Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 749–758
Bioorganic & Medicinal Chemistry

The novel C-5 aryl, alkenyl, and alkynyl substituted uracil derivatives of L-ascorbic acid: Synthesis, cytostatic, and antiviral activity evaluations

Tatjana Gazivoda,[a] Silvana Raić-Malić,[a] Marko Marjanović,[b] Marijeta Kralj,[b] Krešimir Pavelić,[b] Jan Balzarini,[c] Erik De Clercq[c] and Mladen Mintas[a,*]

[a]*Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, HR-10000 Zagreb, Croatia*
[b]*Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička 54, HR-10001 Zagreb, Croatia*
[c]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Received 14 September 2006; revised 18 October 2006; accepted 23 October 2006
Available online 25 October 2006

Abstract—The novel C-5 substituted uracil derivatives of L-ascorbic acid were synthesized by coupling of 5-iodouracil-4,5-didehydro-5,6-dideoxy-L-ascorbic acid with unsaturated stannanes under Stille reaction conditions. The new compounds were evaluated for their antitumoral and antiviral activities. Among all compounds evaluated the 5-propynyl substituted uracil derivative of L-ascorbic acid (**7**) exhibited the most pronounced cytostatic activities against all examined tumor cell lines (IC_{50}: 0.2–0.78 μM). However, this compound was also cytotoxic to human normal fibroblasts WI 38. The 5-(phenylethynyl)uracil-2,3-di-*O*-benzylated L-ascorbic acid derivative (**4**) exhibited an albeit slight (IC_{50}: 55–108 μM), but selective inhibitory effect toward all tumor cell lines except for cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), laryngeal carcinoma (Hep-2), and colon carcinoma (SW 620), and no cytotoxicity to normal human fibroblast (WI 38). Compound **7** showed some, not highly specific, inhibitory potential against vesicular stomatitis virus, Coxsackie B4 virus, and Sindbis viruses (EC_{50}: 1.6 μM).

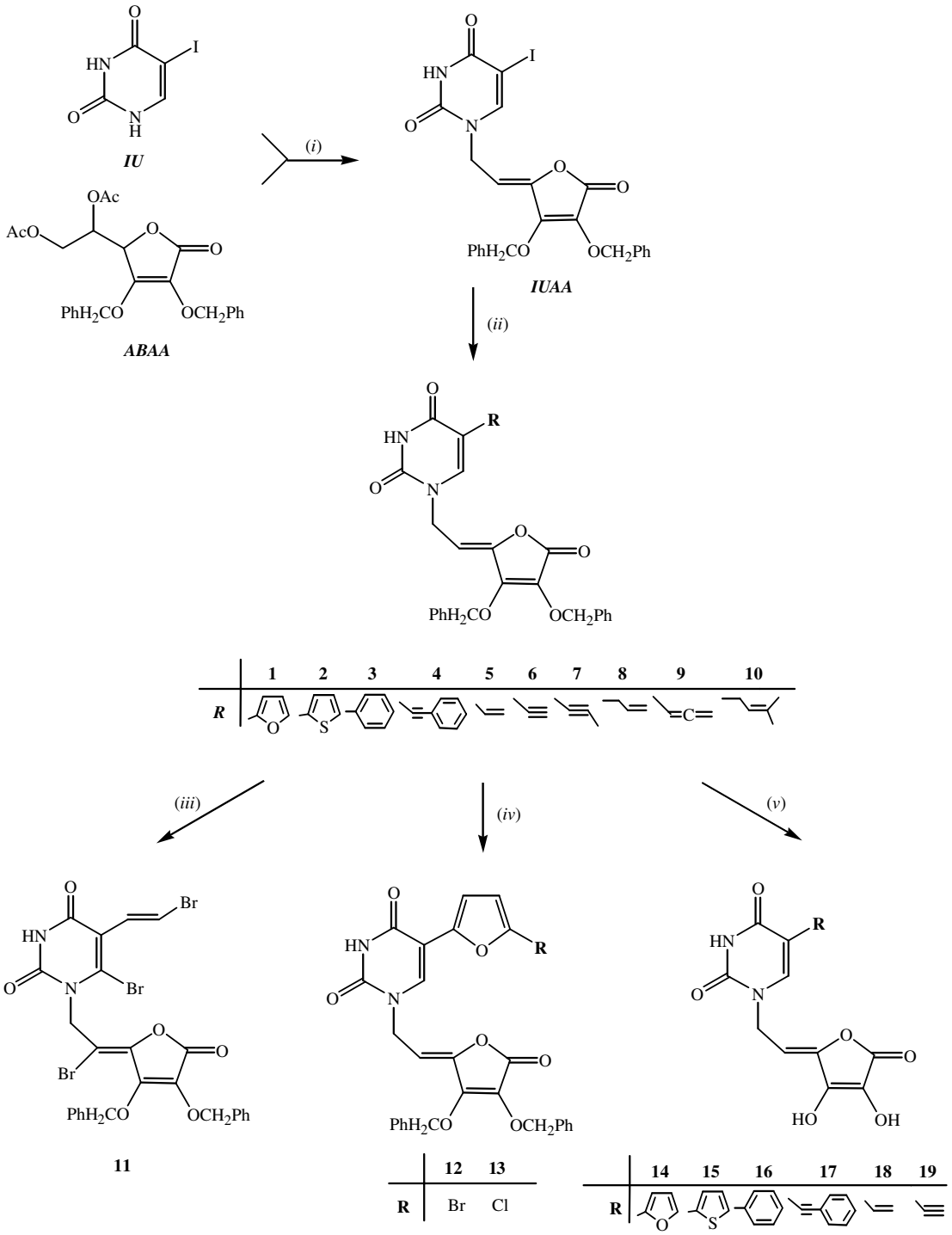
1. Introduction

Vitamin C has been considered as a useful synthetic precursor to many molecules of potential biological utility on account of its inherent, varied chemical functionality.[1–3]

