) gave products **26** + **27** (18 mg, 23%). The isomeric ratio **26/27** was 2:1.

C. One-Pot Alkylation/Elimination Procedure Using K_2CO_3. A mixture of adenine (**18**, 810 mg, 6.0 mmol), (*E*)- and (*Z*)-ethyl 2-bromo-2-(bromomethyl)cyclopropane-1-carboxylates (**24** and **25**, 2.14 g, 7.5 mmol) and flame-dried K_2CO_3 (4.98 g, 36.0 mmol) in DMF (30 mL) was stirred at 100 °C under nitrogen for 22 h. After cooling, the insoluble portion was filtered off, and it was washed with a mixture of CH_2Cl_2−MeOH (4:1, 2 × 50 mL). The filtrate was evaporated, and the residue was flash-chromatographed on a silica gel column with CH_2Cl_2−MeOH (95:5) as an eluent. Evaporation of appropriate fractions gave a mixture of products **26** and **27** (610 mg, 39.2%, mp 166−175 °C in the ratio of 2:1).

D. Using Separated 21, 22, and Potassium *tert*-Butoxide. Two separate experiments were performed with *E*- and *Z*-isomer **21** or **22** (17 mg, 50 μmol) and tBuOK (8.4 mg, 75 μmol) in DMF (1 mL) as described in method A. The solvent was evaporated, and the crude product was chromatographed on 2 mm thick silica gel TLC plate (10 × 10 cm) in CH_2Cl_2−MeOH (9:1). The major UV-absorbing band was eluted with the same solvent to give 8−10 mg of **26** and **27**. The ^1H NMR showed the ratio of **26/27** obtained from *E*-isomer **21** was 1.5:1 whereas that from *Z*-isomer **22** gave the ratio of 1:1.5.

E. Using Separated 21, 22, and K_2CO_3. The experiments were performed as described in method D with *E*- and *Z*-isomer **21** or **22** (31 mg, 0.09 mmol) and K_2CO_3 (38 mg, 0.27 mmol) instead of tBuOK at 100 °C for 3 h. After cooling, the insoluble portion was filtered off using a Celite pad, and it was washed with DMF (3 × 3 mL). The volatile components were removed in vacuo, and the residue was dissolved in CD_3SOCD_3. The ^1H NMR indicated the presence of **21** and products **26**, **27** (45:55) and the ratio of **26/27** was 2.5:1. With the *Z*-isomer **22** the reaction was run to the completion of elimination (100 °C, 10 h), and the ratio of **26/27** was 2:1.

Synadenol (14) and *(E)*-9-((2-(Hydroxymethyl)cyclopropylidene)methyl)adenine (16). DIBALH in THF (1 M, 27.2 mL, 27.2 mmol) was added dropwise into a solution of esters **26** + **27** (881 mg, 3.4 mmol) in THF (50 mL) at 0 °C over 10 min with stirring under N_2. The stirring at 0 °C was continued for 3.5 h. Saturated aqueous NH_4Cl (25 mL) was then added, and the gel-like solid was filtered off using a Celite bed. It was washed with CH_2Cl_2−MeOH (1:1, 5 × 40 mL). The combined filtrate and washings were evaporated, and the residue was chromatographed on a silica gel column using CH_2Cl_2−MeOH (9:1 and 85:15) as eluents. The appropriate fractions were evaporated to give a mixture of compounds **14** and **16** (557 mg, 75%).

A solution of this mixture (2.56 mmol) in DMF (30 mL) and *N,N*-dimethylformamide dimethyl acetal (0.51 mL, 3.85 mmol) was stirred at room temperature for 14 h. Evaporation of the

volatile components in vacuo at room temperature (oil pump) left a mixture of compounds **28** and **29** as a syrup which was flash-chromatographed on a silica gel column using dichloromethane–methanol (92:8 and 85:15). The appropriate fractions were pooled and evaporated to give *Z*-isomer **28** (264 mg, 37.8% yield) as a white solid and *E*-isomer **29** (260 mg, 37.2% yield) as a foam. A small portion of unresolved **28** and **29** (63 mg, 9.0% yield) was also recovered.

Z-isomer **28**: mp 172–178 °C; UV λ_{max} 311 (ϵ 31 300), 233 nm (ϵ 27 600); IR 3500–3180 (OH), 1638 and 1600–1540 (olefin and N^6-((dimethylamino)methylene)adenine), 1060, 1030, 830, and 810 cm^{-1} (cyclopropane ring); ^1H NMR ∂ 1.21 (t, 1H, $^2J = {}^3J_{trans}$ = 6.8 Hz) and 1.49 (t, 1H, $^2J = {}^3J_{cis}$ = 8.7 Hz, $H_{3'}$), 2.14 (dq, 1H, $^3J_{cis}$ = 7.5 Hz, $^3J_{trans}$ = 5.7 Hz, $H_{4'}$), 3.10 and 3.17 (2s, 6H, $N(CH_3)_2$), 3.29 (t, overlapped with H_2O, 1H, ^2J = 10.8 Hz) and 3.74 (dt, 1H, ^2J = 10.8 Hz, ^3J = 5.3 Hz, $H_{5'}$), 5.11 (t, 1H, ^3J = 5.1 Hz, OH), 7.43 (s, 1H, $H_{1'}$), 8.42 (s, 1H, H_2), 8.86 (s, 1H, H_8), 8.90 (s, 1H, N=C**H**$N(CH_3)_2$); ^{13}C NMR 6.74 ($C_{3'}$), 19.73 ($C_{4'}$), 35.00 and 41.13 ($N(CH_3)_2$), 63.38 ($C_{5'}$), 110.60 ($C_{1'}$), 116.18 ($C_{2'}$), 152.77 (N=**C**H$N(CH_3)_2$), 125.38 (C_5), 139.98 (C_8), 150.40 (C_4), 158.53 (C_2), 159.66 (C_6); FAB-MS 381 (M + thioglycerol + H, 5.7), 273 (M + H, 7.4), 191 (8.2), 149 (29.8), 91 (100.0).

