Med Chem Res (2011) 20:280–286
DOI 10.1007/s00044-010-9318-1

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, cytostatic and anti-viral activity evaluation of the novel acyclic nucleoside analogues containing a sterically constrained (*Z*)-4-amino-2-butenyl moiety

Karlo Wittine · Krešimir Benci · Sandra Kraljević Pavelić · Krešimir Pavelić · Siniša Bratulić · Karlo Hock · Jan Balzarini · Mladen Mintas

Received: 4 May 2009 / Accepted: 12 February 2010 / Published online: 27 February 2010

Abstract A series of the novel pyrimidine (**3–6**) and purine (**12–15, 18–21**) acyclic nucleoside analogues in which the sugar moiety was replaced by a sterically constrained *Z*-4-amino-, 4-aminohydrochloride-2-butenyl, or aliphatic 4-aminohydrochloride-2-butyl moiety were synthesized and evaluated for their anti-viral and cytostatic activity potency. Cytostatic evaluation of the novel compounds on selected panel of human tumour-cell lines showed that the majority of compounds exerted a non-specific anti-proliferative effect at the highest tested concentration (i.e. 1×10^{-4} M) against all cell lines. Nevertheless, a rather moderate but selective anti-proliferative effects on HeLa cell cultures in comparison to normal fibroblasts WI 38, were observed for compounds **15** and **21**. No anti-viral activity was observed, except for compounds **3**, **4**, **5** and **19** that showed anti-HIV activity at 50% effective concentration ranging between 10 and 96 μM.

Keywords Acyclic nucleoside analogues · Purine and pyrimidine derivatives · Cytostatic activity · Anti-viral activity

Introduction

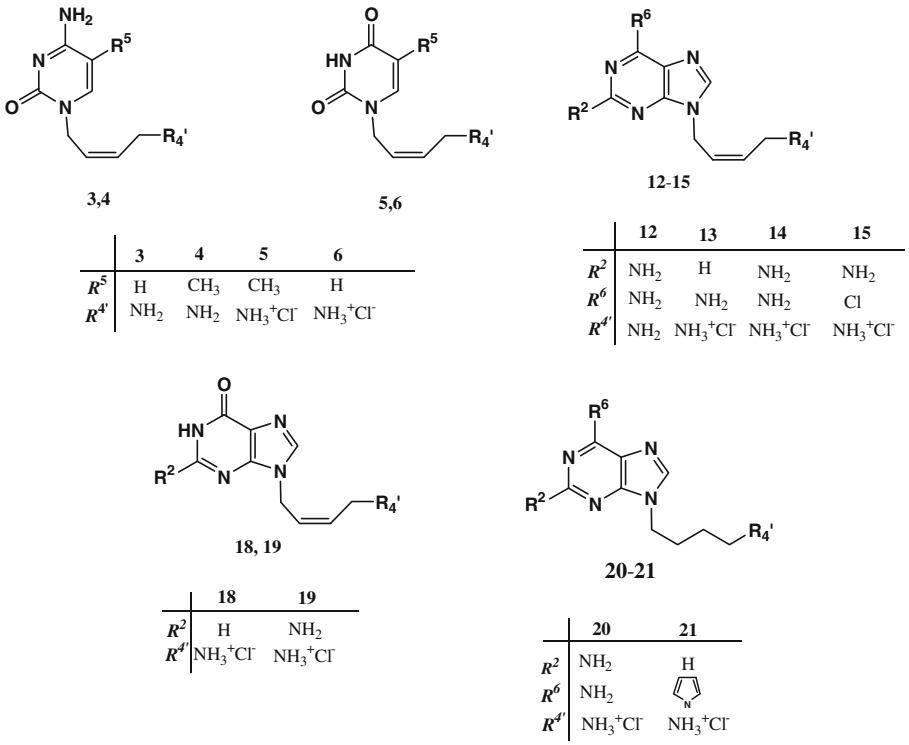
Nucleoside analogues have been the cornerstone of anti-viral chemotherapy over the past decades. There is considerable evidence that introduction of a sterically constrained structural element into the nucleoside or carbocyclic nucleoside structure can lead to effective anti-viral nucleoside analogues (Wu and Hong, 2005; Haines *et al*., 1987). Thus, acyclic nucleoside analogues of 5′-*O*-tritylthymidine containing constrained butenyl spacer showed selective inhibitory activity for either human mitochondrial thymidine kinase (TK) or phylogenetically close HSV-1 TK. (Hernández *et al.*, 2003). Furthermore, chloropurine derivatives containing conformationally constrained acyclic side chain demonstrated inhibitory activity against a wide range of cancer cell lines (Chen *et al.*, 2005). According to this and related to our previous studies on unsaturated acyclic and epoxide nucleoside analogues (Krištafor *et al.*, 2006) and unsaturated acyclic C-5 pyrimidine nucleoside analogues (Gazivoda *et al.*, 2008), we have synthesized a series of the novel acyclic pyrimidine (**3–6**) and purine (**12–21**) nucleoside analogues containing a sterically constrained *Z*-4-amino-2-butenyl, 4-aminohydrochloride-2-butenyl or aliphatic 4-aminohydrochloride-2-butyl moiety (Fig. 1). The principal goal of this study was thus to evaluate the cytostatic and anti-viral activity potency of the novel compounds.