On the other hand, many pyrimidine nucleoside analogues substituted at the 5-position of the heterocycle are known to possess potent biological properties and have been investigated as anticancer and antiviral drugs.[4–6] The most active congeners among the 5-substituted 2′-deoxyuridines are *E*-5-(2-halogenovinyl)-2′-deoxyuridines and 5-heteroaryl-2′-deoxyuridines. These compounds have emerged as potent and selective inhibitors of herpes viruses (HSV-1 and HSV-2) and varicella-zoster virus (VZV).[7–10] The structure–activity relationship (SAR) studies seem to indicate that the nature of C-5 substituents likely to confer activity are those which are electron-withdrawing and conjugated to the pyrimidine base.[11,12] In addition, unsaturated analogues of nucleosides have also been developed as antitumoral and antiviral agents.[13–16] We have found that some pyrimidine and purine derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid possess pronounced cytostatic activities against some malignant human tumor cell lines.[17–19] It has also been presumed that the biological activity of such type of derivatives of L-ascorbic acid has its origin in the reactivity of their double bond conjugated with the lactone ring toward biological nucleophiles.[20] The intense search for clinically useful nucleoside derivatives has resulted in a wealth of new approaches for their synthesis. One of the most useful synthetic approaches involves the use of palladium-catalyzed addition and substitution reactions.[21] Taking into account the pharmacological potential of this class of compounds, we have synthesized by Stille cross-coupling the novel C-5 substituted pyrimidine derivatives of 2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (Scheme 1) and evaluated their cytostatic and antiviral activities.

Keywords: L-Ascorbic acid; C-5 substituted uracil derivatives; Cytostatic activities; Antiviral activities.
* Corresponding author. Tel.: +385 1 4597 214; fax: +385 1 4597 224; e-mail: mladen.mintas@fkit.hr

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.10.046



Scheme 1. Synthesis of C-5 pyrimidine substituted 4,5-didehydro-5,6-dideoxy-L-ascorbic acid derivatives. Reagents and conditions: (i) HMDS, $(NH_4)_2SO_4$, reflux, then TMS-triflate, CH_3CN, 60 °C; (ii) R-$SnBu_3$, $(PPh_3)_2PdCl_2$, THF, reflux; (iii) Br_2, CCl_4, rt, 30 min; (iv) NBS/NClS, pyridine, 90 °C, 2 h; (v) 1 M BCl_3/CH_2Cl_2, −40 °C to rt, 24 h.

2. Results and discussion

2.1. Synthesis

Condensation of 5,6-di-*O*-acetyl-2,3-di-*O*-benzyl-L-ascorbic acid (ABAA)[22,23] and 5-iodouracil (IU) gave 5-iodouracil-2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (IUAA)[18] which, by the Stille reaction[24,25] with various unsaturated stannanes, afforded 5-substituted uracil derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid **1**–**10** (Scheme 1).

Bromination of the 5-vinyl-L-ascorbic acid derivative (**5**) with bromine gave 5-(2-bromovinyl)-6-bromouracil-2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**11**) (Scheme 1). Halogenation of the 5-furyluracil derivative of L-ascorbic acid (**1**) with *N*-bromosuccinimide (NBS) and *N*-chlorosuccinimide (NClS) gave 5-(bro-

mofuryl)uracil (**12**) and 5-(chlorofuryl)uracil (**13**) derivatives of L-ascorbic acid, respectively (Scheme 1).

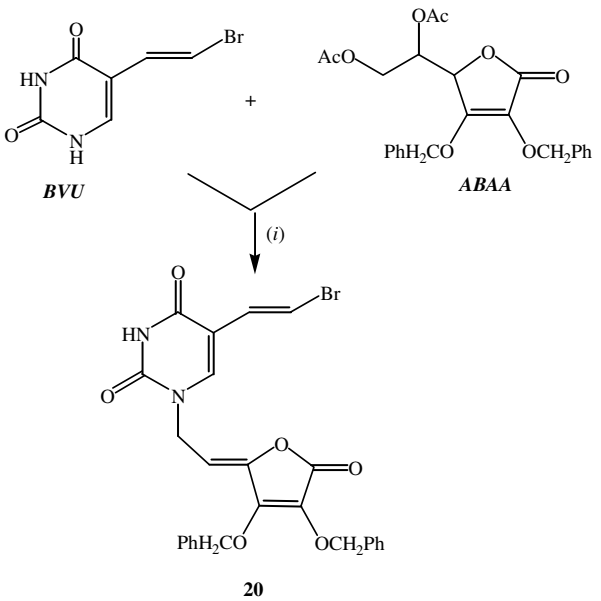
Debenzylation of the condensation products (**1**–**6**) was accomplished by treatment with solution of boron trichloride in dichloromethane affording **14**–**19** (Scheme 1).

(*E*)-5-(2-Bromovinyl)uracil (BVU) needed for the synthesis of L-ascorbic acid congener of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was prepared by a procedure given in the literature.[26] (*E*)-5-(2-Bromovinyl)uracil-2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**20**) was obtained by Vorbrüggen condensation of BVU and ABAA (Scheme 2). Silylation of 5-bromovinyluracil with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and trichloromethylsilane in the presence of ammonium sulfate, and subsequent condensation of the silylated product thus obtained with ABAA in the presence of trimethylsilyltrifluoromethanesulfonate (TMS-triflate) as Friedel–Crafts catalyst, gave **20** (Scheme 2).

2.2. Cytostatic activity

Compounds **1**–**20** were evaluated for their cytostatic activities against malignant human tumor cell lines: murine leukemia (L1210), human T-lymphocytes (Molt4/C8, CEM), cervical carcinoma (HeLa), breast carcinoma (MCF-7), pancreatic carcinoma (MiaPaCa-2), laryngeal carcinoma (Hep-2), and colon carcinoma (SW 620), as well as human fibroblast cells (WI 38) (Table 1).