E-isomer **29**: UV λ_{max} 310 (ϵ 32 300), 231 nm (ϵ 29 500); IR 3550–3200 (OH), 1635 and 1600–1540 (olefin and N^6-((dimethylamino)methylene)adenine), 1030 and 810 cm^{-1} (cyclopropane ring); ^1H NMR ∂ 1.39 (ddd, 1H, ^2J = 8.7 Hz, $^3J_{trans}$= 5.4 Hz, ^4J = 2.4 Hz) and 1.71 (td, 1H, ^2J = 9.0 Hz, $^3J_{cis}$ = 9.0 Hz, ^4J = 2.1 Hz, $H_{3'}$), 1.97 (dqd, 1H, $^3J_{cis}$ = 8.4 Hz, $^3J_{trans}$ = 6.6 Hz, ^4J = 1.5 Hz, $H_{4'}$), 3.11 and 3.17 (2s, 6H, $N(CH_3)_2$), 3.41 (t, 2H, ^3J = 6.2 Hz, $H_{5'}$), 4.82 (t, 1H, ^3J = 5.9 Hz, OH), 7.52 (d, 1H, $H_{1'}$), 8.43 (s, 1H, H_2), 8.56 (s, 1H, H_8), 8.89 (s, 1H, N=C**H**N-$(CH_3)_2$); ^{13}C NMR 7.93 ($C_{3'}$), 18.09 ($C_{4'}$), 35.04 and 41.14 ($N(CH_3)_2$), 63.57 ($C_{5'}$), 110.74 ($C_{1'}$), 116.59 ($C_{2'}$), 152.80 (N=**C**HN-$(CH_3)_2$), 125.40 (C_5), 139.47 (C_8), 150.59 (C_4), 158.48 (C_2), 159.67 (C_6); FAB-MS 381 (M + thioglycerol + H, 51.7), 273 (M + H, 100.0), 191 (69.8).

The separated isomers (250 mg, 0.92 mmol of each) were dissolved in methanolic ammonia saturated at 0 °C (100 mL). The mixtures were stirred at room temperature for 16 h. The volatile components were evaporated, and the residues were recrystallized from methanol to give synadenol (**14**, 170 mg, 85%) and the *E*-isomer **16** (172 mg, 86%).

Synadenol (**14**): mp 238–239 °C (change of modification at 200–231 °C); UV λ_{max} 276 (shoulder, ϵ 9300), 261 (ϵ 13 500), 226 nm (ϵ 29 400); IR 3300 and 3100 (NH_2 and OH), 1670, 1600, and 1580 (adenine ring and olefin), 1047 and 1025 cm^{-1} (cyclopropane ring); ^1H NMR ∂ 1.22 (ddd, 1H, ^2J = 8.0 Hz, $^3J_{trans}$ = 5.3 Hz, ^4J = 1.5 Hz) and 1.50 (dt, 1H, ^2J = 8.5 Hz, $^3J_{cis}$ = 8.5 Hz, ^4J = 1.5 Hz, $H_{3'}$), 2.13 (dqd, 1H, $^3J_{cis}$ = 8.8 Hz, $^3J_{trans}$ = 6.5 Hz, ^4J = 1.5 Hz, $H_{4'}$), 3.33 (dt, overlapped with H_2O, 1H, ^2J = 13.0 Hz, ^3J = 5.5 Hz) and 3.73 (dt, 1H, ^2J = 11.0 Hz, ^3J = 5.3 Hz, $H_{5'}$), 5.11 (td, 1H, ^3J = 4.8 Hz, ^4J = 0.8 Hz, OH), 7.33 (s, 2H, NH_2), 7.38 (d, 1H, ^4J = 1.5 Hz, $H_{1'}$), 8.17 (s, 1H, H_2), 8.74 (s, 1H, H_8); ^{13}C NMR 6.72 ($C_{3'}$), 19.70 ($C_{4'}$), 63.35 ($C_{5'}$), 110.69 ($C_{1'}$), 115.87 ($C_{2'}$), 118.84 (C_5), 138.13 (C_8), 148.13 (C_4), 153.42 (C_2), 156.47 (C_6); EI-MS 217 (M, 31.4), 200 (45.2), 187 (37.0), 173 (6.5), 159 (11.2), 148 (15.3), 135 (adenine, 100.0); HRMS calcd for $C_{10}H_{11}N_5O$ M 217.0964, found M 217.0960. Anal. ($C_{10}H_{11}N_5O$) C, H, N.

