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Synthesis, X-ray crystal structural study, antiviral and cytostatic evaluations of the novel unsaturated acyclic and epoxide nucleoside analogues

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Abstract—A series of the novel purine and pyrimidine nucleoside analogues were synthesised in which the sugar moiety was replaced by the 4-amino-2-butenyl (**2**–**6** and **10**–**18**) and oxiranyl (**8** and **20**) spacer. The *Z*- (**2**–**6**) and *E*-isomers (**10**–**18**) of unsaturated acyclic nucleoside analogues were synthesized by condensation of 2- and 6-substituted purine and 5-substituted uracil bases with *Z*- (**1**) or *E*-phthalimide (**9**) precursors. The oxiranyl nucleoside analogues (**8** and **20**) were obtained by epoxidation of **1** and **9** with *m*-chloroperoxybenzoic acid and subsequent coupling with adenine. The new compounds were evaluated for their antiviral and antitumor cell activities. Among the olefinic nucleoside analogues, *Z*-isomer of adenine containing 4-amino-2-butenyl side chain (**6**) exhibited the best cytostatic activities, particularly against colon carcinoma (SW 620, IC_{50} = 26 μM). Its *E*-isomer **15** did not show any antiproliferative activity against malignant tumor cell lines, except for a slight inhibition of colon carcinoma (SW 620, IC_{50} = 56.5 μM) cells. In general, *Z*-isomers showed better cytostatic activities than the corresponding *E*-isomers. (*Z*)-4-Amino-2-butenyl-adenine nucleoside analogue **6** showed albeit modest but selective activity against HIV-1 (EC_{50} = 4.83 μg mL^{-1}).

1. Introduction

Nucleoside analogues have been the cornerstone of antiviral chemotherapy over the past decades. Since the discovery of 3′-azido-3′-deoxythymidine (AZT) as an antiviral agent for the treatment of acquired immunodeficiency syndrome (AIDS), much attention has been focused on nucleosides as reverse transcriptase inhibitors in the search for more active and less toxic compounds.[1] Although structure–activity relationship studies have not led to a uniform pharmacophore model for the antiviral activities of nucleosides, some structural features have proved to be particularly effective for specific antiviral activities. There is considerable evidence that introduction of a rigid structural element into nucleoside or carbocyclic nucleoside structure can lead to effective antiviral nucleoside analogues.[2,3] Thus, the presence of a double bond in acyclic nucleoside analogues is structural feature important for strong antiherpetic activity of guanine analogue (**1b**, Fig. 1).[4]

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 (**2a**).[5] Thymidine with a 2-butenyl spacer (**3c**) was the first acyclic nucleoside analogue exhibiting potent inhibition of thymidine kinase 2 (TK-2) which catalyzed phosphorylation of antiviral drugs.[6] Its role in mitochondrial DNA synthesis as well as mitochondrial toxicity observed under prolonged treatment with antiviral drugs such as AZT is still under debate.[7]

Keywords: Olefinic nucleoside analogues; Epoxide purine derivatives; Cytostatic activity; Anti-HIV-1 activity.
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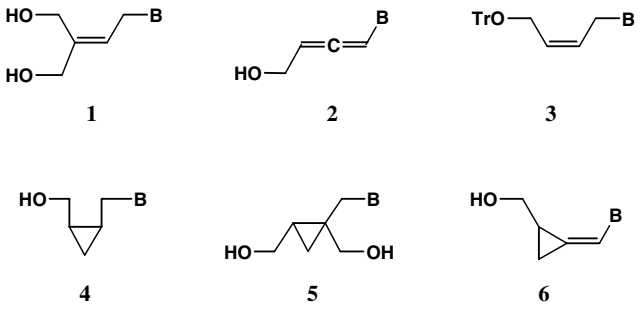


Figure 1. Unsaturated acyclic **1**–**3** and cyclopropyl **4**–**6** nucleoside analogues.

Studies with cyclopentane nucleoside analogues initiated the investigation of ring-constricted analogues containing a three-membered ring. Among them, *Z*-configuration of the cyclopropyl guanine nucleoside (**4b**, Fig. 1) showed antiherpetic potency (HSV-1 and HSV-2) comparable to that of acyclovir.[8] Also, the guanine derivative with an additional hydroxymethyl group at 1′-position (**5b**) showed more potent antiviral activity against HSV-1 than acyclovir.[9] Synadenol (**6a**) and synguanol (**6b**) comprising a methylenecyclopropane moiety exhibited a potent antiviral activity, particularly against HCMV.[10]

In this connection and related to our previous studies on purine 1-amino-1-cyclopropane carboxylic acid,[11] we have prepared now the series of novel purine and pyrimidine nucleoside analogues containing 4-amino-2-butenyl (**2**–**6** and **10**–**18**) and oxyranyl spacer (**8** and **20**) in *Z*- and *E*-configuration (Fig. 2).

2. Results and discussion

2.1. Synthesis

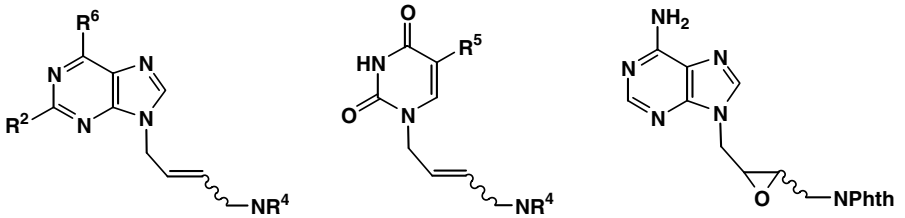
The *cis*-olefinic (**6**) and *cis*-epoxide (**8**) purine nucleoside analogues, as well as *cis*-olefinic (**4** and **5**) pyrimidine nucleoside analogues were obtained by condensation of the *Z*-4-chloro-2-butenyl (**1**) and *Z*-4-chloro-2,3-epoxide (**7**) derivatives of phthalimide, respectively, with the purine or 5-substituted pyrimidine bases (Scheme 1).

The series of *trans*-olefinic purine (**15**) and pyrimidine (**16**–**18**) nucleoside analogues, as well as *trans*-epoxide purine (**20**) nucleoside analogue were prepared by coupling of the *E*-4-bromo-2-butenyl (**9**) and *E*-4-bromo-2,3-epoxide (**19**) derivatives of phthalimide with adenine, 6-chloropurine and 5-substituted pyrimidine derivatives (Scheme 2).

The key intermediates *Z*-4-chloro-2-butenyl (**1**) and *E*-4-bromo-2-butenyl (**9**) derivatives of phthalimide were synthesized by using a classical Gabriel reaction.[12] Condensations of **1** with purine derivatives and 5-substituted pyrimidines using K_2CO_3 or NaH as a base gave (*Z*)-*N*-phthalimide protected 4-amino-2-butenyl purine (**2a**,**b** and **3a**,**b**) and pyrimidine derivatives (**4** and **5**) (Scheme 1), whereas the condensation of **9** with purine and pyrimidine bases gave (*E*)-*N*-phthalimide protected 4-amino-2-butenyl purine (**10** and **11a**,**b**) and pyrimidine derivatives (**12**–**14**) (Scheme 2).

It should be noted that the condensations of **1** and **9** with silylated pyrimidine bases did not yield the desired products. Condensation reactions of *Z*-isomer **1** with purine bases gave mixture of N-9 (**2a**) and N-7 (**2b**) regioisomers of adenine (N-9:N-7 = 4:1) and guanine (**3a** and **3b**, N-9:N-7 = 5:1) derivatives. *E*-isomer **9** with 6-chloropurine also gave the mixture of N-9 (**11a**) and N-7 isomers of (**11b**) (N-9:N-7 = 3:1), while reaction of **9** with adenine afforded only N-9 isomer **10** (Scheme 2).