Of all evaluated compounds, 5-propynyluracil derivative of 2,3-di-*O*-benzylated L-ascorbic acid (**7**) showed the best cytostatic activity against all evaluated tumor cell lines (IC_{50}: 0.2–0.78 μM) (Fig. 1). However, this compound exhibited also cytotoxic effect on normal human fibroblasts (WI 38). Moreover, 5-furyluracil-(**1**), 5-vinyluracil-(**5**), 5-ethynyluracil- (**6**), and 5-isopentenyluracil (**10**) derivatives of 2,3-di-*O*-benzylated-L-ascorbic acid and 5-(phenylethynyl)uracil-2,3-dihydroxy-L-ascorbic acid derivative (**17**) exhibited rather marked inhibitory activity against the growth of all tumor cell lines (IC_{50} within the concentration range of 1–8.3 μM), but also cytotoxic activities toward normal human fibroblasts (WI 38). However, the 5-(phenylethynyl)uracil-2,3-di-*O*-benzylated L-ascorbic acid derivative (**4**) showed slight (IC_{50}: 55–108 μM) cytostatic activity toward all tumor cell lines except for cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), laryngeal carcinoma (Hep-2), and colon carcinoma (SW 620), and no cytotoxicity to normal human fibroblast (WI 38). Introduction of a halogen in position 5 of the furan moiety of 5-bromofuryluracil- (**12**) and 5-chlorofuryluracil (**13**) derivatives of L-ascorbic acid decreases their cytostatic activities compared to the corresponding unsubstituted 5-furyluracil derivative (**1**). The 6-bromo-5′-bromo-5-(2-bromovinyl)uracil-2,3-di-*O*-benzylated L-ascorbic acid derivative (**11**) showed better inhibitory activity than its structural congener **20** without bromines at positions C-6 and C-5′. Comparison of cytostatic and cytotoxic activities of compounds **1**–**20** (Table 1) indicated low selectivity of most compounds in their cytostatic potency.



Scheme 2. The synthesis of (*E*)-5-(2-bromovinyl)uracil-2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**20**). Reagents and conditions: (i) HMDS, $(NH_4)_2SO_4$, Cl_3MeSi, reflux, then TMS-triflate, CH_3CN, 60 °C.

2.3. Antiviral activity

The compounds **1**–**20** were also evaluated against herpes simplex virus type 1 and 2, vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, Sindbis virus, Punta Toro virus, respiratory syncytial virus, parainfluenza-3 virus, and reovirus-1, and their activities were compared with those of 9-(3-hydroxyethoxymethyl)guanine (acyclovir), 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (brivudin), 9-(1,3-dihydroxypropyl)adenine [(*S*)-DHPA], and 1-(β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (ribavirin). The results presented in Tables 2 and 3 show that 2,3-di-*O*-benzylated derivative of L-ascorbic acid containing a propynyl side chain at C-5 of the uracil ring (**7**) displayed borderline activity against vesicular stomatitis virus, Coxsackie B4 virus, and Sindbis viruses (IC_{50}: 1.6 μM). Furthermore, the 5-isopentenyluracil-2,3-di-*O*-benzylated L-ascorbic acid derivative (**10**) showed moderate activity against HSV-1, HSV-2, vaccinia virus, and Punta Toro virus (IC_{50}: 4 μM) (Tables 2 and 3), while 5-phenylethynyluracil-2,3-dihydroxy-L-ascorbic acid (**17**) showed also a comparable activity against HSV-2, vaccinia virus, and vesicular stomatitis virus (IC_{50}: 4 μM) (Table 3). The 5-ethynyluracil derivative of 2,3-dihydroxy-L-ascorbic acid (**19**) showed possible activity only against vaccinia virus (IC_{50}: 4 μM) (Table 2). Determination of the cytotoxicity of **1**–**20** indicates that all compounds showed relatively low selectivity in their antiviral potencies. The antiviral activity levels were usually close to the cytotoxic activity, and therefore it is unclear whether the observed activity is due to a selective antiviral activity, or to a toxic activity against the cell cultures.

Table 1. Inhibitory effects of compounds **1**–**20** on the growth of malignant tumor cell lines and diploid fibroblasts (WI 38)

Compound	IC_{50}[a] (μM)								
	L1210	Molt4/C8	CEM	HeLa	MCF-7	MiaPaCa-2	Hep-2	SW 620	WI 38
1	6.8 ± 0.4	6.6 ± 0.2	7.0 ± 0	2 ± 0.03	2 ± 0.4	1.6 ± 0.05	2.2 ± 1	2.4 ± 0.4	1.8[b]
2	142 ± 27	149.8 ± 13.6	117 ± 9.7	18 ± 0.5	17 ± 4	16.5 ± 2.5	18 ± 0.1	21 ± 0.08	19[b]
3	142 ± 37	159.4 ± 7.9	122 ± 11.8	25 ± 8	18 ± 1	18 ± 1	22 ± 5	21 ± 2	28[b]
4	108 ± 1	108 ± 6	101 ± 11	>100	55 ± 43	>100	>100	>100	>100
5	7.2 ± 0	7.6. ± 0.2	7.2 ± 0.6	14.5 ± 0.7	3.8 ± 0.6	22 ± 0.4	14.2 ± 1.9	15.6 ± 4.6	14.3 ± 8.6
6	6.8 ± 0.6	7.2 ± 0	4.6 ± 0.2	3.2 ± 0.2	2.9 ± 1.3	8.7 ± 2.7	4.6 ± 1.2	5 ± 2	1.5 ± 0.2
7	0.47 ± 0.27	0.78 ± 0.06	0.77 ± 0.18	0.2 ± 0.12	0.2 ± 0.03	0.5 ± 0.1	0.7 ± 0.09	0.3 ± 0.2	0.2 ± 0.07
8	20 ± 1	20 ± 1	20 ± 1	2.9 ± 1.8	4.7 ± 1.3	5 ± 1	4.7 ± 1.3	2.7 ± 1.6	10.4 ± 5.5
9	>250	>250	>250	>100	77 ± 21	>100	>100	>100	>100
10	8.2 ± 0.2	8.3 ± 0.3	7.7 ± 0.2	2 ± 0.4	4 ± 2	5 ± 0.2	2.6 ± 1	4 ± 0.8	2 ± 0.3
11	32 ± 19	110 ± 8.7	91 ± 11.6	22 ± 7	15 ± 4	20 ± 2	22 ± 4	⩾100	49[b]
12	115 ± 21	125 ± 6.9	134 ± 10	24 ± 6	29 ± 6	27 ± 9	28 ± 4	49 ± 48	16[b]
13	165 ± 1.9	157.9 ± 26.3	162 ± 26	⩾100	⩾100	⩾100	95 ± 2	>100	42[b]
14	⩾500	⩾500	436 ± 90	>100	>100	>100	>100	>100	>100
15	>500	>500	>500	>100	>100	>100	>100	>100	>100
16	354 ± 205	322 ± 84	282 ± 2	>100	>100	>100	>100	>100	>100
17	8.1 ± 0.2	8.2 ± 0.1	7.6 ± 0.2	2 ± 1	1 ± 0.07	3 ± 0.5	3 ± 0.6	4 ± 3	1 ± 0.09
18	309 ± 111	309 ± 11	284 ± 75	40 ± 6	36 ± 5	⩾100	46 ± 14	>100	36[b]
19	15 ± 1	37 ± 1	23 ± 6	13 ± 5	13 ± 2	13 ± 3	12 ± 1	17 ± 3	11 ± 2
20	196 ± 54.1	⩾200	233 ± 60	45 ± 5	40 ± 15	>100	40 ± 4	>100	38[b]

[a] IC_{50}—50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Only one experiment was carried out.