E-isomer **16**: mp 208–210 °C; UV λ_{max} 277 (shoulder, ϵ 8900), 261 (ϵ 12 200), 226 nm (ϵ 27 600); IR 3340 and 3180 (NH_2 and OH), 1666, 1612, and 1578 cm^{-1} (adenine ring and olefin); ^1H NMR ∂ 1.38 (ddd, 1H, ^2J = 8.0 Hz, $^3J_{trans}$ = 5.5 Hz, ^4J = 2.5 Hz) and 1.71 (dt, 1H, $^2J = {}^3J_{cis}$ = 8.9 Hz, ^4J = 1.5 Hz, $H_{3'}$), 1.97 (dqd, 1H, $^3J_{cis}$ = 8.6 Hz, $^3J_{trans}$ = 6.5 Hz, ^4J = 1.5 Hz, $H_{4'}$), 3.41 (t, 2H, ^3J = 6.3 Hz, $H_{5'}$), 4.82 (td, 1H, ^3J = 5.6 Hz, ^4J = 0.5 Hz, OH), 7.34 (s, 2H, NH_2), 7.48 (q, 1H, ^4J = 2.0, $H_{1'}$), 8.17 (s, 1H, H_2), 8.48 (s, 1H, H_8); ^{13}C NMR 9.68 ($C_{3'}$), 18.06 ($C_{4'}$), 63.59 ($C_{5'}$), 110.81 ($C_{1'}$), 116.24 ($C_{2'}$), 118.85 (C_5), 137.61 (C_8), 148.68 (C_4), 153.48 (C_2), 156.49 (C_6); EI-MS 217 (M, 15.0), 200 (78.5), 172 (25.9), 148 (11.3), 136 (adenine + H, 43.1), 41 (100.0); HRMS calcd for $C_{10}H_{11}N_5O$ M 217.0964, found M 217.0954. Anal. ($C_{10}H_{11}N_5O$) C, H, N.

***(Z)*-2-Amino-6-chloro-9-((2-carbethoxycyclopropylidene)methyl)purine (31) and *(E)*-2-Amino-6-chloro-9-((2-carbethoxycyclopropylidene)methyl)purine (32).** A mixture of 2-amino-6-chloropurine (**30**, 2.04 g, 12 mmol), dibromo esters **24** + **25** (5.29 g, 15 mmol), and flame-dried K_2CO_3 (8.29 g, 60 mmol) in DMF (60 mL) was stirred at 100 °C (bath temperature) for 20 h under N_2. Chromatography on a silica gel column (80 g) using CH_2Cl_2–MeOH (98.5:1.5 and 97:3) gave a mixture of esters **31** and **32** (1.98 g, 56.2%) in the ratio of 2:1 as indicated by ^1H NMR: mp 177–196 °C after recrystallization from benzene or 171–187 °C after recrystallization from ethanol; UV λ_{max} 311 (ϵ 7400), 230 (ϵ 26 800), 210 nm (shoulder, ϵ 16 100); IR 3420, 3325, and 3220 (NH_2), 1725 (C=O, ester), 1646, 1615, and 1565 (purine ring and olefin), 1000 cm^{-1} (cyclopropane ring); *Z*-isomer **31**: ^1H NMR ∂ 1.10 (t, 3, ^3J = 7.0 Hz, CH_3), 1.91 (ddd, 1H, ^2J = 8.8 Hz, $^3J_{trans}$ =5.0 Hz, ^4J = 2.0 Hz) and 2.00 (td, 1H, $^2J = {}^2J_{cis}$ = 8.5 Hz, ^4J = 2.0 Hz, $H_{3'}$), 2.90 (ddd, 1H, $^3J_{cis}$ = 8.3 Hz, $^3J_{trans}$ = 5.0 Hz, ^4J = 2.0 Hz, $H_{4'}$), 4.02–4.12 (m, 2H, OCH_2), 6.99 (s, 2H, NH_2), 7.35 (q, 1H, ^4J = 2.2 Hz, $H_{1'}$), 8.19 (s, 1H, H_8); ^{13}C NMR 10.75 ($C_{3'}$), 14.42 (CH_3), 19.76 ($C_{4'}$), 61.17 (OCH_2), 111.17 ($C_{1'}$), 113.74 ($C_{2'}$), 170.58 (C=O), 123.49 (C_5), 139.75 (C_8), 150.27 (C_4), 152.95 (C_2), 160.55 (C_6). *E*-isomer **32**: ^1H NMR ∂ 1.18 (t, 3H, ^3J = 7.0 Hz, CH_3), 2.07 (ddd, 1H, ^2J = 9.3 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 2.8 Hz) and 2.17 (ddd, 1H, ^2J = 9.5 Hz, $^3J_{cis}$ = 8.5 Hz, ^4J = 2.5 Hz, $H_{3'}$), 2.63 (ddd, 1H, $^3J_{cis}$ = 8.5 Hz, $^3J_{trans}$ = 5.0 Hz, ^4J = 1.8 Hz, $H_{4'}$), 4.02–4.12 (m, 2H, OCH_2), 7.04 (s, 2H, NH_2), 7.42 (q, 1H, ^4J = 2.2 Hz, $H_{1'}$), 8.46 (s, 1H, H_8); ^{13}C NMR 13.45 ($C_{3'}$), 14.50 (CH_3), 17.67 ($C_{4'}$), 61.09 (OCH_2), 111.84 ($C_{1'}$), 114.11 ($C_{2'}$), 170.14 (C=O), 123.57 (C_5), 140.23 (C_8), 150.19 (C_4), 153.10 (C_2), 160.55 (C_6); EI-MS 293 and 295 (M 89.4, 29.9), 264 and 266 (30.8, 11.5), 248 and 250 (22.7, 8.7), 236 and 238 (10.9, 4.0), 221 and 223 (100.0, 32.9), 208 and 210 (5.9, 3.2), 184 (25.3), 169 and 171 (39.1, 14.1); HRMS calcd for $C_{12}H_{12}{}^{35}ClN_5O_2$ M 293.06795, found M 293.0680. Anal. ($C_{12}H_{12}ClN_5O_2$) C, H, Cl, N.