Removal of the phthalimide protecting group the final stage of Gabriel amine synthesis was achieved using hydrazine hydrate in ethanol.[13] Deprotection of adenine phthalimide derivatives **2** and **10** gave adenine derivatives with free amino group in the side chain (**6** and **15**, Schemes 1 and 2). Because of low solubility of compounds **2** and **10** in ethanol, these reactions were carried out by heating the mixtures under reflux for 3 days. The pyrimidine derivatives with free amino group were obtained only as *E*-isomers (**16**–**18**, Scheme 2). All reactions proceeded smoothly at room temperature with very good yields, particularly the deprotection of **13** and **14**, which gave the desired products **17** and **18** in a few hours. Easier deprotection of these two compounds compared to uracil derivative **12** can be explained by significantly higher solubility of 5-fluoro-

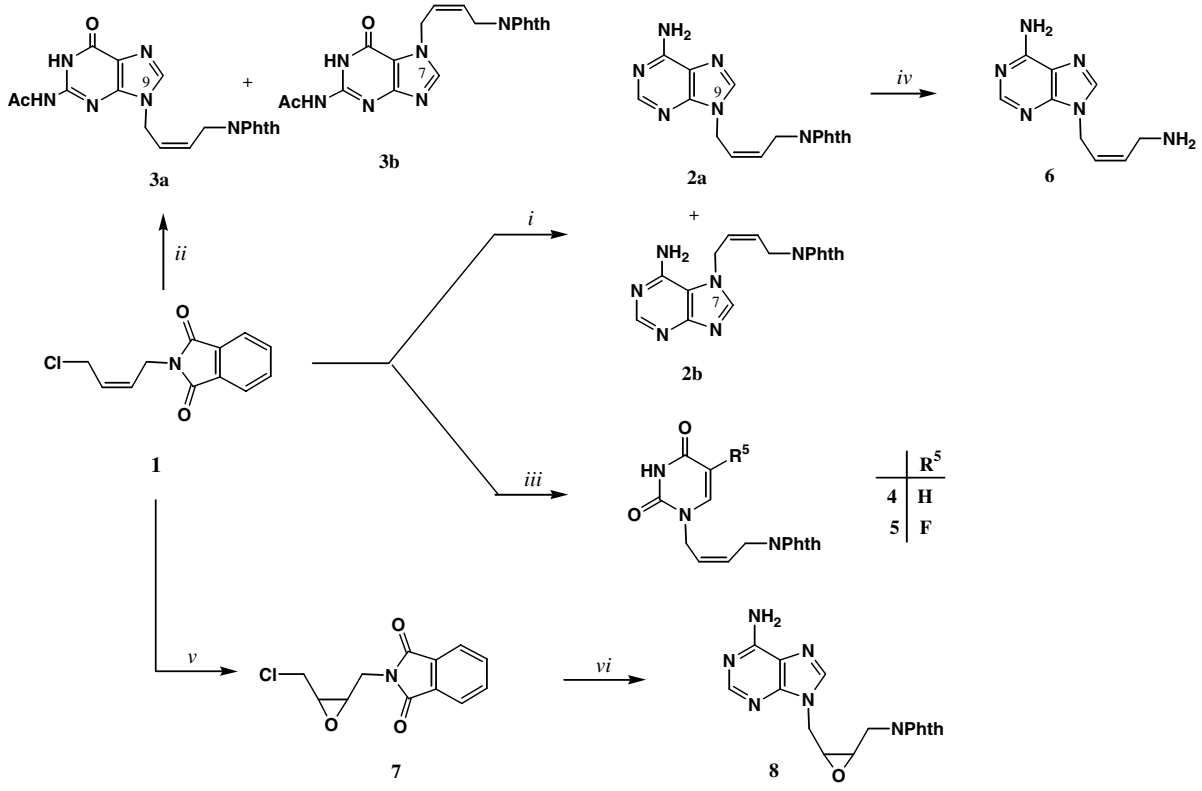


2, **3**, **6**, **10**, **11**, **15** **4**, **5**, **12-14**, **16-18** **8**, **20**

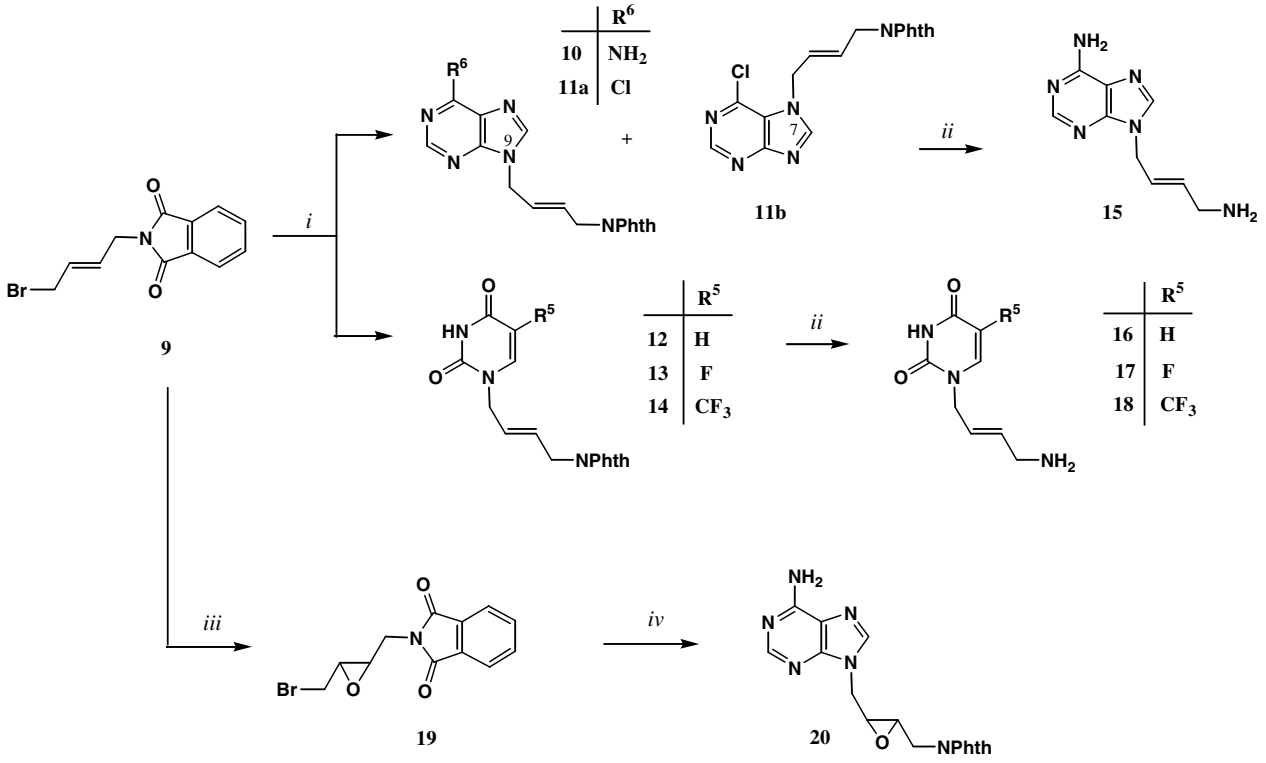
R^2 = H, NHAc R^4 = H, Phth

R^6 = NH_2, O, Cl R^5 = H, F, CF_3

Figure 2. The unsaturated acyclic (**2**–**6** and **10**–**18**) and epoxide (**8** and **20**) nucleoside analogues.



Scheme 1. Synthesis of *cis*-olefinic purine (**2a**, **b**, **3a**, **b** and **6**) and pyrimidine (**4** and **5**), and oxiranyl (**8**) adenine nucleoside analogues. Reagents: (i) adenine, K_2CO_3 in DMF; (ii) N-acetylated guanine, 60% NaH in DMF; (iii) pyrimidine bases, K_2CO_3 in DMF; (iv) $N_2H_4 \times H_2O$ in EtOH; (v) 70% *m*-CPBA in CH_2Cl_2; (vi) K_2CO_3 in DMF.



Scheme 2. Synthesis of *trans*-olefinic purine (**10**,**11a**, **b** and **15**) and pyrimidine (**12**–**14** and **16**–**18**), and oxiranyl (**20**) adenine nucleoside analogues. Reagents: (i) purine or pyrimidine base, K_2CO_3 or NaH in DMF; (ii) $N_2H_4 \times H_2O$ in EtOH; (iii) 70% *m*-CPBA in CH_2Cl_2; (iv) K_2CO_3 in DMF.

uracil (**13**) and 5-(trifluoromethyl)uracil (**14**) derivatives than uracil (**12**).

The epoxidation reactions of the *Z*- (**1**) and *E*-precursors (**9**) (Schemes 1 and 2) were performed with *m*-chloroperoxybenzoic acid. This specific reagent was selected as an epoxidating agent because (4-amino-2,3-epoxy)butyl derivatives (**7** and **19**, Schemes 1 and 2) completely retain their starting *Z*- or *E*-configuration. Condensations of adenine with **7** and **19** in the presence of K_2CO_3 as a base proceeded also with the retention of configuration giving *Z*-**8** and *E*-**20** (Schemes 1 and 2).