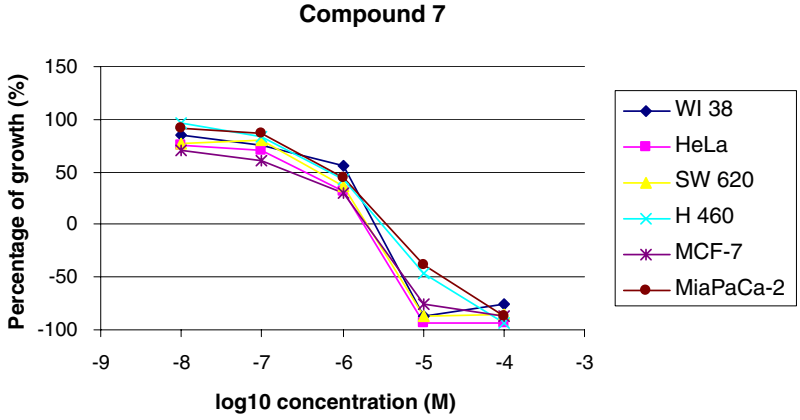


Figure 1. Dose–response profile for compound **7** tested in vitro.

3. Conclusion

The new C-5 substituted uracil L-ascorbic acid derivatives (**1**–**10**) were synthesized by palladium-catalyzed cross-coupling Stille reaction of 5-iodouracil-2,3-di-*O*-benzyl-4,5-didehydro-5,6-dideoxy-L-ascorbic acid and unsaturated aliphatic and aryl stannanes. Bromination of the 5-vinyluracil derivative (**5**) with bromine gave the tribrominated compound (**11**), while bromination of 5-furyluracil derivative (**1**) with *N*-halosuccinimide afforded corresponding monohalogenated compounds **12** and **13**. Compounds with deprotected positions 2 and 3 of the lactone ring (**14**–**19**) were obtained by debenzylation of **1**–**6** with borontrichloride. The (*E*)-5-(2-bromovinyl)uracil derivative of L-ascorbic acid (**20**) was prepared by condensation of silylated uracil base and diacetylated derivative of L-ascorbic acid. Among all evaluated compounds the 5-propynyl substituted uracil derivative of L-ascorbic acid (**7**) exhibited the most pronounced cytostatic activities against all examined tumor cell lines (IC_{50}: 0.2–0.78 μM), but it was also cytotoxic to human normal fibroblasts WI 38. The 5-propynyluracil derivative of L-ascorbic acid (**7**) had low activity against Sindbis virus, Coxsackie virus B4, and vesicular stomatitis virus (EC_{50}: 1.6 μM). Moreover, the 5-isopentenyluracil-2,3-di-*O*-benzylated- (**10**) and 5-(phenylethynyl)uracil- (**17**) and 5-ethynyluracil- (**19**) 2,3-dihydroxy-L-ascorbic acid derivatives showed borderline activity (EC_{50}: 4 μM against herpes simplex virus type 1 and 2, vaccinia virus, and Punta Toro virus).

4. Experimental

4.1. General methods

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

Table 2. Antiviral activity and cytotoxicity of compounds **1**–**20** in E_6SM and HEL[c] cell cultures

Compound	Minimum inhibitory concentration[a] (μM)					Minimum cytotoxic concentration[b] (μM)
	Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV	
1	>6.4	>6.4	>6.4	>6.4	> 6.4	32
2	>156	>156	>156	>156	>156	780
3	>157	>157	95	>157	>157	787
4	>40	>40	>40	>40	>40	200
5[c]	>18	>18	>18	>18	>18	87
6[c]	>18	>18	>18	>18	>18	⩾18
7	>0.4	>0.4	>0.4	>0.4	>0.4	2
8	>8	>8	>8	>8	>8	40
9	>8	>8	>8	>8	>8	40
10[c]	4	4	4	>4	>4	20
11	>116	>116	>116	>116	>116	578
12	>70	>70	>70	>70	>70	347
13	>150	>150	>150	>150	>150	752
14[c]	>100	>100	>100	>100	>100	>100
15[c]	>100	>100	>100	>100	>100	>100
16[c]	>100	>100	>100	>100	>100	>100
17[c]	>4	4	4	4	>4	20
18	>144	>144	>144	>144	>144	720
19[c]	>4	>4	4	>4	>4	20
20	>150	>150	>150	>150	>150	746
Brivudin	0.48	300	2.4	>500	300	>500
Ribavirin	500	300	100	>500	500	>500
Acyclovir	0.48	0.8	300	>500	60	>500
Ganciclovir	0.096	0.096	60	>100	0.8	>100

[a] Required to reduce virus-induced cytopathogenicity by 50%.
[b] Required to cause a microscopically detectable alteration of normal cell morphology.
[c] Cytotoxicity and antiviral activity in HEL cell cultures.