***(Z)*-2-Amino-6-chloro-9-((2-(hydroxymethyl)cyclopropylidene)methyl)purine (33) and *(E)*-2-Amino-6-chloro-9-((2-(hydroxymethyl)cyclopropylidene)methyl)purine (34).** DIBALH in cyclohexane (1 M, 19.4 mL, 19.4 mmol) was added dropwise into a solution of esters **31** + **32** (1.90 g, 6.47 mmol) in THF (100 mL) during 10 min with stirring at 0 °C under N_2. Two additional portions of hydride (6.47 mL, 6.47 mmol) were added in 30 min intervals. The stirring at 0 °C was continued for a total of 2 h. The reaction was quenched by adding aqueous methanol (50%, 10 mL) with stirring, which was then continued for 30 min at 0 °C and overnight at room temperature. The precipitated gel was filtered off, and it was washed with CH_2Cl_2–MeOH (4:1, 2 × 50 mL). The combined filtrate and washings were evaporated, and the crude product was chromatographed on a silica gel column using CH_2Cl_2–MeOH (97:3 and 92:8). The separated products **33** and **34** were rechromatographed to give *Z*-isomer **33** (680 mg, 41.8%) and *E*-isomer **34** (380 mg, 23.3%). Analytical samples of both isomers were obtained by recrystallization from ethanol.

Z-isomer **33**: mp 214–216 °C; UV λ_{max} 310 (ϵ 8000), 233 nm (ϵ 31 800); IR 3440 (shoulder), 3330 and 3200 (OH and NH_2), 1640, 1610, and 1570 (purine ring and olefin), 1040 and 1005 cm^{-1} (cyclopropane ring); ^1H NMR ∂ 1.21 (ddd, 1H, ^2J = 8.5 Hz, $^3J_{trans}$ = 5.3 Hz, ^4J = 2.0 Hz) and 1.49 (td, 1H, $^2J = {}^3J_{cis}$ = 8.3 Hz, ^4J = 1.5 Hz, $H_{3'}$), 2.13 (dqd, 1H, $^3J_{cis}$ = 8.3 Hz, $^3J_{trans}$ = 5.5 Hz, ^4J = 2.0 Hz, $H_{4'}$), 3.28 (ddd, 1H, ^2J = 11.3 Hz, ^3J = 7.8 Hz, ^3J = 6.0 Hz) and 3.73 (dt, 1H, ^2J = 10.5 Hz, ^3J = 5.0 Hz, $H_{5'}$), 5.06 (dd, 1H, ^3J = 6.0 Hz, ^3J = 4.5 Hz, OH), 7.01 (s, 2H, NH_2), 7.20 (q, 1H, $H_{1'}$), 8.70 (s, 1H, H_8); ^{13}C NMR 6.80 ($C_{3'}$), 19.73 ($C_{4'}$), 63.25 ($C_{5'}$), 110.24 ($C_{1'}$), 116.81 ($C_{2'}$), 123.52 (C_5), 140.36 (C_8), 150.00 (C_4), 152.81 (C_2), 160.49 (C_6); EI-MS 251 and 253 (M, 41.4, 15.1), 234 and 236 (50.9, 19.8), 221 and 223 (18.6, 8.9), 198 (26.2), 170 and 172 (100.0, 38.5). Anal. ($C_{10}H_{10}ClN_5O$) C, H, Cl, N.

E-isomer **34**: mp 201–204 °C; UV λ_{max} 310 (ϵ 8100), 231 nm (ϵ 33 700); IR 3460, 3340, and 3220 (OH and NH_2), 1650–1610 and 1568 (purine ring and olefin), 1010 cm^{-1} (cyclopropane ring); ^1H NMR ∂ 1.37 (ddd, 1H, ^2J = 9.0 Hz, $^3J_{trans}$ = 5.5

Hz, ^4J = 2.5 Hz) and 1.71 (td, 1H, ^2J = $^3J_{cis}$ = 9.0 Hz, ^4J = 2.5 Hz, $H_{3'}$), 1.96 (dqd, 1H, $^3J_{cis}$ = 8.6 Hz, $^3J_{trans}$ = 5.3 Hz, ^4J = 1.5 Hz, $H_{4'}$), 3.40 (AB, 2H, $H_{5'}$), 4.82 (t, 1H, ^3J = 6.0 Hz, OH), 7.01 (s, 2H, NH_2), 7.31 (q, 1H, ^4J = 2.0 Hz, $H_{1'}$), 8.48 (s, 1H, H_8); ^{13}C NMR 9.79 ($C_{3'}$), 18.16 ($C_{4'}$), 63.52 ($C_{5'}$), 110.44 ($C_{1'}$), 117.37 ($C_{2'}$), 123.51 (C_5), 140.00 (C_8), 150.05 (C_4), 152.99 (C_2), 160.48 (C_6); EI-MS 251 and 253 (M, 36.1, 12.3), 234 and 236 (84.0, 29.7), 222 and 224 (8.6, 3.4), 198 (24.6), 170 and 172 (100.0, 37.8). Anal. ($C_{10}H_{10}ClN_5O$) C, H, Cl, N.