2.2. ^{1}H and ^{13}C NMR spectra

Structures of the newly synthesized compounds were determined from their ^{1}H and ^{13}C NMR and mass spectra. The assignment of ^{1}H NMR spectra was performed on the basis of the chemical shifts, substituent induced chemical shifts, signal intensities, magnitude and multiplicity of H–H and H–F (**5**, **13**, and **17**) coupling constants. The ^{1}H NMR data are given in Table 1. The most important difference between *Z*- (**2**–**8**) and *E*-series (**10**–**20**) of nucleoside analogues is seen for methine protons H-2′ and H-3′. Each of these protons gives regular doublet of triplets, which are well distinguished in *E*-series of nucleoside analogues but are merged into multiplet in *Z*-series. Coupling constant for H-2′ and H-3′ in *Z*-isomer (**3**) is 10.6 Hz, while in *E*-series (**10**, **11**, **13**–**15**, **17** and **18**) they are in the range 15.3–15.6 Hz what is in accord with values found for structurally related nucleoside analogues.[10] The spectra of 5-fluorouracil derivatives (**5**, **13**, and **17**) additionally confirm structures by H–F coupling constants (6.3–6.7 Hz) for the H-6 proton. Chemical shift for the H-6 proton in uracil (**12**), 5-fluorouracil (**13**) and 5-(trifluoromethyl)uracil (**14**) derivatives decreases in the series: δ(H-6) in **14** > δ(H-6) in **13** > δ(H-6) in **12** as a consequence of the deshielding effect of the fluorine atom. Chemical shifts for N-7 regioisomers (**2b**, **3b** and **11b**) are in accordance with the literature data for N-7 substituted purine derivatives.[14,15]

2.3. X-ray crystal structure study

The X-ray structure analysis has been used to unambiguously determine the structure of compound **14**. In **14** (Fig. 3), the pyrimidine and phthalimide rings are bonded via a butenyl bridge. The bond lengths in this structure present no unexpected features. The bond angle C2–N3–C4 in the pyrimidine ring is widened and amounts to 127.1(1)°. However, the sum of the endocyclic bond angles is 720°, as expected for unpuckered aromatic ring. The C7 and C10 atoms are disposed in an *antiperiplanar* fashion; the C7–C8–C9–C10 torsion angle is 177.0(2)°. The phthalimide ring is almost planar, since the biggest deviation of one of the atoms from the nine-membered ring mean plane is 0.024(3) Å. The pyrimidine and phthalimide rings are mutually parallel, with the dihedral angle between their mean planes of 3.5(1)°. Furthermore, both rings are similarly oriented towards the plane defined by the C7–C8–C9–C10 atoms; the corresponding dihedral angles are 69.1(3) and 65.6(3)° for pyrimidine and phthalimide rings, respectively.

The molecules of **14** are joined by one N–H···O hydrogen bond, thus forming centrosymmetric dimers via eight-membered rings (Fig. 4; Table 2). The action of this hydrogen bond is reinforced by one C–H···F hydrogen bond and three C–H···O hydrogen bonds, so generating three-dimensional network.

2.4. Cytostatic activity

Compounds **2a**–**8** and **10**–**20** were evaluated for their cytostatic activity against several malignant tumor cell lines: murine leukemia (L1210), human T-lymphocyte (Molt4/C8 and CEM), cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7), lung carcinoma (H 460) and normal (diploid) human fibroblasts (WI 38, control cell line) (Table 3).

In the series of olefinic nucleoside analogues, *Z*-isomer of 9-(4-amino-2-butenyl)adenine (**6**) exhibited the best cytostatic effects, particularly against colon carcinoma (IC_{50} = 26 μM). Its *E*-isomer (**15**) did not show antiproliferative activity against malignant cell lines, except for a slight inhibition of the proliferation of colon carcinoma cells (IC_{50} = 56.5 μM). Comparison of the cytostatic activity of other *Z*- and *E*-isomers of acyclic nucleoside analogues revealed that *Z*-isomers (**2a** and **5**) had better inhibitory activities than corresponding *E*-isomers (**10** and **13**). Generally, 2-butenyl pyrimidine derivatives exhibited lower antiproliferative activity than 2-butenyl purine derivatives. Compound **19** showed rather modest activity against murine leukemia L1210 (IC_{50} = 16 μM), human T-lymphocyte Molt4/C8 (IC_{50} = 16 μM) and CEM (IC_{50} = 15 μM) cell lines.

The results of the cytostatic activity indicated that the oxyranyl spacer in **8** and **20** as compared to the 2-butenyl spacer did not improve cytostatic effect, except for a slight inhibitory affect against HeLa (IC_{50} = 45 μM) cells.

2.5. Antiviral activity

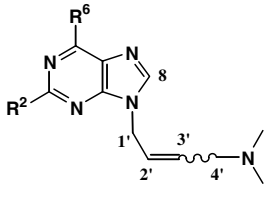
Compounds **2a**–**8** and **10**–**20** were evaluated against human immunodeficiency virus (HIV-1 and HIV-2) (Table 4), varicella zoster virus (VZV), human cytomegalovirus (HCMV), parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, herpes simplex virus type 1 and 2, vaccinia virus, vesicular stomatitis virus and respiratory syncytial virus.

No specific antiviral effects were noted for the new compounds against any of the viruses evaluated. Only exception was the adenine containing 4-amino-2-butenyl side chain (**6**), which showed some selective activity against HIV-1 (EC_{50} = 4.83 μg/mL) (Table 4).

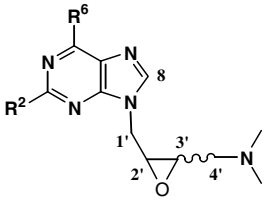
3. Conclusions

The novel purine and pyrimidine nucleoside analogues, in which the ribofuranose moiety was replaced by the conformationally restricted olefinic (**2a**–**6** and **10**–**18**)

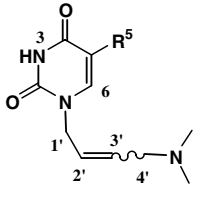
Table 1. ^{1}H NMR chemical shifts (δ/ppm)[a] and H–H coupling constants (J/Hz) in ^{1}H NMR spectra for compounds **2**–**20** (cf. Schemes 1 and 2)



2, 3, 6, 10, 11, 15



8, 20



4, 5, 12-14, 16-18

	H-1′	H-2′	H-3′	H-4′	H-Phth	NH_2-4′	H-2	H-8	NH_2	H-5	H-6	NH-3
2a N-9	5.00 (d, 2H, J_3 = 6.7)	5.64–5.80 (m, 2H)		4.46 (d, 2H, J_3 = 6.4)	7.82–7.92 (m, 4H)	/	8.08 (s, 1H)	8.18 (s, 1H)	7.27 (s, 2H)	/	/	/
2b N-7	5.17 (d, 2H, J_3 = 6.8)	5.80–5.92 (m, 1H)	5.66–5.77 (m, 1H)	4.53 (d, 2H, J_3 = 6.7)	7.85–7.89 (m, 4H)	/	7.73 (s, 1H)	8.43 (s, 1H)	7.92 (s, 2H)	/	/	/
3a[b]N-9	5.07 (d, 2H, J_3 = 6.7)	5.72 (dt, 1H, J_3 = 7.1, J_{cis} = 10.6)	5.56 (dt, 1H, J_3 = 7.0, J_{cis} = 10.6)	4.35 (d, 2H, J_3 = 6.2)	7.74–7.81 (m, 4H)	/	/	8.13 (s, 1H)	/	/	/	/
3b[b]N-7	4.91 (d, 2H, J_3 = 6.5)	5.74 (dt, 1H)	5.67 (dt, 1H)	4.41 (d, 2H, J_3 = 6.5)	7.79–7.91 (m, 4H)	/	/	8.00 (s, 1H)	/	/	/	/
4	4.44 (d, 2H, J_3 = 6.3)	5.50–5.60 (m, 2H)		4.30 (d, 2H, J_3 = 6.3)	7.76–7.83 (m, 4H)	/	/	/	/	5,53 (d, 1H, J_3 = 7.8)	7.60 (d, 1H, J_3 = 7.8)	11.22 (s, 1H)
5	4.27 (d, 1H, J_3 = 4.4) 4.19 (d, 1H, J_3 = 4.9)	5.47–5.70, (m, 2H)		4.11 (d, 1H, J_3 = 4.1) 4.06 (d, 1H, J_3 = 4.3)	7.74–7.83 (m, 4H)	/	/	/	/	/	7.99 (d, 1H, J_{HF} = 6.2)	11.27 (s, 1H)
6	4.72 (d, 2H, J_3 = 6.7)	5.43–5.66 (m, 2H)			3.30 (m, 2H)	/	3.30 (br)	8.04 (s, 1H)	8.06 (s, 1H)	7.11 (s, 2H)	/	/
7[a]	3.70–3.79 (m, 1H) 3.96 (dd, 1H, J_2 = 14.6, J_3 = 6.1)	3.35–3.39 (m, 1H)	3.24–3.29 (m, 1H)	3.70–3.79 (m, 2H)	7.70 (dd, 2H, J_3 = 3.0, 5.2) 7.82 (dd, 2H, J_3 = 3.1, 5.2)	/	/	/	/	/	/	/
8	4.35 (dd, 1H, J_2 = 14.8, J_3 = 4.0) 4.56 (dd, 2H, J_2 = 14.8, J_3 = 7.4)	3.35–3.39 (m, 1H)	3.30 (1H)	3.88 (dd, 1H, J_2 = 14.8, J_3 = 6.0) 3.94 (dd, 2H,J_2 = 14.8, J_3 = 5.5)	7.79 (dd, 2H, J_3 = 3.0, 4.9) 7.85 (dd, 2H, J_3 = 2.9, 4.9)	/	8.02 (s, 1H)	8.12 (s, 1H)	7.21 (s, 2H)	/	/	/
10	4.73 (d, 2H, J_3 = 5.0)	5.86 (dt, 1H, J_3 = 5.8, J_{trans} = 15.5)	5.65 (dt, 1H, J_3 = 5.3, J_{trans} = 15.5)	4.17 (d, 2H, J_3 = 5.2)	7.82–7.90 (m, 4H)	/	8.06 (s, 1H)	8.10 (s, 1H)	7.19 (s, 2H)	/	/	/