Electronic supplementary material The online version of this article (doi:10.1007/s00044-010-9318-1) contains supplementary material, which is available to authorized users.

K. Wittine · K. Benci · M. Mintas (✉)
Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia
e-mail: mladen.mintas@fkit.hr

S. Kraljević Pavelić · K. Pavelić · S. Bratulić · K. Hock
Division of Molecular Medicine, Laboratory for systems biomedicine, Ruđer Bošković Institute, Bijenička cesta 54, P. O. Box 1016, 10001 Zagreb, Croatia

J. Balzarini
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium



	3	4	5	6
R^5	H	CH_3	CH_3	H
$R^{4'}$	NH_2	NH_2	$NH_3^+Cl^-$	$NH_3^+Cl^-$

	12	13	14	15
R^2	NH_2	H	NH_2	NH_2
R^6	NH_2	NH_2	NH_2	Cl
$R^{4'}$	NH_2	$NH_3^+Cl^-$	$NH_3^+Cl^-$	$NH_3^+Cl^-$

	18	19
R^2	H	NH_2
$R^{4'}$	$NH_3^+Cl^-$	$NH_3^+Cl^-$

	20	21
R^2	NH_2	H
R^6	NH_2	
$R^{4'}$	$NH_3^+Cl^-$	$NH_3^+Cl^-$

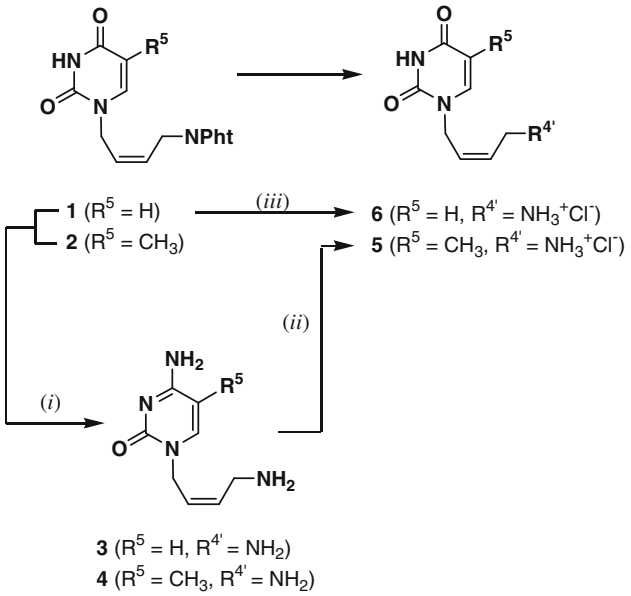
Fig. 1 The unsaturated acyclic pyrimidine (**3–6**), purine (**12–19**) and saturated acyclic purine (**20** and **21**) nucleoside analogues