Table 3. Antiviral activity and cytotoxicity of compounds **1**–**20** in Vero and HeLa[c] cell cultures

Compound	Minimum inhibitory concentration[a] (μM)				Minimum cytotoxic concentration[b] (μM)
	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Vesicular stomatitis virus[c]	
1	>6.4	>6.4	>6.4	>6.4	32
2	>156	>156	>156	>156	780
3	>157	>157	>157	>157	787
4	120	>200	>200	>200	>200
5	>18	>18	>18	>87	87
6	>18	>18	>18	>18	⩾18
7	1.6	1.6	>1.6	1.6	8
8	24	24	>40	24	200
9	>200	>200	>200	>40	>200
10	>4	>4	4	>4	20
11	>116	>116	>116	>116	578
12	>70	>70	>70	70	347
13	>150	>150	>150	>150	752
14	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100
17	>4	>4	>4	>4	20
18	>29	>29	>29	120	720
19	>4	>4	4	>4	20
20	>150	>150	>150	>150	746
Brivudin	>500	>500	>500	>500	>500
(*S*)-DHPA	>500	>500	300	>500	>500
Ribavirin	100	>500	300	100	>500

[a] Required to reduce virus-induced cytopathogenicity by 50%.
[b] Required to cause a microscopically detectable alteration of normal cell morphology.
[c] Antiviral activity in HeLa cell cultures.

Precoated E. Merck Silica Gel 60F-254 plates were used for thin-layer chromatography (TLC) and for preparative chromatography, and the spots were detected under UV light (254 nm). Column chromatography (CLC) was performed using silica gel (0.063–0.2 mm) Fluka; glass column was slurry-packed under gravity. Compounds' purity was analyzed by HPLC with DAD detector. 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 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. General procedure for the preparation of 5-substituted uracil derivatives of L-ascorbic acid (1–10). To a solution of 5-iodouracil-2,3-di-*O*-benzyl-L-ascorbic acid (0.27 mmol) in tetrahydrofuran (15 mL), organostannane (2.7 mmol) and catalytic amounts of dichlorobis(triphenylphosphine)palladium (0.027 mmol) were added. The reaction mixture was refluxed for 2–20 h, then the reduced palladium was filtered off and solvent was evaporated under reduced pressure. The oily residue was twice purified by column chromatography (CH_2Cl_2/MeOH = 40:1) to yield the oil which was then additionally purified by thin-layer preparative chromatography (CH_2Cl_2/MeOH = 60:1).

4.2.1.1. 1-[5-(Furan-2-yl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (1). The procedure was carried out using 2-(tributylstannyl)furan (2.5 mL, 7.9 mmol) for 2 h which gave orange powder (216 mg, 33.2%, mp 71–73 °C). ^{1}H NMR (DMSO-d_6): δ 11.30 (s, 1H, NH), 7.91 (s, 1H, H-6), 5.48 (t, J = 6.54 Hz, 1H, H-5′), 4.51 (d, J = 6.74 Hz, 2H, H-6′), 5.31 and 5.14 (s, 2H, H-7′ and H-7″), 7.66–7.54 (m, 10H, Ph), 6.60 (d, J = 3.08 Hz, 1H, H-3″), 6.46 (dd, J = 1.70, 3.10 Hz, 1H, H-4″), 7.72 (dd, J = 1.80, 3.09 Hz, 1H, H-5″) ppm. ^{13}C NMR (DMSO-d_6): δ 163.76 (C-1′), 122.89 (C-2′), 133.41 (C-3′), 142.35 (C-4′), 67.40 (C-5′), 42.76 (C-6′), 150.82 (C-2), 159.57 (C-4), 101.25 (C-5), 145.41 (C-6), 74.04 and 73.00 (C-7′ and C-7″), 135.76 and 135.42 (C-8′ and C-8″ Ph), 131.57–128.58 (C–Ph), 148.14 (C-2″), 109.11 (C-3″), 113.23 (C-4″), 134.70 (C-5″) ppm. MS m/z 498.5 ($M^{+\cdot}$).

4.2.1.2. 1-[5-(Thiophen-2-yl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (2). The procedure was carried out using 2-(tributylstannyl)thiophene (899 mg, 2.7 mmol) for 2 h yielding brown powder (47 mg, 33.9%, mp 145–147 °C). ^{1}H NMR (DMSO-d_6): δ 11.61 (s, 1H, NH), 8.23 (s, 1H, H-6), 5.51 (t, J = 7.12 Hz, 1H, H-5′), 4.43 (d, J = 7.24 Hz, 2H, H-6′), 5.24 and 5.07 (s, 2H, H-7′ and H-7″), 7.38–7.25 (m, 10H, Ph), 7,48 (d, J = 3.08 Hz, 1H, H-3″), 6,98 (t, J = 3.10 Hz, 1H, H-4″), 7,55 (dd, J = 1.70, 3.10 Hz, 1H, H-5″) ppm. ^{13}C NMR (DMSO-d_6): δ 163.72 (C-1′), 122.76 (C-2′), 138.89 (C-3′), 142.14 (C-4′), 68.93 (C-5′), 43.39 (C-6′), 149.76 (C-2), 161.77 (C-4), 104.00 (C-5), 140.89 (C-6), 73.00 and 73.98 (C-7′ and C-7″), 135.75 and 135.39 (C-8′ and C-8″ Ph), 128.77–128.52 (C–Ph), 137.28 (C-2″), 119.32 (C-3″), 121.48 (C-4″), 126.61 (C-5″) ppm. MS m/z 514.6 ($M^{+\cdot}$).

4.2.1.3. 1-(5-Phenyluracil-1-yl)-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (3). The procedure was carried out using (tributylstannyl)benzene (734 mg, 2 mmol) for 2 days to yield colorless oil (41 mg, 31%). ^{1}H NMR (DMSO-d_6): δ 11.48 (s, 1H, NH), 7.84 (s, 1H, H-6), 5.50 (t, J = 6.12 Hz, 1H, H-5′), 4.06 (d, J = 6.81 Hz, 2H, H-6′), 5.15 and 4.92 (s, 2H, H-7′ and H-7″), 7.56–7.21 (m, 10H, Ph) ppm. ^{13}C NMR (DMSO-d_6): δ 163.71 (C-1′), 121.88 (C-2′), 142.21 (C-3′), 147.98 (C-4′), 101.22 (C-5′), 43.24 (C-6′), 149.12 (C-2), 161.66 (C-4), 106.26 (C-5), 142.91 (C-6), 73.90 and 72.86 (C-7′ and C-7″), 135.74 and 135.32 (C-8′ and C-8″ Ph), 128.76–128.54 (C–Ph), 126.51 (C-1″) ppm. MS m/z 508.5 ($M^{+\cdot}$).