11a N-9	4.90 (d, 2H, J_3 = 5.4)	5.90 (dt, 1H, J_3 = 5.8, J_{trans} = 15.5)	5.73 (dt, 1H, J_3 = 5.5, J_{trans} = 15.5)	4.18 (d, 2H, J_3 = 4.4)	7.81–7.88 (m, 4H)	/	8.64 (s, 1H)	8.76 (s, 1H)	/	/	/	/
11b N-7	5.02 (d, 2H, J_3 = 4.4)	5.87 (dt, 1H)	5.42 (dt, 1H)	4.11 (d, 2H, J_3 = 4.1)	7.65-7.73 (m, 4H)	/	8.70 (s, 1H)	8.72 (s, 1H)	/	/	/	/
12	4.16 (d, 2H, J_3 = 3.0)	5.58–5.62 (m, 2H)		4.10 (d, 2H, J_3 = 3.0)	7.74–7.82 (m, 4H)	/	/	/	/	5.47 (d, 1H, J_3 = 7.5)	7.46 (d, 1H, J_3 = 7.8)	11.18 (s, 1H)
13	4.14–4.22 (m, 2H)	5.72 (dt, 1H, J_3 = 4.7, J_{trans} = 15.5)	5.64 (dt, 1H J_3 = 5.7, J_{trans} = 15.5)	4.14–4.22 (m, 2H)	7.80–7.90 (m, 4H)	/	/	/	/	/	7.94 (d, 1H, J_3 = 6.5)	11.79 (br s, 1H)
14	4.26 (d, 2H, J_3 = 5.3)	5.70 (dt, 1H, J_3 = 4.9, J_{trans} = 15.6)	5.63 (dt, 1H J_3 = 5.6, J_{trans} = 15.6)	4.11 (d, 2H, J_3 = 4.5)	7.75–7.85 (m, 4H)	/	/	/	/	/	8.23 (s, 1H)	11.77 (s, 1H)
15	4.71 (d, 2H,J_3 = 5.3)	5.75 (dt, 1H, J_3 = 5.6, J_{trans} = 15.3)	5.65 (dt, 1H J_3 = 5.4, J_{trans} = 15.3)	3.10 (d, 2H, J_3 = 4.0)	/	2.90 (br, 2H)	8.09 (s, 1H)	8.11 (s, 1H)	7.21 (s, 2H)	/	/	/
16	4.19 (d, 2H, J_3 = 4.0)	5.56–5.69 (m, 2H)		3.16 (br, 2H)	/	3.86 (br, 2H)	/	/	/	5.50 (d, 1H, J_3 = 7.8)	7.50 (d, 1H, J_3 = 7.7)	/
17	4.12 (d, 2H, J_3 = 5.6)	5.66 (dt, 1H, J_3 = 5.1, J_{trans} = 15.5)	5.53 (dt, 1H J_3 = 5.7, J_{trans} = 15.5)	3.09 (d, 2H, J_3 = 3.9)	/	4.30 (br, 2H)	/	/	/	/	7.90 (d, 1H, J_{HF} = 6.7)	/
18	4.33 (d, 2H, J_3 = 5.1)	5.77 (dt, 1H, J_3 = 5.0, J_{trans} = 15.4)	5.66 (dt, 1H, J_{trans} = 15.4)	3.19 (d, 2H, J_3 = 3.5)	/	5.30 (br, 2H)	/	/	/	/	8.30 (s, 1H)	/
19[a]	3.74 (dd, 1H, J_2 = 14.4, J_3 = 4.8) 3.94 (dd, 1H, J_2 = 14.5, J_3 = 5.2)	3.19–3.23 (m, 1H)	3.11–3.15 (m, 1H)	3.26–3.33 (m, 2H)	7.69 (dd, 2H, J_3 = 3.0, J_3 = 5.2) 7.81 (dd, 2H, J_3 = 3.0, J_3 = 5.1)	/	/	/	/	/	/	/
20	4.26 (dd, 1H, J_2 = 14.8, J_3 = 5.4) 4.38 (dd, 1H, J_2 = 14.8, J_3 = 4.1)	3.30 (1H)	3.12–3.17 (m, 1H)	3.71 (dd, 1H, J_2 = 14.7, J_3 = 4.7) 3.82 (dd, 1H, J_2 = 14.7, J_3 = 4.8)	7.83–7.88 (m, 4H)	/	7.99 (s, 1H)	8.03 (s, 1H)	7.20 (s, 2H)	/	/	/

[a] DMSO-d_6 as a solvent for all compounds except for **7** and **19** which were recorded in $CDCl_3$; chemical shifts are referred to TMS. Multiplicity of coupling and number of protons are given in parentheses: s, singlet; d, doublet; m, complex multiplet; br, broad.

[b] Compound **3a**: signal for $-CH_3$: 2.08 ppm (s, 3H); signal for –NH: 11.48 ppm (s, 1H), 12.05 ppm (s, 1H). Compound **3b**: signal for $-CH_3$: 2.19 ppm (s, 3H); signal for –NH: 11.60 ppm (s, 1H), 12.02 ppm (s, 1H).

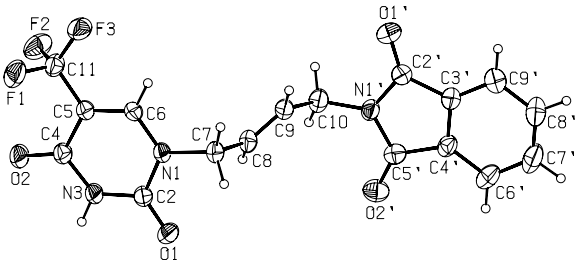


Figure 3. The molecular structure and labelling of **14**. Displacement ellipsoids are drawn at the 30% probability level.

and oxiranyl (**8** and **20**) moieties were synthesized. *Z*-(**2a**–**6**) and *E*-isomers (**10**–**18**) of nucleoside analogues with butenyl spacer between free and protected amino group and heterocyclic ring were synthesized by condensation of 2- and 6-substituted purine and 5-substituted uracil derivatives with *Z*-4-chloro-2-butenyl (**1**) or *E*-4-bromo-2-butenyl (**9**) phthalimide synthetic precursors. Purines containing 4-(amino-2,3-epoxy)butyl side chain (**8** and **20**) were obtained by epoxidation of **1** and **9** with *m*-chloroperoxybenzoic acid and subsequent coupling with adenine. The final step of Gabriel amine synthesis