Materials and methods

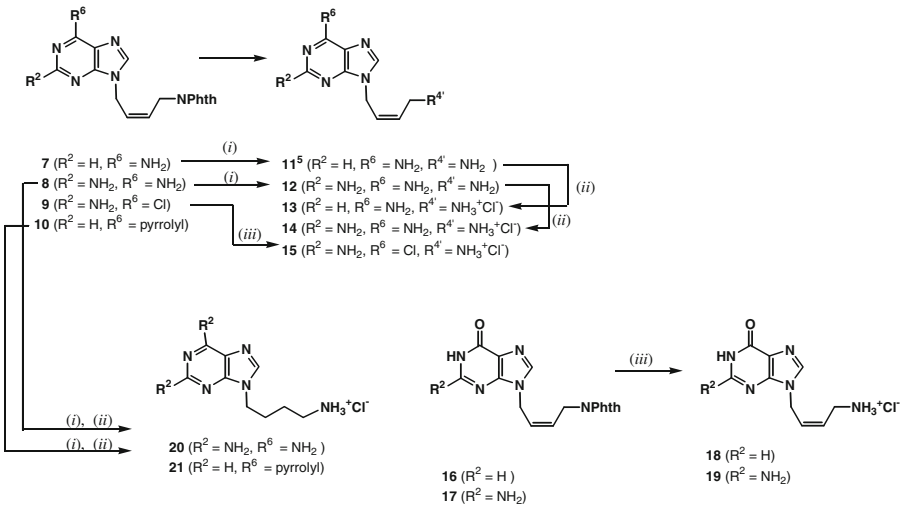
Melting points were determined on a Kofler micro hot-stage apparatus (Reichert, Wien) and are uncorrected. Precoated Merck silica gel 60F-254 plates were used for thin layer chromatography (TLC), and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (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 ^1H 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 DMSO-d_6 and measured in 5 mm NMR tubes. The ^1H and ^{13}C NMR chemical shift values (δ) are expressed in ppm referred to TMS and coupling constants (J) in Hz.

Chemistry

The starting (*Z*)-*N*-phthalimide protected 4-amino-2-butenyl pyrimidine (**1** and **2**) and purine (**7–10** and **16**, **17**) derivatives were prepared by procedures described in our previous paper (Krištafor *et al.*, 2006). The novel pyrimidine (**3** and **4**) and purine (**12**) derivatives containing a primary amino group in the acyclic moiety were prepared by Gabriel amine synthesis while the corresponding ammonium hydrochloride salts (**5**, **6** and **13–15**) were obtained by acidifying either their 4-amino-2-butenyl- or *N*-phthalimido precursors (Schemes 1 and 2). In this reaction the purine derivatives containing saturated 4-aminobutyl side chain (**20** and **21**, Scheme 2) were also obtained.



Scheme 1 Synthesis of *cis*-olefinic pyrimidine (**3–6**) nucleoside analogues:(i) hydrazine hydrate in EtOH; (ii) 0.1 M HClaq; (iii) 2.5 M HClaq



Scheme 2 Synthesis of unsaturated (**12**–**19**) and saturated (**20**–**21**) purine nucleoside analogues: (i) hydrazine hydrate in EtOH; (ii) 0.1 M HClaq; (iii) 2.5 M HClaq

Compounds preparation

*General procedure for the preparation of (Z)-1-(4-Amino-2-butenyl)- pyrimidine (**3** and **4**) and purine (**12**) derivatives*

To a stirred solution of (*Z*)-1-[4-(*N*-Phthalimido)-2-butenyl]- pyrimidine (**1** and **2**) or purine (**8**) derivatives in EtOH (20–30 ml) was added hydrazine hydrate. The reaction mixture was stirred for 24 h under heating at reflux temperature, evaporated to dryness and purified by column chromatography (MeOH: CH_2Cl_2: Et_3N = 1:10:0.5).