Synguanol (15) and *(E)*-9-((2-(Hydroxymethyl)cyclopropylidene)methyl)guanine (17). The solution of the *Z*-isomer **33** (179 mg, 0.78 mmol) in HCO_2H (80%, 10 mL) was heated at 80 °C (bath temperature) with stirrring for 3.5 h. After cooling, the volatile components were evaporated, the crude product was dissolved in water (20 mL), and the solution was lyophilized. The resultant white solid was suspended in MeOH−NH_4OH (3:1, 40 mL), and the mixture was stirred overnight at room temperature. The solvents were evaporated, and a suspension of the residue in MeOH (70 mL) was refluxed for 2 h. The mixture was kept overnight at 0 °C to give synguanol (**15**, 155 mg, 85%): mp >300 °C; UV λ_{max} 271 (ϵ 11 600), 229 nm (ϵ 29 400); IR 3450 (flat peak), 3320 and 3170 (OH and NH_2), 1725, 1690, 1640, and 1600 (guanine ring and olefin), 1020 cm^{-1} (cyclopropane ring); ^{1}H NMR ∂ 1.17 (ddd, 1H, ^2J = 8.3 Hz, $^3J_{trans}$ = 5.5 Hz, ^4J = 2.0 Hz) and 1.45 (td, 1H, ^2J = $^3J_{cis}$ = 8.6 Hz, ^4J = 1.5 Hz, $H_{3'}$), 2.07 (dqd, 1H, $^3J_{cis}$ = 9.0 Hz, $^3J_{trans}$ = ^3J = 5.5 Hz, $H_{4'}$), 3.32 (t, overlapped with H_2O, 1H, ^2J = 10.0 Hz) and 3.68 (dd, 1H, ^2J = 11.0 Hz, ^3J = 5.5 Hz, $H_{5'}$), 5.04 (broad s, 1H, OH), 6.51 (s, 2H, NH_2), 7.11 (d, 1H, $H_{1'}$), 8.31 (s, 1H, H_8), 10.65 (s, 1H, NH); ^{13}C NMR 6.60 ($C_{3'}$), 19.56 ($C_{4'}$), 63.23 ($C_{5'}$), 110.59 ($C_{1'}$), 115.44 ($C_{2'}$), 116.68 (C_5), 134.70 (C_8), 150.12 (C_4), 154.33 (C_2), 157.11 (C_6); FAB-MS 342 (M + thioglycerol + H, 10.4), 234 (M + H, 3.9), 233 (M, 0.9), 232 (M − H, 3.9), 216 (6.6), 197 (15.3), 181 (52.0). Anal. $C_{10}H_{11}N_5O_2$ (C, H, N).

The procedure described above was used for the synthesis of the *E*-isomer **17** (120 mg, 83% yield) starting from the chloropurine intermediate **34** (150 mg, 0.60 mmol): mp >300 °C; UV λ_{max} 270 (ϵ 11 900), 228 nm (ϵ 30 300); IR 3440−3300 and 3150 (OH and NH_2), 1710−1640 and 1610 (guanine ring and olefin), 1030 cm^{-1} (cyclopropane ring); ^{1}H NMR ∂ 1.31 (ddd, 1H, ^2J = 9.0 Hz, $^3J_{trans}$ = 5.3 Hz, ^4J = 2.5 Hz) and 1.66 (td, 1H, ^2J = $^3J_{cis}$ = 9.0 Hz, ^4J = 2.5 Hz, $H_{3'}$), 1.92 (dqd, 1H, $^3J_{cis}$ = 8.6 Hz, $^3J_{trans}$ = ^3J = 5.5 Hz, ^4J = 1.5 Hz, $H_{4'}$), 3.37 (AB, 2H, J_{AB} = 14.54 Hz, ^3J = 6.5 Hz, $H_{5'}$), 4.80 (broad s, 1H, OH), 6.52 (s, 2H, NH_2), 7.21 (q, 1H, ^2J = 2.0 Hz, $H_{1'}$), 8.04 (s, 1H, H_8), and 10.66 (s, 1H, NH); ^{13}C NMR 9.55 ($C_{3'}$), 17.91 ($C_{4'}$), 63.58 ($C_{5'}$), 110.75 ($C_{1'}$), 115.89 ($C_{2'}$), 116.68 (C5), 134.16 (C_8), 150.29 (C_4), 154.35 (C_2), and 157.10 (C_6); FAB-MS 342 (M + thioglycerol + H, 44.5), 234 (M + H, 35.5), 216 (9.6), 197 (10.3), 181 (41.8), 152 (46.0), 91 (64.8), 73 (100.0). Anal. ($C_{10}H_{11}N_5O_2$·0.5 H_2O) C, H, N.

Adenosine Deaminase (ADA) Assay.[23] Compound **14** or **16** (2.6 μmol) was incubated with ADA from calf intestine (0.36 units) in 0.05 M Na_2HPO_4 (pH 7.5, 0.4 mL) at room temperature. Aliquots were periodically withdrawn and examined by TLC in CH_2Cl_2:MeOH (9:1). The spots of starting compound and product of deamination were eluted with ethanol and the UV spectra were taken. Analogue **16** was deaminated quantitatively in less than 24 h. Deamination of synadenol (**14**) gave the ratio of **14** and deaminated product approximately 1:1 after 48 h. This ratio was not changed after a total of 120 h of incubation.

AMP Deaminase (AMPDA) Assay.[41] Compound **14** (2.6 μmol) was incubated with AMPDA from *aspergillus* sp. (0.76 units) in 0.1 M Na_2HPO_4 (pH 6.5, 0.4 mL) at room temperature. TLC in CH_2Cl_2−MeOH (9:1) showed that deamination was >95% complete after 4.5 h.

Biological Assays. Cell Culture Procedures. The routine growth and passage of KB and HFF cells was performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum or 10% fetal bovine serum (FBS) for HFF cells. The sodium bicarbonate concentration was varied to meet the buffering capacity required. These and other adherent cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures by using 0.05% trypsin plus 0.02% EDTA in a HEPES-buffered salt solution.[42] Similar suspension culture conditions were employed for CEM cells.