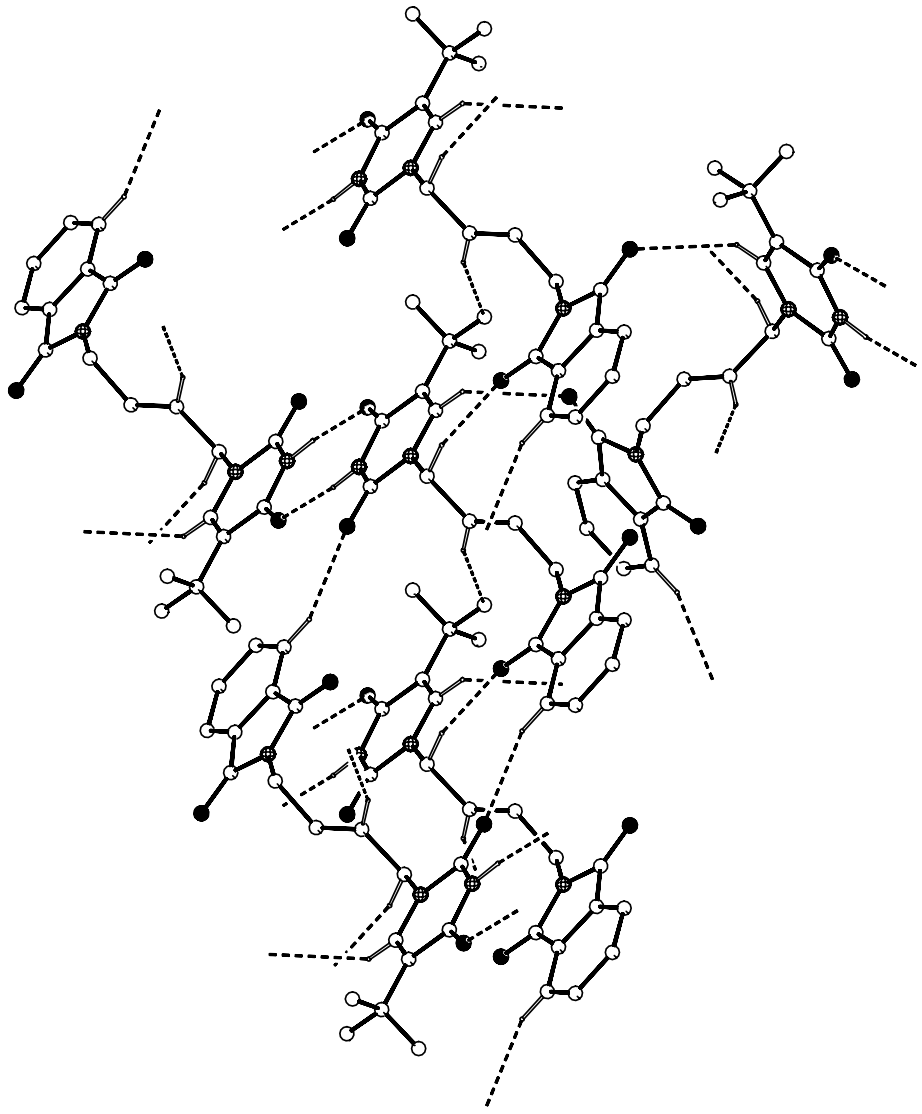


Figure 4. Part of the crystal structure of **14**, showing the hydrogen bonds that link the molecules of **14** into three-dimensional network. The unit-cell outline and hydrogen atoms not involved in the hydrogen-bonding have been omitted for clarity. Hydrogen bonds are indicated by dashed lines.

Table 2. Hydrogen-bonding geometry for **14**

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)	Symmetry codes
N3–H3···O2	0.92(4)	1.93(4)	2.838(3)	171(3)	$-x, -y, 1-z$
C6–H6···O1′	0.93	2.59	3.222(4)	126	$1/2-x, 1/2+y, 1/2-z$
C6′–H6′···O1	0.93	2.44	3.270(4)	149	$1/2-x, -3/2-y, 1-z$
C7–H7B···O2′	0.97	2.34	3.284(4)	164	$x, 1+y, z$
C8–H8···F3	0.93	2.48	3.334(3)	153	$x, -1+y, z$

Table 3. Inhibitory effects of the **2**–**8** and **10**–**20** on the growth of malignant tumor cell lines and normal diploid fibroblasts (WI 38)

Compound	IC_{50}[a] (μM)								
	L1210	Molt4/C8	CEM	HeLa	MiaPaCa-2	SW 620	MCF-7	H 460	WI 38
2a	124 ± 13	134 ± 20	102 ± 7	>100	>100	>100	>100	>100	27.7 ± 20
3a	>200	182 ± 25	183 ± 24	91.5 ± 97.8	>100	>100	>100	>100	>100
4	>200	>200	>200	>100	>100	>100	>100	>100	>100
5	101 ± 1	117 ± 50	86 ± 32	66.2 ± 18	>100	>100	85 ± 12	>100	>100
6	93 ± 36	>200	75 ± 32	73 ± 21	62 ± 15	26 ± 1.9	79 ± 11	88 ± 13	34.3 ± 17
7	>200	171 ± 41	151 ± 69	63.6 ± 27.6	>100	>100	>100	>100	⩾100
8	>200	>200	174 ± 37	45 ± 0.68	>100	>100	>100	>100	>100
10	>200	>200	>200	>100	>100	>100	>100	>100	>100
11a	120 ± 8	91 ± 7	89 ± 5	55 ± 1.7	>100	>100	>100	>100	>100
12	>200	>200	>200	>100	>100	>100	>100	>100	>100
13	80 ± 14	>200	>200	>100	>100	>100	>100	>100	>100
14	>200	>200	>200	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	56.5 ± 14	>100	>100	>100
16	>100	>100	>100	>100	>100	>100	95 ± 85	>100	>100
17	>100	>100	>100	>100	>100	>100	>100	>100	>100
18	>200	>200	>200	>100	>100	>100	>100	>100	>100
19	16 ± 2	16 ± 0	15 ± 1	>100	>100	>100	>100	>100	⩾100
20	128 ± 0	121 ± 8	104 ± 10	>100	>100	>100	>100	>100	>100

[a] Compound concentration required to inhibit tumor cell proliferation by 50%.

Table 4. Anti-HIV-1 and -HIV-2 activity of the compounds **2**–**8** and **10**–**20** in human T-lymphocyte (CEM) cells

Compound	EC_{50}[a] (μg/mL)		Cytotoxicity (μg/mL)	
	HIV-1	HIV-2	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
2a	>100	>100	>100	>50
3a	>100	>100	>100	>50
4	>100	>100	>100	>50
5	>100	>100	>100	>50
6	4.83 ± 2.84	⩾20	>100	5.7
7	>100	>100	>100	>50
8	>4	>20	>100	>50
10	>4	>20	>100	>50
11a	>100	>100	>100	>50
12	>100	>100	>100	>50
13	>20	>100	>100	>50
14	>100	>100	>100	>50
15	>50	>50	>100	3.1
16	>100	>100	>100	>50
17	>100	>100	>100	12.6
18	>50	>50	>100	50
19	>20	>20	20	9.2
20	>100	>100	>100	>50

[a] Effective concentration or concentration required to protect by 50% CEM cells against the cytopathogenicity of HIV.
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

was removal of the phthalimide protecting group using hydrazine hydrate to give adenine (**6** and **15**) and pyrimidine derivatives (**16**–**18**) with free amino functionality. ^{1}H and ^{13}C NMR spectra indicated that the products **2**, **3** and **11** exist as a mixture of N-9 and N-7 regioisomers in which N-9 isomers prevail. *E*-isomer of the phthalimide precursor **9** in the reaction with adenine afforded only N-9 isomer **10**.

The compounds **2a**–**8** and **10**–**20** were evaluated for their antiviral and antitumor cell activities. Among the olefinic nucleoside analogues, (*Z*)-9-(4-amino-2-butenyl)adenine (**6**) exhibited the best cytostatic effects, particularly against colon carcinoma (IC_{50} = 26 μM). Its *E*-isomer **15** did not show any antiproliferative activity against malignant cell lines, except for a slight inhibition of colon carcinoma (IC_{50} = 56.5 μM) cells. In general, *Z*-isomers had better cytostatic activities than the corresponding *E*-isomers. The adenine derivative containing *Z*-4-amino-2-butenyl side chain (**6**), showed rather modest but selective activity against HIV-1 (EC_{50} = 4.83 μg/mL).

4. Experimental

4.1. General methods

Melting points were determined on a Kofler micro hot-stage apparatus (Reichert, Wien) and are uncorrected.