*General procedure for the preparation of (Z)-1-(4-Aminohydrochloride-2-butenyl)- pyrimidine (**5** and **6**) and purine (**13**–**15**, **18** and **19**) derivatives*

The reaction procedure with compounds (*Z*)-1-(4-Amino-2-butenyl)- pyrimidine (**4**) and purine (**11** and **12**) derivatives were carried out using a 0.1 M HCl (10–15 ml) solution to achieve (**5** and **13**, **14**), while the (*Z*)-1-[4-(*N*-Phthalimido)-2-butenyl]- pyrimidine (**1**) and purine (**9**, **16** and **17**) derivatives were carried out using a 2.5 M HCl (ml) solution to achieve (**6** and **15**, **18** and **19**). The reaction mixtures were stirred under heating at 100°C for 24 h, evaporated to dryness and the residues were purified with EtOH, then additionally if necessary with MeOH and filtered off.

*(Z)-1-(4-Amino-2-butenyl)cytosine (**3**)*

The procedure was carried out using (*Z*)-1-[4-(*N*-Phthalimido)-2-butenyl]uracil (**1**) (1333 mg, 4.29 mmol), hydrazine hydrate (0.42 ml, 8.60 mmol). Pure **3** (40 mg, 3%) was obtained as white solid. mp = 130°C; MS *m/z* 182 $[M + 2H]^+$; ^{13}C NMR (DMSO) δ: 164.64 (C-4), 151.78 (C-2), 136.64 (C-6), 128.64 and 128.02 (*C*=H), 95.25 (C-5), 49.22 (C-1′), 38.60 (C-4′).

*(Z)-1-(4-Amino-2-butenyl)-5-methylcytosine (**4**)*

The procedure was carried out using (*Z*)-1-[4-(*N*-Phthalimido)-2-butenyl]tymine (**2**) (1087 mg, 3 mmol), hydrazine hydrate (0.44 ml, 9 mmol). Pure **4** (60 mg, 5.5%) was obtained as white solid. mp = 196°C; MS *m/z* 196 $[M + 2H]^+$; ^{13}C NMR (DMSO) δ: 164.72 (C-4), 151.31 (C-2), 141.50 (C-6), 128.87 and 128.39 (*C*=H), 109.31 (C-5), 44.29 (C-1′), 36.66 (C-4′), 12.37 (CH_3).

*(Z)-1-(4-Ammoniumhydrochloride-2-butenyl)tymine (**5**)*

The procedure was carried out using (*Z*)-1-(4-Amino-2-butenyl)tymine (**4**) (40 mg, 0,17 mmol), 0.1 M HCl solution. Pure **5** (18 mg, 45%) was obtained as white solid. mp = 260°C; MS *m/z* 196 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 164.71 (C-4), 151.32 (C-2), 141.52 (C-6), 131.23 and 128.78 (*C*=H), 108.94 (C-5), 44.25 (C-1′), 36.06 (C-4′), 12.35 (CH_3).

*(Z)-1-(4-Ammoniumhydrochloride-2-butenyl)uracil (**6**)*

The procedure was carried out using (*Z*)-1-[4-(*N*- Phthalimido)-2-butenyl]uracil (**1**) (300 mg, 0.96 mmol), 2.5 M HCl solution. Pure **6** (15 mg, 5%) was obtained as white solid. mp = 230°C; MS *m/z* 183 $[M + 2H\text{–}HCl]^+$; ^{13}C NMR (DMSO) δ: 164.14 (C-4), 151.37 (C-2), 145.17 (C-6), 129.91 and 126.49 (*C*=H), 101.64 (C-5), 44.53 (C-1′), 36.06 (C-4′).

*(Z)-9-(4-Amino-2-butenyl)-2,6-diaminopurine (**12**)*

The procedure was carried out using (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]-2,6-diaminopurine (1640 mg, 4.70 mmol), hydrazine hydrate (0.34 ml, 7 mmol). Pure **12** (400 mg, 24%) was obtained as white solid. mp = 122–125°C; MS

m/z 220 $[M + 2H]^+$; ^{13}C NMR (DMSO) δ: 160.71 (C-6), 156.57 (C-4), 151.94 (C-2), 137.54 (C-8), 135.46 and 124.69 (*C*=H), 113.60 (C-5), 40.81 (C-1′), 38.47 (C-4′).