Antiviral Assays. A. HCMV Plaque and Yield Reduction Assays. These assays used the Towne strain, plaque-purified isolate P_o of HCMV, kindly provided by Dr. Mark Stinski, University of Iowa. The HFF cells in 24-well cluster dishes were infected with approximately 100 plate-forming units (pfu) of HCMV per cm^2 cell sheet using the procedures detailed earlier.[43] Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 8−10 days, cell sheets were fixed and stained with crystal violet and microscopic plaques enumerated. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug. For yield reduction assay, the following procedure[44] was used. The HFF cells were planted at 12 500 cells/well in 96-well cluster dishes, incubated overnight, and infected with HCMV at a multiplicity of infection (moi) of 0.5−1 pfu/cell in a volume of 0.1 mL/well. After virus adsorption, fresh medium (0.1 mL) containing test compounds was added to the wells in a manner so that six compounds could be tested in duplicate on a single plate with concentrations from 100 to 0.14 μM. Plates were incubated at 37 °C for 4 days and subjected to one cycle of freezing and thawing; and aliquots from each of the wells were transferred to the first column of a fresh 96-well monolayer culture of HFF cells and serially diluted across the plate. Each column of the original drug-containing plate was diluted across a new separate plate in this manner. After virus adsorption, media were replaced, cultures were incubated, plaques were enumerated, and titers calculated as described.[44]

B. HSV-1 ELISA. An enzyme-linked immunosorbent assay (ELISA)[45] was employed to detect HSV-1. Briefly, 96-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 μL per well of MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in triplicate and HSV-1 (KOS strain kindly provided by Dr. Sandra K. Weller, University of Connecticut) at a concentration of 100 pfu/well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked, rinsed, and horseradish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody-containing solution, plates were rinsed and then developed by adding a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4, and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

C. HSV-1, HSV-2, VZV, and MCMV Plaque Reduction Assays. 1. HFF and MEF Cells. Two days prior to use, HFF or MEF cells were plated into six-well plates and incubated at 37 °C with 5% CO_2 and 90% humidity. On the date of assay, drug was made up in MEM with 2% FBS and then serially diluted 1:5 in MEM using six concentrations of drug. The initial starting concentration was usually 100 μg/mL down to 0.03 μg/mL. The virus used for infection was diluted in MEM containing 10% FBS to a desired concentration which gave 20−30 plaques per well. The media was aspirated from the wells and 0.2 mL of virus added to each well in triplicate with 0.2 mL of media being added to drug toxicity wells. The plates were then incubated for 1 h with shaking every 15 minutes. After incubation, MEM containing the various drug concentrations was added to appropriate wells in duplicate and in a volume of 2.0 mL. For HSV-1 and HSV-2, pooled human globulin obtained from Baxter Health Care Corp. was diluted 1:500 and added to the media that the drug was diluted in to prevent extracellular spread of HSV. For VZV, no antibody in the overlay was utilized. For the HSV

assay the cultures were incubated for 3 days. For VZV assays additional media was added on day 5 and incubated for a total of 10 days. MCMV assays had no additional media added, and they were incubated for 7 days. At the end of the incubation period for HSV-1 and -2, 1.0 mL of 0.1% crystal violet in 20% methanol was added to each well and incubated for 10 min. The liquid was then aspirated off, monolayers were washed, and plaques were enumerated using a stereomicroscope. For VZV, HCMV, and MCMV assays the cells were stained with 1.5% neutral red solution for MCMV and 5% for HCMV and VZV. The cells were incubated for 6 h, the liquid was removed, and plaques were enumerated. The results were compared with control wells and IC_{50} values calculated as described below.

2. Vero Cells.[46] Vero cells were seeded onto 24-well dishes at 1×10^5 cells per well using RPMI + 5% FBS + 0.1% methylcellulose (15 cps). The cells were incubated for 24 h at 37 °C in a CO_2 incubator until they were confluent. The media were then removed, and virus (100 μL, HSV-1, KOS strain or HSV-2, 333 strain) was added at 20 or 100 pfu per well in RPMI + 2% dialyzed FBS. The dishes were incubated at 37 °C in the atmosphere of 5% CO_2 for 1 h, rotating every 15 min to ensure that virus covers the walls. Drug solutions were prepared with acyclovir as a control in RPMI + 2% dialyzed FBS + 0.1% methylcellulose (4000 cps) and appropriate amounts were added to the wells. The mixtures were then incubated in 5% CO_2 for 48–72 h. The media were then removed, and staining was performed with 0.8% crystal violet in 50% ethanol for 15 min at room temperature. The dishes were rinsed with water and allowed to dry before determining the plaque number.

D. HSV, HCMV Cytopathic Effect (CPE) Inhibition Assay. Low passage HFF cells were seeded into 96-well tissue culture plates 24 h prior to use at a cell concentration of 2.5×10^5 cells/mL in 0.1 mL of MEM supplemented with 10% FBS. The cells were incubated for 24 h at 37 °C in a CO_2 incubator. After incubation, the medium was removed, and 100 μL of MEM containing 2% FBS was added to all but the first row. In the first row, 125 μL of experimental drug was added in triplicate wells. Medium alone was added to both cell and virus control wells. The drug in the first row of wells was then diluted serially 1:5 throughout the remaining wells by transferring 25 μL using the Cetus Liquid Handling Machine. After a 1 h incubation, 100 μL of the appropriate virus concentration was added to each well, excluding cell control wells which received 100 μL of MEM. For HSV-1 and HSV-2 assays, the virus concentration utilized was 1000 pfu per well. For CMV assays, the virus concentration added was 2500 pfu per well. The plates were then incubated at 37 °C in a CO_2 incubator for 3 days for HSV-1 or HSV-2 and for 14 days for CMV. An additional 50 μL of MEM was added on day 7 for CMV assays only. After the incubation period, media was aspirated, and the cells were stained with a 5% crystal violet/ethanol/formaldehyde solution for 4 h. The stain was then removed, and the plates were rinsed with tap water until all excess stain was removed. The plates were allowed to dry for 24 h and then read on a Bio-Tek Instruments Microplate reader at 630 nm.