Precoated Merck silica gel 60 F-254 plates were used for thin-layer chromatography (TLC), and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0.05–0.2 mm, Merck); glass column was slurry packed under gravity. The electron impact mass spectra were recorded with an EXTREL FT MS 2002 instrument with ionizing energy of 70 eV. High field one- and two-dimensional ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C resonance. The samples were dissolved in $CDCl_3$ or DMSO-d_6 and measured in 5 mm NMR tubes. The ^{1}H and ^{13}C NMR chemical shift values (δ) are expressed in ppm referred to TMS and coupling constants (J) in Hz.

4.2. Compounds preparation

4.2.1. (*Z*)-4-Chloro-2-butenyl-*N*-phthalimide (1) and (*E*)-4-bromo-2-butenyl-*N*-phthalimide (9). Compounds **1** and **9** were prepared as described previously.[12]

4.2.2. (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]adenine (2a) and (*Z*)-7-[4-(*N*-phthalimido)-2-butenyl]adenine (2b). To a stirred mixture of adenine (401 mg, 2.97 mmol) and K_2CO_3 (411 mg, 2.97 mmol) in DMF (25 mL), **1** (500 mg, 2.12 mmol) was added. The reaction mixture was heated at 70 °C for 4.5 h under N_2 atmosphere and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 10:1) afforded **2a** (246 mg, 35%) as white crystals and **2b** (61 mg). **2a**: mp = 225–227 °C; MS *m*/*z* 335.1213 $[MH]^+$; ^{13}C NMR (DMSO) δ: 168.08 (C=O), 156.30 (C-6), 152.77 (C-2), 149.74 (C-4), 134.91 (*C*H-phth), 132.20 (C_{quat}-phth), 128.27 and 128.06 (*C* = H), 123.58 (*C*H-phth), 118.85 (C-5), 40.31 (C-1′), 34.87 (C-4′); **2b**: ^{13}C NMR (DMSO) δ: 167.57 (C=O), 154.91 (C-6), 152.42 (C-2), 149.59 (C-4), 143.10 (*C*-8), 134.39 (*C*H-phth), 131.71 (C_{quat}-phth), 128.58 and 126.74 (*C* = H), 123.06 (*C*H-phth), 120.37 (C-5), 45.83 (C-1′), 34.52 (C-4′).

4.2.3. (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]-2-(*N*-acetylamino)purin-6-on (3a) and (*Z*)-7-[4-(*N*-phthalimido)-2-butenyl]-2-(*N*-acetylamino)purin-6-on (3b). To a stirred mixture of N-acetylated guanine (1.147 g, 5.90 mmol) and 60% NaH (260 mg, 5.90 mmol) in DMF (50 mL), **1** (1 g, 4.25 mmol) was added. The reaction mixture was stirred under N_2 atmosphere overnight at room temperature, 2 h at 55 °C and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 15:1) afforded **3** (860 mg, 52%) as white crystals.

Compound **3a**: mp = 244–247 °C; MS *m*/*z* 393.1358 $[MH]^+$; 173.81 ($C{=}O_{acetyl}$), 168.01 ($C{=}O_{phth}$), 157.60 (C-6), 153.12 (C-2), 147.38 (C-4), 144.40 (C-8), 134.96 (*C*H-phth), 132.13 (C_{quat}-phth), 128.32 and 128.23 (*C* = H), 123.56 (*C*H-phth), 111.69 (C-5), 43.54 (C-1′), 34.91 (C-4′), 24.18 (CH_3).

Compound **3b**: ^{13}C NMR (DMSO) δ: 173.90 ($C{=}O_{acetyl}$), 168.07 ($C{=}O_{phth}$), 155.50 (C-6), 149.04 (C-2), 148.16 (C-4), 139.77 (C-8), 134.92 (*C*H-phth), 132.07 (C_{quat}-phth), 128.48 and 127.57 (*C* = H), 123.57 (*C*H-phth), 120.48 (C-5), 40.57 (C-1′), 34.99 (C-4′), 24.19 (CH_3).

4.2.4. (*Z*)-1-[4-(*N*-Phthalimido)-2-butenyl]uracil (4). To a stirred mixture of uracil (333 mg, 2.97 mmol) and K_2CO_3 (411 mg, 2.97 mmol) in DMF (20 mL), **1** (500 mg, 2.12 mmol) was added. The reaction mixture was heated at 85 °C for 5 h under N_2 atmosphere and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 20:1) afforded **4** (295 mg, 45%) as white crystals. Mp = 182–184 °C; MS *m*/*z* 312.0878 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.52 (C=O), 163.62 (C-4), 150.86 (C-2), 145.05 (C-6), 134.41 (*C*H-phth), 131.66 (C_{quat}-phth), 127.97 and 127.42 (*C* = H), 123.07 (*C*H-phth), 101.24 (C-5), 44.04 (C-1′), 34.44 (C-4′).

4.2.5. (*Z*)-1-[4-(*N*-Phthalimido)-2-butenyl]-5-fluorouracil (5). To a stirred mixture of 5-fluorouracil (718 mg, 5.52 mmol) and 60% NaH (241 mg, 5.52 mmol) in DMF (25 mL), **1** (1 g, 4.25 mmol) was added. The reaction mixture was stirred under N_2 atmosphere overnight at room temperature and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 60:1) afforded **5** (753 mg, 54%) as white crystals. Mp = 194–197 °C; MS *m*/*z* 330.1914 $[MH]^+$;^{13}C NMR (DMSO) δ: 167.43 (C=O), 156.25 (C-4), 149.05 (C-2), 139.07 (C-5, J_{CF} = 227.60 Hz), 134.43 (*C*H-phth), 131.57 (C_{quat}-phth), 128.37 and 126.97 (*C* = H), 125.77 (C-6, J_{CF} = 31.53 Hz), 123.09 (*C*H-phth), 49.29 (C-1′), 38.32 (C-4′).

4.2.6. (*Z*)-9-(4-Amino-2-butenyl)adenine (6). Compound **2** (138 mg, 0.41 mmol) was suspended in EtOH (7 mL) and heated at 65 °C for 2 h. Hydrazine hydrate (0.06 mL, 1.24 mmol) was then added to the reaction mixture, which was refluxed for 2 h and then stirred overnight at room temperature. The precipitate was filtered off and washed with EtOH. The filtrate was evaporated and the residue purified by column chromatography (MeOH/CH_2Cl_2/Et_3N = 10:1:0.5) afforded **6** (70 mg, 83%) as yellowish crystals. Mp = 160–164 °C; UV (MeOH): λ_{max} ($\log\varepsilon$) = 262.0 nm (4.08), 208.0 nm (4.31); MS *m*/*z* 205.1200 $[MH]^+$; ^{13}C NMR (DMSO) δ: 156.41 (C-6), 152.89 (C-2), 149.71 (C-4), 140.96 (C-8), 135.75 and 124.32 (*C* = H), 119.12 (C-5), 44.56 (C-1′), 42.95 (C-4′).

4.2.7. (*Z*)-4-Chloro-2,3-epoxy-1-*N*-phthalimide (7). Compound **1** (929 mg, 3.94 mmol) was dissolved in CH_2Cl_2 (15 mL) and the solution was cooled to 0 °C. After 30 min, 70% *m*-chloroperoxybenzoic acid (*m*-CPBA, 972 mg, 3.94 mmol) was added. The reaction mixture was stirred 5 days at room temperature and then at 40 °C (CH_2Cl_2 reflux) for 24 h. Saturated aqueous solution of $NaHCO_3$ was then added and the reaction mixture extracted with CH_2Cl_2 (3 × 30 mL). Combined organic extracts were dried (Na_2SO_4) and the solvent was evaporated. Purification of the residue by column chromatography (CH_2Cl_2) afforded the compound **7** (590 mg, 60%) as white crystals. Mp = 87–88 °C; ^{13}C NMR ($CDCl_3$) δ: 167.31 (C=O), 133.76 (*C*H-phth),

131.45 (C_{quat}-phth), 123.04 (*C*H-phth), 55.70 (C-3), 53.97 (C-2), 40.95 (C-4), 35.51 (C-1).

4.2.8. (*Z*)-9-[4-(*N*-Phthalimido)-2,3-epoxy-butyl]adenine (8). To a stirred mixture of adenine (336 mg, 2.50 mmol) and K_2CO_3 (343 mg, 2.50 mmol) in DMF (20 mL), **7** (500 mg, 1.99 mmol) was added. The reaction mixture was heated at 70 °C for 6 h under nitrogen atmosphere and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 10:1) afforded **8** (131 mg, 19%) as white crystals. Mp = 202–205 °C; MS *m*/*z* 351.1205 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.66 (C=O), 155.99 (C-6), 152.53 (C-2), 149.58 (C-4), 140.62 (C-8), 134.49 (*C*H-phth), 131.68 (C_{quat}-phth), 123.16 (*C*H-phth), 118.63 (C-5), 54.47 (C-2′), 53.80 (C-3′), 41.78 (C-1′), 35.99 (C-4′).