*(Z)-9-(4-Ammoniumhydrochloride-2-butenyl)adenine (**13**)*

The procedure was carried out using (*Z*)-1-(4-Amino-2-butenyl)adenine (220 mg, 0.65 mmol), 0.1 M HCl solution. Pure **13** (74 mg, 17%) was obtained as white solid. mp = 261–263°C; MS *m/z* 205 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 154.02 (C-6), 151.39 (C-4), 148.81 (C-2), 129.01 (C-8), 128.10 and 127.22 (*C*=H), 118.50 (C-5), 40.94 (C-4′), 36.08 (C-1′).

*(Z)-9-(4-Ammoniumhydrochloride-2-butenyl)-2,6-diaminopurine (**14**)*

The procedure was carried out using (*Z*)-1-(4-Amino-2-butenyl)-2,6-diaminopurine (150 mg, 0.69 mmol), 0.1 M HCl solution. Pure **14** (25 mg, 0.17%) was obtained as white solid. mp = 264–265°C; MS *m/z* 220 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 154.83 (C-6), 152.83 (C-4), 150.53 (C-2), 130.13 (C-8), 128.92 and 127.11 (*C*=H), 111.42 (C-5), 40.80 (C-4′), 36.06 (C-1′).

*(Z)-9-(4-Aminohydrochloride-2-butenyl)-6-chloropurine (**15**)*

The procedure was carried out using (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]-6-chloropurine (200 mg, 0.54 mmol), 2.5 M HCl solution. Pure **15** (22 mg, 0.11%) was obtained as white solid. mp = 257–260°C; MS *m/z* 239 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 151.20 (C-4), 149.32 (C-2), 130.26 (C-8),129.06 and 127.00 (*C*=H), 39.13 (C-1′), 36.16 (C-4′).

*(Z)-9-(4-Ammoniumhydrochloride-2-butenyl)hypoxanthine (**18**)*

The procedure was carried out using (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]hypoxanthine (130 mg, 0.39 mmol), 2.5 M HCl solution. Pure **18** (55 mg, 42%) was obtained as white solid. mp = 223–226°C; MS *m/z* 206 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 156.61 (C-6), 154.75 (C-4), 145.72 (C-2), 134.97 (C-8), 130.37 and 126.84 (*C*=H), 115.16 (C-5), 43.92 (C-4′), 36.16 (C-1′).

*(Z)-9-(4-Ammoniumhydrochloride-2-butenyl)quanine (**19**)*

The procedure was carried out using (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]quanine (553 mg, 1.5 mmol), 2.5 M HCl solution. Pure **19** (96 mg, 0.17%) was obtained as white solid. mp = 235–240°C; MS *m/z* 221 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 155.84 (C-6), 154.27 (C-4), 150.16 (C-2), 131.24 (C-8), 128.01 and 127.88 (*C*=H), 109.26 (C-5), 41.43 (C-4′), 36.07 (C-1′).

*(Z)-9-(4-Ammoniumhydrochloride-2-butanyl)-2,6-diaminopurine (**20**)*

The procedure was carried out using (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]-2,6-diaminopurine (1600 mg, 4.57 mmol), hydrazine hydrate (6.65 ml, 137.14 mmol), then in situ 0.1 M HCl solution.

Pure **21** (40 mg, 2.5%) was obtained as brownish solid. mp = 125–127°C; MS *m/z* 221 $[M\text{-}HCl]^+$; ^{13}C NMR (DMSO) δ: 152.73 (C-4), 111.42 (C-5), 42.85 (C-4′), 38.36 (C-1′), 26.50 (C-2′), 24.37 (C-3′).

*(Z)-9-(4-Ammoniumhydrochloride-2-butyl)-6-pyrrolilpurine (**21**)*

The procedure was carried out using (*Z*)-9-[4-(*N*-Phthalimido)-2-butenyl]-6-pyrrolilpurin (630 mg, 1.64 mmol), hydrazine hydrate (0.16 ml, 3.28 mmol), then in situ 0.1 M HCl solution.