E. EBV Immunofluorescence Assay. A concentration of 1×10^6 Daudi cells were infected with 0.1–0.2 moi of P3HR-1 strain of EBV in RPMI-1640 medium for 45 min at 37 °C in a CO_2 incubator. After virus adsorption, the cells were washed and pelleted, and the supernatant was discarded. The drugs to be tested were serially diluted in RPMI-1640 and added to the appropriate tubes. The cultures were incubated for 2 days in complete medium to allow viral gene expression. Following the 48 h incubation period, a number of cells of each sample were counted and washed in phosphate-buffered saline (PBS), and slides were prepared. The slides were dried overnight. The dried cells were fixed with acetone. A monoclonal antibody to viral capsid antigen (VCA) was then added to the cells, incubated for 30 min at 37 °C, and washed in PBS. This was followed by a fluorescein-conjugated goat anti-mouse IgG antibody. The cells were counterstained with Evans Blue. A total of 500 negative and fluorescence positive cells were counted. The total number of VCA positive cells in the cultures was calculated and IC_{50} values determined using Microsoft Excel Software.

F. EBV DNA Hybridization Assay. (1) Cell Cultures. A high-yield EBV-producing cell line H-1 derived from human P3 HR1 cells was used in this study. The cells were cultured in RPMI 1640 medium supplemented with dialyzed fetal bovine serum (10%) and kanamycin (100 μg/mL), and they were grown at 37 °C in a humidifed incubator containing CO_2 (5%).

(2) Exposure of H-1 Cells to Drugs. The H-1 cells were maintained in a logarithmic phase of growth for 2 days prior to the initiation of treatment. They were seeded in 24-well plates at the density of 2×10^5 cells/well in fresh medium (2 mL) with or without drug and incubated at 37 °C for 5 days. After treatment with drug, the cells were pelleted and the inhibitory effect of drug on EBV DNA was determined by slot–blot analysis.

(3) Slot–Blot Assay. A total of 4×10^5 H-1 cells treated with drugs were lysed in Tris·HCl (400 μL, 10 mM, pH 7.5) solution by freezing and thawing three times. The lysate was treated with RNase A (at a final concentartion of 50 μg/mL) at 37 °C for 30 min and then with proteinase K (at a final concentration of 100 μg/mL) at 55 °C for 2 h. The samples were denatured by adding NaOH (0.4 M) and EDTA (10 mM), pH 8.2. After heating at 100 °C (water bath) for 10 min, the samples were spotted on a positively charged nylon membrane using a manifold. The α-^{32}P-dCTP-labeled EBV ECORIC fragment was then used as a probe for DNA hybridization. The same mebranes were reprobed with human Alu DNA (BAM H-1 fragment) after stripping the EBV ECORIC probe. Autoradiographic results were analyzed by densitometry. The amount of EBV DNA in treated H-1 cells was determined from the ratio of EBV DNA to Alu DNA used as comparison with untreated control H-1 cells. The same membranes were used for assessment of toxicity to mitochondria by rehybridization with a mitochondrial DNA probe after removing Alu DNA probe.

G. HHV-6. An ELISA assay was performed using covalent amine plates (Costar, Cambridge, MA). The plates were activated by the addition of a homobifunctional cross-linking agent, bis(sulfosuccinimidyl) suberate, and then washed with PBS. Samples consisting of 150 μL of suspended HSB-2 cells infected with HHV-6 strain GS (obtained through the courtesy of Dr. Robert C. Gallo, N. I. H.) and previously incubated with selected concentrations of drug on a separate plate were solubilized in Triton X-100 in coating buffer. The plate was covered and incubated for 1 h at 37 °C in a 5% CO_2 atmosphere. These binding conditions facilitated covalent attachment of the antigen to the free end of the cross-linker.

After covalent binding, the antigen solution was decanted and the plate was washed four times in HEPES-buffered saline[42] with 0.05% Tween 20 (HBS-T), soaking for 3 min for each wash. Unbound sites on the plate were blocked, the blocker was decanted, and diluted primary monoclonal antibody, specific for HHV-6 (GS) glycoprotein gp116, was added. The plate was covered and incubated for 1 h at 37 °C. The plate was washed again, blocker again added, and horseradish peroxidase-labeled rabbit anti-mouse antibody added to each well. The plate was incubated for 1 h at 37 °C, washed again as described above, and developed using TMB-Turbo (Pierce, Rockford, IL) for 30 min at room temperature. The reaction was stopped with 2 M H_2SO_4. Absorbance in each well was determined at 450/570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