4.2.9. (*E*)-9-[4-(*N*-Phthalimido)-2-butenyl]adenine (10). To a stirred mixture of adenine (338 mg, 2.50 mmol) and K_2CO_3 (346 mg, 2.50 mmol) in DMF (20 mL), **9** (500 mg, 1.79 mmol) was added. The reaction mixture was heated at 70 °C for 3 h under nitrogen atmosphere and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 10:1) afforded **10** (307 mg, 51%) as white crystals. Mp = 215–217 °C; MS *m*/*z* 335.1218 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.61 (C=O), 156.08 (C-6), 152.60 (C-2), 149.43 (C-4), 140.64 (C-8), 134.58 (*C*H-phth), 131.75 (C_{quat}-phth), 127.70 and 127.17 (*C* = H), 123.25 (*C*H-phth), 118.72 (C-5), 43.81 (C-1′), 38.44 (C-4′).

4.2.10. (*E*)-9-[4-(*N*-Phthalimido)-2-butenyl]-6-chloropurine (11a) and (*E*)-7-[4-(*N*-phthalimido)-2-butenyl]-6-chloropurine (11b). To a stirred mixture of 6-chloropurine (665 mg, 4.30 mmol) and 50% NaH (206 mg, 4.30 mmol) in DMF (40 mL), **9** (1 g, 3.57 mmol) was added. Reaction mixture was stirred at 40 °C overnight under argon atmosphere. The solvent was evaporated and the oily residue was dissolved in EtOAc (100 mL) and extracted with saturated NH_4Cl (100 mL). The organic layer was dried over $MgSO_4$, the solvent was evaporated and the oily residue purified by column chromatography (CH_2Cl_2/MeOH = 30:1), which afforded **11** (634 mg, 50%) as white crystals.

Compound **11a**: mp = 233–236 °C; MS *m*/*z* 354.0651 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.64 (C=O), 151.92 (C-6), 149.20 (C-4), 134.62 (*C*H-phth), 131.83 (C_{quat}-phth), 128.69 and 126.18 (*C* = H), 123.31 (*C*H-phth), 44.77 (C-1′), 38.47 (C-4′).

Compound **11b**: ^{13}C NMR (DMSO) δ: 167.90 (C=O), 156.53(C-6), 155.66 (C-4), 152.11 (C-2), 151.25 (C-8), 134.90 (*C*H-phth), 132.07 (C_{quat}-phth), 128.28 and 127.85 (*C* = H), 123.56 (*C*H-phth), 122.49 (C-5), 47.80 (C-1′), 38.77 (C-4′).

4.2.11. (*E*)-9-[4-(*N*-Phthalimido)-2-butenyl]uracil (12). To a stirred mixture of uracil (280 mg, 2.50 mmol) and K_2CO_3 (345 mg, 2.50 mmol) in DMF (20 mL), **9** (500 mg, 1.79 mmol) was added. The reaction mixture was heated at 80 °C for 3 h under nitrogen atmosphere and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 20:1) afforded **12** (360 mg, 55%) as white crystals. Mp = 200–203 °C; MS *m*/*z* 312.0871 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.43 (C=O), 163.60 (C-4), 150.66 (C-2), 145.15 (C-6), 134.43 (*C*H-phth), 131.58 (C_{quat}-phth), 127.60 and 126.62 (*C* = H), 123.09 (*C*H-phth), 101.13 (C-5), 47.85 (C-1′), 38.33 (C-4′).

4.2.12. (*E*)-9-[4-(*N*-Phthalimido)-2-butenyl]-5-fluorouracil (13). To a stirred mixture of 5-fluorouracil (557 mg, 4.30 mmol) and 50% NaH (206 mg, 4.30 mmol) in DMF (40 mL), **9** (1.000 g, 3.57 mmol) was added. Reaction mixture was stirred under nitrogen atmosphere for 24 h at room temperature and then at 40 °C overnight. The solvent was evaporated, saturated NH_4Cl (100 mL) was added and the solution was extracted with EtOAc (3 × 70 mL). Combined organic extracts were dried over $MgSO_4$. The solvent was evaporated and the residue purified by column chromatography (CH_2Cl_2/MeOH = 30:1), which afforded **13** (834 mg, 71%) as white crystals. Mp = 227–231 °C; MS *m*/*z* 330.1919 $[MH]^+$;^{13}C NMR (DMSO) δ: 169.72 (C=O), 159.64 (C-4, J_{CF} = 25.96 Hz), 151.51 (C-2), 141.81 (C-5, J_{CF} = 229.49 Hz), 136.69 (*C*H-phth), 133.71 (C_{quat}-phth), 131.79 (C-6, J_{CF} = 33.38 Hz), 130.22 and 128.22 (*C* = H), 125.31 (*C*H-phth), 50.42 (C-1′), 40.53 (C-4′).

4.2.13. (*E*)-9-[4-(*N*-Phthalimido)-2-butenyl]-5-(trifluoromethyl)uracil (14). To a stirred mixture of 5-(trifluoromethyl)uracil (500 mg, 2.78 mmol) and 60% NaH (120 mg, 3.00 mmol) in DMF (30 mL), **9** (840 mg, 3.00 mmol) was added. The reaction mixture was stirred under nitrogen atmosphere 4 h at room temperature. The solvent was evaporated, the residue was dissolved in CH_2Cl_2 (100 mL) and extracted with saturated aqueous solution of NH_4Cl (100 mL). The organic layer was dried over $MgSO_4$, the solvent was evaporated and the residue purified by column chromatography (CH_2Cl_2/MeOH = 40:1), which afforded **14** (646 mg, 68%) as white crystals. Mp = 204–206 °C; MS *m*/*z* 380.0856 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.41 (C=O), 159.29 (C-4), 149.88 (C-2), 146.86 (C-6), 134.42 (*C*H-phth), 131.56 (C_{quat}-phth), 128.42 and 125.95 (*C* = H), 123.06 (*C*H-phth), 122.66 (CF_3,J_{CF} = 269.12 Hz), 102.25 (C-5, J_{CF} = 31.85 Hz), 48.88 (C-1′), 38.31 (C-4′).

4.2.14. (*E*)-9-(4-Amino-2-butenyl)adenine (15). Compound **10** (132 mg, 0.40 mmol) was suspended in EtOH (7 mL) and hydrazine hydrate (0.04 mL, 0.80 mmol) was added into the reaction mixture which was then stirred for 4 days at room temperature. The precipitate was filtered off and washed with EtOH. The filtrate was evaporated and the residue purified by column chromatography (MeOH/CH_2Cl_2/Et_3N = 10:1:0.5). Pure **15** (75 mg, 93%) was obtained as white crystals. Mp = 155–159 °C; UV (MeOH): λ_{max} ($\log\varepsilon$) = 262.0 nm (4.15), 210.0 nm (4.26); MS *m*/*z* 205.1210 $[MH]^+$; ^{13}C NMR (DMSO) δ: 155.93 (C-6), 152.43 (C-2), 149.32 (C-4), 140.48 (C-8), 136.00 and 123.29 (*C* = H), 118.65 (C-5), 44.11 (C-1′), 42.66 (C-4′).

4.2.15. (*E*)-9-(4-Amino-2-butenyl)uracil (16). Compound **12** (600 mg, 1.93 mmol) was suspended in EtOH (25 mL) and hydrazine hydrate (0.15 mL, 2.96 mmol) was added into the reaction mixture, which was then stirred for 24 h at room temperature. The solvent was evaporated and the residue purified by column chromatography ($MeOH/CH_2Cl_2/Et_3N$ = 20:1:1). Compound **17** (250 mg, 72%) was obtained as white crystals. Mp = 191–194 °C; UV (MeOH): λ_{max} ($\log\varepsilon$) = 265.0 nm (3.99), 208.0 nm (4.08); MS *m*/*z* 182.0857 $[MH]^+$; ^{13}C NMR (DMSO) δ: 163.69 (C-4), 150.73 (C-2), 145.25 (C-6), 132.30 and 125.18 (*C* = H), 101.07 (C-5), 48.20 (C-1′).