4.2.1.4. 1-[5-(Phenylethynyl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (4). The procedure was carried out using 2-phenyl(tributylstannyl)ethine (938 mg, 2.4 mmol) for 24 h which gave yellow oil (62 mg, 32.4%). ^{1}H NMR (DMSO-d_6): δ 11.51 (s, 1H, NH), 8.15 (s, 1H, H-6), 5.33 (d, J = 6.92 Hz, 1H, H-5′), 4.41 (d, J = 7.12 Hz, 2H, H-6′), 5.20 and 5.08 (s, 2H, H-7′ and H-7″), 7.48–7.35 (m, 15H, Ph) ppm. ^{13}C NMR (DMSO-d_6): δ 164.12 (C-1′), 121.41 (C-2′), 137.53 (C-3′), 146.59 (C-4′), 99.17 (C-5′), 42.11 (C-6′), 152.43 (C-2), 161.31 (C-4), 100.73 (C-5), 148.13 (C-6), 73.61 and 73.18 (C-7′ and C-7″), 136.13 and 135.62 (C-8′ and C-8″ Ph), 127.16–128.56 and 131.42–132.00 (C–Ph), 88.21(C-1″), 92.63 (C-2″), 133.05 (C-3″) ppm. MS m/z 532.5 ($M^{+\cdot}$).

4.2.1.5. 1-(5-Vinyluracil-1-yl)-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (5). The procedure was carried out using (tributylstannyl)ethene (634 mg, 2 mmol) for 4 h under reflux and additionally stirred for 24 h at room temperature. Compound **5** was obtained as white powder (55 mg, 40%, mp 156–158 °C). ^{1}H NMR (DMSO-d_6): δ 11.36 (s, 1H, NH), 7.80 (s, 1H, H-6), 5.45 (t, J = 6.67 Hz, 1H, H-5′), 4.47 (d, J = 6.63 Hz, 2H, H-6′), 5.24 and 5.08 (s, 2H, H-7′ and H-7″), 7.40–7.31 (m, 10H, Ph), 6.26 (dd, J = 11.55, 6.1 Hz, 1H, H-1″), 5.45 (dd, J = 1.9, 11.5 Hz, 2H, H-2″) ppm. ^{13}C NMR (DMSO-d_6): δ 163.71 (C-1′), 122.96 (C-2′), 142.25 (C-3′), 148.08 (C-4′), 103.80 (C-5′), 43.04 (C-6′), 149.93 (C-2), 162.57 (C-4), 110.48 (C-5), 142.96 (C-6), 73.99 and 72.99 (C-7′ and C-7″), 135.76 and 135.38 (C-8′ and C-8″ Ph), 128.83–128.52 (C–Ph), 127.92 (C-1″), 113.84 (C-2″) ppm. MS m/z 458.5 ($M^{+\cdot}$).

4.2.1.6. 1-(5-Ethynyluracil-1-yl)-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (6). The procedure was carried out using (tributylstannyl)ethine (630 mg, 2 mmol) for 4 h under reflux and additionally stirred for 24 h at 50 °C. Compound **6** was isolated as yellow-brown oil (52 mg, 43.8%). ^{1}H NMR (DMSO-d_6): δ 11.56 (s, 1H, NH), 8.02 (s, 1H, H-6), 5.47 (t, J = 6.60 Hz, 1H, H-5′),

4.47 (d, J = 6.63 Hz, 2H, H-6′), 5.33 and 5.07 (s, 2H, H-7′ and H-7″), 7.37–7.25 (m, 10H, Ph), 4.03 (s, 1H, H-2″) ppm. ^{13}C NMR (DMSO-d_6): δ 169.03 (C-1′), 122.98 (C-2′), 142.27 (C-3′), 149.82 (C-4′), 103.60 (C-5′), 43.32 (C-6′), 157.85 (C-2), 163.68 (C-4), 97.03 (C-5), 149.47 (C-6), 73.98 and 72.88 (C-7′ and C-7″), 135.75 and 135.40 (C-8′ and C-8″ Ph), 128.83–127.70 (C–Ph), 83.49 (C-1″), 75.08 (C-2″) ppm. MS m/z 456.5 ($M^{+\cdot}$).

4.2.1.7. 1-(5-Propynyluracil-1-yl)-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (7). The procedure was carried out using (tributylstannyl)-1-propine (789 mg, 2.4 mmol) for 4 h yielding brown oil (55 mg, 32.5%). ^{1}H NMR (DMSO-d_6): δ 11.40 (s, 1H, NH), 8.13 (s, 1H, H-6), 5.43 (t, J = 6.64 Hz, 1H, H-5′), 4.43 (d, J = 7.01 Hz, 2H, H-6′), 5.25 and 4.98 (s, 2H, H-7′ and H-7″), 7.56–7.42 (m, 10H, Ph), 2.23 (s, 3H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): δ 164.28 (C-1′), 122.87 (C-2′), 142.13 (C-3′), 144.56 (C-4′), 101.54 (C-5′), 42.78 (C-6′), 149.77 (C-2), 162.32 (C-4), 100.11 (C-5), 143.73 (C-6), 72.55 and 72.41 (C-7′ and C-7″), 134.67 and 134.52 (C-8′ and C-8″ Ph), 128.60–127.44 (C–Ph), 82.33 (C-1″), 97.09 (C-2″), 24.65 (C-3″) ppm. MS m/z 470.5 ($M^{+\cdot}$).

4.2.1.8. 1-(5-Allyluracil-1-yl)-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (8). The procedure was carried out using (tributylstannyl)-2-propene (794 mg, 2.4 mmol) for 2 days under reflux to yield yellow oil of **8** (48 mg, 28.2%). ^{1}H NMR (DMSO-d_6): δ 11.31 (s, 1H, NH), 7.86 (s, 1H, H-6), 5.37 (t, J = 6.68 Hz, 1H, H-5′), 4.43 (d, J = 6,98 Hz, 2H, H-6′), 5.22 and 5.06 (s, 2H, H-7′ and H-7″), 7.36–7.22 (m, 10H, Ph), 2.89 (m, 2H, H-1″), 6.02 (m, 1H, H-2″), 4.71 (m, 2H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): δ 165.14 (C-1′), 123.06 (C-2′), 140.12 (C-3′), 147.22 (C-4′), 100.76 (C-5′), 43.21 (C-6′), 155.34 (C-2), 162.88 (C-4), 98.66 (C-5), 142.75 (C-6), 73.21 and 73.07 (C-7′ and C-7″), 135.74 and 135.28 (C-8′ and C-8″ Ph), 128.40-127.25 (C-Ph), 33.12 (C-1″), 126.23 (C-2″), 109.84 (C-3″) ppm. MS m/z 472.2 ($M^{+\cdot}$).