Pure **21** (80 mg, 13%) was obtained as brownish solid. mp = 145–150°C; MS 256.9 *m/z* $[M\text{-}HCl]^{2+}$; ^{13}C NMR (DMSO) δ: 153.22 (C-6), 146.38 (C-4), 121.16 (C-5), 120.04 (C-2), 112.42 (C-8), 42.72 (C-4′), 38.01 (C-1′), 26.1550 (C-2′), 23.95 (C-3′).

Anti-tumour cell activity assays

Cell culturing

The suspension cell lines L1210, Molt4/C8, HeLa (cervical carcinoma), SW 620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep G2 (hepatocarcinoma), MCF-7 (breast carcinoma) and WI 38 (normal diploid human fibroblasts), were cultured as monolayers by using standard cell culturing procedures.

Proliferation assays

The cytostatic activity against L1210, Molt4/C8 and CEM cells was measured in 200 µl-wells of a 96-well microtiter plate (initial cell number: 5–7.5 × 10^4 cells/well) essentially as originally described. After 48 (L1210) or 72 h (CEM, Molt4/C8), the tumour-cell number was determined by a Coulter counter. For the anti-proliferative assays, a panel of monolayer tumour cell lines (hepatocellularcarcinoma Hep G2, cervical carcinoma HeLa, breast

carcinoma MCF-7, pancreatic carcinoma MiaPaCa-2, colon carcinoma SW 620 and human normal fibroblasts (WI 38) was used for a standard MTT assay as described previously (Gazivoda *et al.*, 2005).

The IC_{50} and LC_{50} values for each compound were calculated from dose–response curves using linear regression analysis as described previously (Gazivoda *et al.*, 2005).

Anti-viral activity assays

The anti-viral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4 and Punta Toro virus), HeLa (vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus) or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (one $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, … μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The methodology of the anti-HIV assays was as follows: human CEM cells ($\sim 3 \times 10^5$ cells/ml) were infected with 100 $CCID_{50}$ of HIV(III_B) or HIV-2(ROD)/ml and seeded in 200-μl wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37°C, HIV-induced giant cell formation was examined microscopically.

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 by 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.

Results and discussion

^1H and ^{13}C NMR spectra

Structures of the newly synthesized compounds were determined by analysis of their ^1H and ^{13}C NMR as well as mass spectra. The assignment of ^1H NMR spectra was performed on the basis of the chemical shifts, substituent induced chemical shifts and signal intensities, magnitude and multiplicity of H–H coupling constants. The ^1H and ^{13}C NMR data given in Table 1 and the experimental part are in full agreement with the proposed structures.

Biological activity

Cytostatic activity

The compounds were evaluated for their cytostatic activity against several malignant tumour cell lines as described per "Materials and methods" section (Supplementary table 2). The results of in vitro screening for the anti-proliferative effect of 12 acyclic nucleoside analogues (compounds **3–5**, **12–15** and **18**, **19**, **21**) showed that the majority of compounds demonstrated a non-specific anti-proliferative effect at the highest tested concentration of 1×10^{-4} M on all cell lines. Compounds **15** (**45** in supplementary figure S1) and **21** (**40** in supplementary figure S1) inhibited the growth of HeLa cells in a dose-dependent manner (supplementary figure S1).

Anti-viral activity

Compounds **3–6**, **12–15** and **18–21** were also evaluated for their activity against a broad variety of DNA and RNA viruses, in cell culture. Unfortunately, none of the compounds showed pronounced anti-viral activity at subtoxic concentrations, except compounds **3**, **4**, **5** and **19** that showed anti-HIV-1(III_B) activity at an EC_{50} of 96 ± 13, 36 ± 12, 29 ± 0.0 and 10 ± 0.0 μM and anti-HIV-2(ROD) activity at an EC_{50} of 78 ± 17, 38 ± 21, 41 ± 13 and 10 ± 0.0 μM, respectively. Compounds **15** and **18** showed marginal anti-HIV-1 and -HIV-2 activity (EC_{50}: ≥100 μM). Compounds **3**, **4** and **5** were evaluated for their inhibitory activity against recombinant HIV-1 reverse transcriptase using poly rA.dT, poly rC.dG and poly rI.dC as the template, but were found inactive at 500 μM. The mechanism of anti-viral activity is currently unclear and subject of further investigation.