4.2.16. (*E*)-9-(4-Amino-2-butenyl)-5-fluorouracil (17). Compound **13** (650 mg, 0.40 mmol) was suspended in EtOH (20 mL) and hydrazine hydrate (0.15 mL, 2.96 mmol) was added into the reaction mixture, which was stirred for 30 h at room temperature. The solvent was evaporated and the residue purified by column chromatography ($MeOH/CH_2Cl_2/Et_3N$ = 20:1:1) to give **17** (240 mg, 61%) as white crystals. Mp = 202–206 °C; UV (MeOH): λ_{max} ($\log\varepsilon$) = 273.0 nm (3.87), 208.0 nm (4.17); MS *m*/*z* 200.0834 $[MH]^+$;^{13}C NMR (DMSO) δ: 157.69 (C-4, J_{CF} = 25.53 Hz), 149.62 (C-2), 139.69 (C-5, J_{CF} = 229.82 Hz), 129.43 (C-6, J_{CF} = 33.22 Hz), 135.85 and 122.93 (*C* = H), 48.51 (C-1′), 42.50 (C-4′).

4.2.17. (*E*)-9-(4-Amino-2-butenyl)-5-(trifluoromethyl)uracil (18). To a solution of**14** (505 mg, 1.33 mmol) in EtOH (30 mL) hydrazine hydrate (0.10 mL, 2.0 mmol) was added. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the residue purified by column chromatography ($MeOH/CH_2Cl_2/Et_3N$ = 10:1:0.5) yielded **18** (320 mg, 61%) as white crystals. Mp = 168–170 °C; UV (MeOH): λ_{max} ($\log\varepsilon$) = 266.1 nm (3.95), 205.0 nm (4.09); MS *m*/*z* 250.0874 $[MH]^+$; ^{13}C NMR (DMSO) δ: 160.28 (C-4), 150.75 (C-2), 147.29 (C-6, J_{CF} = 5.70 Hz), 135.69 and 123.89 (*C* = H), 123.30 (CF_3, J_{CF} = 269.04 Hz), 102.69 (C-5, J_{CF} = 31.20 Hz), 49.67 (C-1′), 42.74 (C-4′).

4.2.18. (*E*)-4-Bromo-2,3-epoxy-1-*N*-phthalimide (19). Compound **9** (1.007 g, 3.60 mmol) was dissolved in CH_2Cl_2 (15 mL) and the solution was cooled to 0 °C. After 30 min, 70% *m*-chloroperoxybenzoic acid (*m*-CPBA, 887 mg, 3.60 mmol) was added. The reaction mixture was stirred 4 days at 40 °C. Analogous workout to that for **7**, afforded **19** (790 mg, 74%) as white crystals. Mp = 93–96 °C; ^{13}C NMR ($CDCl_3$) δ: 167.35 (C═O), 133.75 (*C*H-phth), 131.39 (C_{quat}-phth), 123.05 (*C*H-phth), 56.11 (C-3), 55.96 (C-2), 38.22 (C-4), 35.51 (C-1).

4.2.19. (*E*)-9-[4-(*N*-Phthalimido)-2,3-epoxy-butyl]adenine (20). To a stirred mixture of adenine (319 mg, 2.40 mmol) and K_2CO_3 (327 mg, 2.40 mmol) in DMF (20 mL), **19** (500 mg, 1.89 mmol) was added. The reaction mixture was heated at 70 °C for 3 h under nitrogen atmosphere and then concentrated to dryness. Purification of the residue by column chromatography (CH_2Cl_2/MeOH = 10:1) afforded **20** (364 mg, 62%) as white crystals. Mp = 206–208 °C; MS *m*/*z* 351.1226 $[MH]^+$; ^{13}C NMR (DMSO) δ: 167.67 (C═O), 156.07 (C-6), 152.57 (C-2), 149.69 (C-4), 140.97 (C-8), 134.64 (*C*H-phth), 131.63 (C_{quat}-phth), 123.27 (*C*H-phth), 118.60 (C-5), 54.69 (C-2′), 54.05 (C-3′), 43.82 (C-1′), 38.31 (C-4′).

4.3. X-ray determination of 14

Single crystal of **14** suitable for X-ray single crystal analysis was obtained at room temperature by partial evaporation from ethanol solution (96%). A colourless crystal with dimensions $0.07 \times 0.59 \times 0.71$ mm^3 was selected for X-ray structure analysis. The intensities were collected at 293 K on a Oxford Diffraction Xcalibur2 diffractometer using graphite-monochromated MoK_α radiation (λ = 0.71073 Å), and with the ω-scan mode. The data collection and reduction were carried out with the CrysAlis[16] programs. The crystal structure was solved by direct methods. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares calculations based on F^2. The hydrogen atom attached to the N3 atom was found in a difference Fourier map and its coordinates and isotropic thermal parameter have been refined freely. All other hydrogen atoms were located in difference maps and than treated using appropriate riding models. The final difference maps contained no significant features ($\Delta\rho_{max}/\Delta\rho_{min}$ = 0.195/−0.220 eÅ^{-3}). For structure solution, refinement and analysis were used following programmes: *SHELXS97*,[17] *SHELXL97*[18] and *PLATON*.[19] The molecular and crystal structure drawings were prepared by *PLATON*[19] program. CCDC 600017 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request @ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Crystal data: $C_{17}H_{12}F_3N_3O_4$, M_r = 379.30, monoclinic space group *C* 2/c (No. 15); *a* = 27.248(3), *b* = 6.9029(17), *c* = 18.024(3) Å, β = 96.893(11)°; *V* = 3365.6(11) Å^3; *Z* = 8; *F*(000) = 1552; d_x = 1.497 g cm^{-3}; μ (MoK_α) = 0.130 mm^{-1}; *S* = 1.118; *R*/*wR* = 0.0688/0.1809 for 248 parameters and 2520 reflections with $I \geqslant 2\sigma(I)$, *R*/*wR* = 0.0787/0.1925 for all 2920 independent reflections measured in the range 3.86°–*θ*–25°.

4.4. Antitumor cell activity assays

The HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW 620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep-2 (laryngeal carcinoma) and WI 38 (diploid fibroblasts) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

The growth inhibition activity was assessed according to the slightly modified procedure performed at the National Cancer Institute, Developmental Therapeutics

Program.[20] The cells were seeded into a series of standard 96-well microtiter plates on day 0. The cell concentrations were adjusted according to the cell population doubling time (PDT): 1×10^4/mL for HeLa, Hep-2, MiaPaCa-2 and SW 620 cell lines (PDT = 20–24 h) 2×10^4/mL for MCF-7 cell lines (PDT = 33 h) and 3×10^4/mL for WI 38 (PDT = 47 h). Test agents were then added in five, 10-fold dilutions (10^{-8} to 10^{-4} M) and incubated for a further 72 h. Working dilutions were freshly prepared on the day of testing. The solvent was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentrations. After 72 h of incubation the cell growth rate was evaluated by the MTT assay,[21] which detects dehydrogenase activity in viable cells. The absorbance (OD, optical density) was measured by a microplate reader at 570 nm. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean OD_{test} − mean OD_{tzero}) $\geqslant 0$ then

$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero}) / (\text{mean } OD_{ctrl} - \text{mean } OD_{tzero})$.

If (mean OD_{test} − mean OD_{tzero}) < 0 then:

$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero}) / OD_{tzero}$.

Where:

Mean OD_{tzero} = the average of optical density measurements before exposure of cells to the test compound.

Mean OD_{test} = the average of optical density measurements after the desired period of time.

Mean OD_{ctrl} = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, a concentration necessary for 50% of inhibition. Each result is a mean value from three separate experiments. The IC_{50} values for each compound were calculated from dose–response curves using linear regression analysis by fitting the test concentrations that gave PG values above and below the reference value (i.e., 50%).

4.5. Antiviral activity assays

Antiviral activity against HIV-1, HIV-2, varicella zoster virus (VZV), human cytomegalovirus (HCMV), vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus was determined essentially as described previously.[22,23] Confluent human embryonic lung (HEL) fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus (HCMV) strains Davis and AD-169 at 100 PFU per well. After a 2 h incubation period, residual virus was removed and the infected cells were further incubated with the medium containing different concentrations of the test compounds (in duplicate). After incubation for 7 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically after ethanol fixation and staining with Giemsa. Antiviral activity was expressed as the EC_{50} or concentration required to reduce virus-induced cytopathogenicity by 50%. EC_{50} values were calculated from graphic plots of the percentage of cytopathogenicity as a function of concentration of the compounds.

4.6. Cytotoxicity assays

Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, the compound concentration required to reduce cell growth by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Cytotoxicity was expressed as minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.07.033.

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