4.2.1.9. 1-(5-Alenyluracil-1-yl)-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (9). The procedure was carried out using (tributylstannyl)alene (789 mg, 2.4 mmol) for 2 days which gave orange oil of **9** (72 mg, 42.6%). ^{1}H NMR (DMSO-d_6): δ 11.43 (s, 1H, NH), 8.40 (s, 1H, H-6), 5.52 (t, J = 7,02 Hz, 1H, H-5′), 4.42 (d, J = 6,88 Hz, 2H, H-6′), 5.18 and 4.96 (s, 2H, H-7′ and H-7″), 7.55–7.31 (m, 10H, Ph), 6.06 (s, 1H, H-1″), 5.12 (s, 2H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): δ 162.55 (C-1′), 121.18 (C-2′), 143.26 (C-3′), 149.33 (C-4′), 102.08 (C-5′), 42.54 (C-6′), 150.69 (C-2), 160.22 (C-4), 106.80 (C-5), 137.83 (C-6), 74.16 and 73.09 (C-7′ and C-7″), 134.18 and 133.65 (C-8′ and C-8″ Ph), 130.75–128.14 (C–Ph), 88.33 (C-1″), 182.44 (C-2″), 81.08 (C-3″) ppm. MS m/z 470.5 ($M^{+\cdot}$).

4.2.1.10. 1-[5-(2-Isopentenyl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (10). The procedure was carried out using (tributylstannyl)-3-methyl-2-butene (862 mg, 2.4 mmol) for 48 h under reflux to yield brown oil of **10** (41 mg, 22.8%). ^{1}H NMR (DMSO-d_6): δ 11.28 (s, 1H, NH), 7.99 (s, 1H, H-6), 5.64 (t, J = 6.88 Hz, 1H, H-5′), 4.48 (d, J = 6,92 Hz, 2H, H-6′), 5.22 and 5.07 (s, 2H, H-7′ and H-7″), 7.47–7.32 (m, 10H, Ph), 2.68 (m, 1H, H-1″), 5.99 (m, 1H, H-2″), 2.21 (m, 6H, H-4″, 4‴) ppm. ^{13}C NMR (DMSO-d_6): δ 162.43 (C-1′), 122.74 (C-2′), 140.52 (C-3′), 145.66 (C-4′), 101.23 (C-5′), 43.14 (C-6′), 151.83 (C-2), 159.65 (C-4), 104.12 (C-5), 142.16 (C-6), 72.38 and 72.23 (C-7′ and C-7″), 135.96 and 135.11 (C-8′ and C-8″ Ph), 129.46–127.21 (C–Ph), 33.38 (C-1″), 119.78 (C-2″), 130.16 (C-3″), 21.26 and 23.65 (C-4″ and C-4‴) ppm. MS m/z 500.5 ($M^{+\cdot}$).

4.2.1.11. 1-[5-(2-Bromovinyl)-6-bromouracil-1-yl]-2-(2,3-di-*O*-benzyl-2-bromobutene-4-olidylidene)ethane (11). To a solution of 5-vinyluracil-2,3-di-*O*-benzyl-L-ascorbic acid **5** (210 mg, 0.46 mmol) in tetrachloromethane (15 mL), bromine (147.2 mg, 0.92 mmol) was added dropwise. Reaction mixture was stirred for 30 min at room temperature and solvent was evaporated in vacuo. The oily residue was purified by column chromatography (CH_2Cl_2/MeOH = 50:1) to yield yellow oil (91.6 mg, 28.6%). ^{1}H NMR (DMSO-d_6): δ 11.42 (s, 1H, NH), 5.11 (s, 1H, H-6′), 5.14 and 5.09 (s, 2H, H-7′ and H-7″), 7.33–7.19 (m, 10H, Ph), 7.45 (m, 2H, H-1″), 7.82 (d, J = 3.9 Hz, 1H, H-2″) ppm. ^{13}C NMR (DMSO-d_6): δ 163.18 (C-1′), 122.25 (C-2′), 142.33 (C-3′), 148.62 (C-4′), 100.87 (C-5′), 42.54 (C-6′), 148.16 (C-2), 160.11 (C-4), 109.72 (C-5), 132.24 (C-6), 73.27 and 73.16 (C-7′ and C-7″), 134.19 and 134.06 (C-8′ and C-8″ Ph), 128.52–127.26 (C–Ph), 132.11 (C-1″), 104.88 (C-2″) ppm. MS m/z 695.2 ($M^{+\cdot}$).

4.2.1.12. 1-[5-(5-Bromofuran-2-yl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (12). To a solution of 5-furyluracil-2,3-di-*O*-benzyl-L-ascorbic acid **1** (200 mg, 0.4 mmol) in pyridine (10 mL), *N*-bromosuccinimide (71.2 mg, 0.4 mmol) was added. The reaction mixture was stirred for 1 h at 90 °C. Additional amount of the *N*-bromosuccinimide (71.2 mg, 0.8 mmol) was then added and reaction was continued for next 1 h at 90 °C. The reaction mixture was stirred at room temperature overnight and solvent was then evaporated under reduced pressure. The oily residue was twice purified by column chromatography (CH_2Cl_2/MeOH = 50:1) to yield yellow oil (41.9 mg, 18.3%). ^{1}H NMR (DMSO-d_6): δ 11.68 (s, 1H, NH), 8.10 (s, 1H, H-6), 5.57 (t, J = 6.47 Hz, 1H, H-5′), 4.65 (d, J = 6.44 Hz, 2H, H-6′), 5.31 and 5.16 (s, 2H, H-7′ and H-7″), 7.43–7.31 (m, 10H, Ph), 6.83 (d, J = 3.36 Hz, 1H, H-3″), 6.62 (d, J = 3.39 Hz, 1H, H-4″) ppm. ^{13}C NMR (DMSO-d_6): δ 163.76 (C-1′), 122.89 (C-2′), 133.41 (C-3′), 142.35 (C-4′), 67.40 (C-5′), 42.76 (C-6′), 150.82 (C-2), 159.57 (C-4), 101.25 (C-5), 145.41 (C-6), 74.04 and 73.00 (C-7′ and C-7″), 135.76 and 135.42 (C-8′ and C-8″ Ph), 131.57–128.58 (C–Ph), 148.14 (C-2″), 109.11 (C-3″), 113.23 (C-4″), 134.70 (C-5″) ppm. MS m/z 577.4 ($M^{+\cdot}$).