Table 1 ^{1}H NMR chemical shifts (δ/ppm)[a] and H–H coupling constants (J/Hz) in ^{1}H NMR spectra for compounds **3–6**, **12–15**, **18–21** (c.f. Schemes 1 and 2)

	H-1′	H-2′	H-3′	H-4′	NH_3^+-4′	H-2	H-8	NH_2-6	NH_2-2	H-5	H-6	NH	CH_3-5
3	4.30 (d, 2H, $J_3 = 6.90$)	5.38–5.36 (m, 2H)		3.28 (d, 2H, $J_3 = 6.63$)	/	/	/	/	/	6.20 (s, 1H)	7.61 (d, 1H, $J_3 = 9.20$)	/	/
4	4.32 (d, 2H, $J_3 = 6.00$)	5.66–5.61 (m, 2H)		3.53 (d, 2H, $J_3 = 6.00$)	/	/	/	/	/	/	7.55 (s, 1H)	/	1.76 (s, 3H)
5	4.34 (d, 2H, $J_3 = 5.20$)	5.67–5.62 (m, 2H)		2.77 (s, 2H, $J_3 = 5.20$)	8.21 (br, 3H)	/	/	/	/	/	7.46 (m, 1H,)	11.26 (s, 1H)	1.72 (s, 3H)
6	4.28 (d, 2H, $J_3 = 4.50$)	5.59–5.64 (m, 2H)		3.52 (m, 2H)	8.22 (br, 3H)	/	/	/	/	5.48 (d, 1H, $J_3 = 6.00$)	7.63 (m, 1H)	11.16 (br, 1H)	/
12	4.60 (d, 2H, $J_3 = 6.54$)	5.65–5.52, (m, 2H)		3.49 (d, 2H, $J_3 = 5.70$)	/	/	7.68 (s, 1H)	5.75 (s, 2H)	6.64 (s, 2H)	/	/	/	/
13	4.97 (d, 2H, $J_3 = 6.87$)	5.93–5.71 (m, 2H)		3.75 (t, 2H, $J_3 = 5.84$)	8.42 (br, 3H)	8.48 (s, 1H)	8.46 (s, 1H)	/	/	/	/	/	/
14	4.76 (d, 2H, $J_3 = 6.45$)	5.81–5.76 (m, 2H)		3.73 (t, 2H, $J_3 = 5.64$)	8.27 (br, 3H)	/	8.15 (s, 1H)	/	7.59 (br, 2H)	/	/	/	/
15	4.76 (d, 2H, $J_3 = 6.18$)	5.86–5.72 (m, 2H)		3.72 (t, 2H, $J_3 = 5.70$)	8.24 (br, 3H)	/	8.13 (s, 1H)	/	7.57 (br, 2H)	/	/	/	/
18	5.11 (d, 2H, $J_3 = 6.96$)	5.91–5.67 (m, 2H)		3.70 (t, 2H, $J_3 = 5.49$)	8.21 (br, 3H)	8.56 (s, 1H)	8.06 (s, 1H)	/	/	/	/	12.56 (br, 1H)	/
19	4.73 (d, 2H, $J_3 = 6.69$)	5.86–5.69 (m, 2H)		3.73 (s, 2H)	8.40 (br, 3H)	/	9.10 (s, 1H)	/	7.35 (br, 2H)	/	7.89 (m, 1H)	11.56 (br, 1H)	/
20	3.98 (t, 2H, $J_3 = 7.62$)	1.82 (td, 2H, $J_3 = 7.23$)	1.46 (td, 2H, $J_3 = 7.95$)	2.79 (s, 2H)	8.07 (br, 3H)	/	7.89 (s, 1H)	6.48 (br, 2H)	7.45 (br, 2H)	/	/	/	/
21[b]	4.34 (t, 2H, $J_3 = 6.78$)	1.95 (t, 2H, $J_3 = 7.50$)	1.56 (t, 2H, $J_3 = 7.11$)	2.81 (d, 2H, $J_3 = 5.16$)	8.12 (br, 3H)	8.73 (s, 2H) ($H_2 + H_8$)		/	/	/	/	/	/