H. HBV. Chronically infected 2.2.15 cells were kindly provided by G. Acs (Mount Sinai Medical Center, New York, NY). Cells were maintained in minimal essential medium (MEM) supplemented with fetal bovine serum (10%, vol/vol) and incubated at 37 °C in a moist atmosphere containing CO_2 (5%). Cells were inoculated in tissue culture plates at a

density of 5×10^4 cells/mL MEME in 3.83 cm^2 well. Medium was changed every 3 days after inoculation. On day 6 and day 9 the compounds studied were added to the medium. The cells were incubated with various concentrations of compounds for 6 days. The medium was then centrifuged (8 min, 1000*g*), and an equal volume of 1 M NaCl in 20% poly(ethylene glycol) was added to the supernatant. The virus was pelleted (20 min, 10000*g*), and the pellet was resuspended in a lysis buffer (0.5 mL, 1% SDS,10 mM Tris pH 7.6, 400 mM NaCl, 10 mM EDTA) with proteinase K (0.1 mg/mL). The suspension was incubated for 3 h at 50 °C, and then it was extracted with a mixture of phenol:chloroform:isoamyl alcohol, 25:24:1 (vol/vol). The precipitated DNA was washed with ethanol, and it was dissolved in TE_{80} (10 mM Tris.HCl, pH 8.0, 1 mM EDTA) and then subjected to electrophoresis in agarose gel (0.8%) followed by blotting onto Hybond-N membrane (Amersham). The blot was hybridized with a ^{32}P-labeled HBV DNA probe, it was washed twice with standard saline citrate (SSC, 0.1% SDS) at room temperature for 15 min followed by 0.1 × SSC, 0.1% SDS at room temperature for 15 min and at 60 °C for 10 min. The bands were subjected to autoradiography, and the bands were quantitated by a scanning densitometer (Molecular Dynamics).

I. HIV. HIV was assayed in supernatants of CEM cells infected with strain III_B (provided through the courtesy of Dr. Louis S. Kucera[47]) by determining the amount of reverse transcriptase (RT) activity. Cells were grown, infected, and incubated in the presence of seven concentrations (one-half log dilutions) beginning at 1 or 100 μM of compounds to be assayed. Procedures and the RT assay were performed as detailed previously by White et al.[48]

Cytotoxicity Assays. Several different assays were used to explore cytotoxicity of selected compounds depending on the nature of the antiviral assay.

A. Visual Cytotoxicity. Cytotoxicity produced in stationary HFF, MEF, CEM, or CEM-SS cells from suspension cultures was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[43]

B. KB Cell Growth. The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[49] Briefly, 96-well cluster dishes were planted with KB cells at 3000−5000 cells per well. After overnight incubation at 37 °C, test compound was added in triplicate at eight concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added and plates read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates.

C. Cell Proliferation Assay. Twenty-four hours prior to assay, cells (HFF or Daudi) were seeded in six-well plates at a concentration of 2.5×10^4 cells per well in MEM containing 10% FBS. On the day of the assay, drugs were diluted serially in MEM containing 10% FBS at increments of 1:5 covering a range from 100 to 0.03 mg/mL. For drugs that had to be solubilized in DMSO, control wells receive MEM containing 10% DMSO. The media from the wells was then aspirated, and 2 mL of each drug concentration was added to each well. The cells were incubated in a CO_2 incubator at 37 °C for 72 h. At the end of this time, the media−drug solution was removed, and the cells were washed. Trypsin−EDTA (0.25%, 1 mL) was added to each well and incubated until the cells started to come off the plate. The cell−media mixture was then pipeted up and down vigorously to break up the cell suspension and 0.2 mL of the mixture added to 9.8 mL of Isoton III and counted using a Coulter Counter. Each sample was counted three times with two replicate wells per sample. The IC_{50} values were calculated using a computer program.

D. Neutral Red Uptake. Twenty-four hours prior to assay, HFF cells were plated into 96-well plates at a concentration of 2.5 × 104 cells/well. After 24 h, the media was aspirated, and 125 mL of media containing drug was added to the first row of wells and then diluted serially 1:5 using the automated Cetus Liquid Handling System in a manner similar to that used in the CPE assay. The plates were then incubated in a CO_2 incubator at 37 °C for 7 days. At this time the media/drug was removed, cells were washed, 200 mL/well of 0.01% neutral red in PBS was added, and the mixture was incubated for 1 h. The dye was removed, and the cells were washed using a Nunc Plate Washer. After the wash was removed, 200 mL/well of 50% ethanol/1% aqueous acetic acid was added. The plates were placed on a rotating shaker for 15 min, and the optical densities were then read at 540 nm using a plate reader.

E. Mitochondrial DNA (MtDNA) Content. CEM human T-lymphoblastoid cells were seeded in RPMI 1640 medium (2 mL) containing dialyzed fetal bovine serum (10%) at a concentration of 2×10^5 cells/mL. Cells were incubated with various concentration of compounds for 4 days, they were then counted, centrifuged (3 min, 1000*g*), and resuspended to a density of 2×10^5 cells/mL with fresh medium plus drugs. On day 6 the cells were counted, centrifuged, and resuspended again as described above. On day 8, 1×10^5 cels were collected by centrifugation, and they were washed with PBS. The cell pellets were resuspended in Tris·HCl (0.2 mL, 25 mM, pH 8.0) and EDTA (1 mM) and they were subjected to five freeze−thaw cycles. The cell lysate was then incubated with RNase A (100 μg/mL) at 37 °C for 1 h. Samples were treated with proteinase K (100 μg/mL) at 50 °C for 3 h, 20 × SSC (0.2 mL) was added to the samples which were then heated at 100 °C for 10 min. DNA was immobilized on Hybond-N membrane by using a slot−blot apparatus (Schleicher and Schuell). MtDNA on the blot was detected with a MtDNA-specific probe.[50]

Data Analysis. Dose−response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls as shown in the tables were used in all assays.

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