[a] DMSO-d_6 as a solvent for all compounds; chemical shifts are referred to TMS. Multiplicity of coupling and number of protons are given in parentheses

[b] H_2-py (2″ + 5″) 8.29 (s, 1H); H_2-py (3″ + 4″) 6.44 (s, 2H)

s singlet, *d* doublet, *m* complex multiplet, *br* broad

Conclusion

The in vitro screening of the novel acyclic nucleoside analogues on selected panel of tumour cell lines showed that compounds **15** and **21** exerted rather moderate but selective anti-proliferative effects on HeLa cells in comparison to normal fibroblasts WI 38.

These compounds are therefore suitable for further biological studies on HeLa cells in order to understand the molecular mechanisms underlying the observed anti-proliferative effect.

Acknowledgements Support for this study was provided by the Ministry of Science of the Republic of Croatia (Projects # 125-0982464-2922, # 098-0982464-2393) and by the "Geconcerteerde Onderzoeksacties" of the Katholieke Universiteit Leuven (project # 05/19). We thank Lizette van Berckelaer for excellent technical assistance in performing part of the anti-tumour cell activity assays, as well as Leen Ingels, Leentje Persoons, Frieda De Meyer, Vicky Broeckx, Anita Camps and Lies Vandenheurck for excellent technical assistance in performing the anti-viral activity assays.

References

Chen L, Kode N, Murthi D, Phadtare S (2005) N9- and N7-(Chloromethyl phenylmethyl)chloropurine derivatives from α, α′-dichloroxylenes: synthesis and anticancer activity. Med Chem Res 14(8/9):445–474

Gazivoda T, Plevnik M, Plavec J, Kraljević-Pavelić S, Kralj M, Pavelić K, Balzarini J, De Clerq E, Mintas M, Raić-Malić S (2005) The novel pyrimidine and purine derivatives of l-ascorbic acid: synthesis, one- and two-dimensional 1H and 13C NMR study, cytostatic and antiviral evaluation. Bioorg Med Chem 13:131–139

Gazivoda T, Raić-Malić S, Krištafor V, Makuc D, Plavec J, Bratulić S, Kraljević-Pavelić S, Pavelić K, Naesens L, Andrei G, Snoeck R, Balzarini J, Mintas M (2008) Synthesis, cytostatic and anti-HIV evaluations of the new unsaturated acyclic C-5 pyrimidine nucleoside analogues. Bioorg Med Chem 16:5624–5634

Haines DR, Tseng CKH, Marquez VE (1987) Synthesis and biological activity of unsaturated carboacyclic purine nucleoside analogs. J Med Chem 30:943–947

Hernández A, Balzarini J, Rodríguez-Barrios F, San-Félix A, Karlsson A, Gago F, Camarasa M, Pérez-Pérez MJ (2003) Improving the selectivity of acyclic nucleoside analogues as inhibitors of human mitochondrial thymidine kinase: replacement of a triphenylmethoxy moiety with substituted amines and carboxamides. Bioorg Med Chem Lett 13:3027–3030

Krištafor V, Raić-Malić S, Cetina M, Kralj M, Pavelić K, Balzarini J, DeClercq E, Mintas M (2006) Synthesis, X-ray crystal structural study, antiviral and cytostatic evaluations of the novel unsaturated acyclic and epoxide nucleoside analogues. Bioorg Med Chem 14:8126–8138

Wu Y, Hong JH (2005) Synthesis and anti-HIV activity of novel phenyl branched cyclopropyl nucleosides. Farmaco 60:739–744