4.2.1.13. 1-[5-(5-Chlorofuran-2-yl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (13). To a solution of 5-furyluracil-2,3-di-*O*-benzyl-L-ascorbic acid **1** (200 mg, 0.4 mmol) in pyridine (10 mL), *N*-chlorosuccinimide (104.8 mg, 0.8 mmol) was added. The reaction

mixture was stirred for 2 h at 90 °C and then continued at room temperature overnight. Solvent was evaporated and the oily residue was twice purified by column chromatography (CH_2Cl_2/MeOH = 50:1) to yield brown oil (99.4 mg, 46.7%). ^{1}H NMR (DMSO-d_6): δ 11.31 (s, 1H, NH), 8.42 (s, 1H, H-6), 5.55 (t, J = 6.70 Hz, 1H, H-5′), 4.38 (d, J = 6.72 Hz, 2H, H-6′), 5.19 and 5.06 (s, 2H, H-7′ and H-7″), 7.38–7.21 (m, 10H, Ph), 6.44 (d, J = 4.13 Hz, 1H, H-3″), 5.99 (d, J = 3.87 Hz, 1H, H-4″) ppm. ^{13}C NMR (DMSO-d_6): δ 164.23 (C-1′), 123.18 (C-2′), 135.37 (C-3′), 141.18 (C-4′), 66.92 (C-5′), 40.98 (C-6′), 151.03 (C-2), 161.12 (C-4), 103.21 (C-5), 145.22 (C-6), 74.27 and 73.66 (C-7′ and C-7″), 134.99 and 134.02 (C-8′ and C-8″ Ph), 130.43–128.27 (C–Ph), 146.07 (C-2″), 114.23 (C-3″), 108.11 (C-4″), 139.65 (C-5″) ppm. MS m/z 532.9 ($M^{+\cdot}$).

4.2.1.14. 1-[5-(Furan-2-yl)uracil-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (14). To a stirred solution of **1** (200 mg, 0.402 mmol) in anhydrous CH_2Cl_2 (10 mL), 1 M solution of BCl_3 in CH_2Cl_2 (0.5 ml) was added under argon at −78 °C. The reaction mixture was stirred at −40 °C for 2 h, and then 1 M solution of BCl_3 in CH_2Cl_2 (0.5 ml) was added. It was additionally stirred at 0 °C for 2 h, then the temperature was raised to room temperature and stirred overnight. A solvent mixture of CH_2Cl_2/MeOH (1:1) was added to deactivate unreacted BCl_3 and the solvent was then removed under reduced pressure. The oily residue was purified by column chromatography (CH_2Cl_2/MeOH = 60:1) to yield yellow oily compound **14** (57 mg, 44.6%). ^{1}H NMR (DMSO-d_6): δ 11.42 (s, 1H, NH), 8.04 (s, 1H, H-6), 5.43 (t, J = 6.55 Hz, 1H, H-5′), 4.42 (d, J = 6.67 Hz, 2H, H-6′), 6.53 (m, 2H, H-3″ and H-4″), 7.72 (d, J = 3.64 Hz, 1H, H-5″) ppm. ^{13}C NMR (DMSO-d_6): δ 164.15 (C-1′), 123.29 (C-2′), 134.72 (C-3′), 142.38 (C-4′), 69.04 (C-5′), 42.88 (C-6′), 151.11 (C-2), 160.24 (C-4), 99.39 (C-5), 144.67 (C-6), 147.59 (C-2″), 108.41 (C-3″), 111.33 (C-4″), 132.16 (C-5″) ppm. MS m/z 318.2 ($M^{+\cdot}$).

4.2.1.15. 1-[5-(Thiophen-2-yl)uracil-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (15). Compound **2** (260 mg, 0.498 mmol) was treated according to a procedure that was analogous to that for the preparation of compound **1** to give **14**. The residue was purified by column chromatography (CH_2Cl_2/MeOH = 60:1) to yield brown oily compound **15** (96.6 mg, 55.7%). ^{1}H NMR (DMSO-d_6): δ 11.39 (s, 1H, NH), 8.14 (s, 1H, H-6), 5.28 (t, J = 6.96 Hz, 1H, H-5′), 4.46 (d, J = 6.68 Hz, 2H, H-6′), 7.19 (d, J = 3.10 Hz, 1H, H-3″), 6.71 (t, J = 3.10 Hz, 1H, H-4″), 7.58 (d, 3.10 Hz, 1H, H-5″) ppm. ^{13}C NMR (DMSO-d_6): δ 163.81 (C-1′), 123.14 (C-2′), 136.57 (C-3′), 142.54 (C-4′), 70.12 (C-5′), 42.31 (C-6′), 150.22 (C-2), 161.34 (C-4), 102.53 (C-5), 140.81 (C-6), 138.33 (C-2″), 120.07 (C-3″), 124.52 (C-4″), 128.44 (C-5″) ppm. MS m/z 334.3 ($M^{+\cdot}$).

4.2.1.16. 1-[5-Phenyluracil-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (16). Compound **3** (200 mg, 0.394 mmol) was treated according to a procedure that was analogous to that for the preparation of compound **14**. The oily residue was purified by column chromatography (CH_2Cl_2/MeOH = 60:1) to yield oily compound **16** (64.3 mg, 49.4%). ^{1}H NMR (DMSO-d_6): δ 11.51 (s, 1H, NH), 7.99 (s, 1H, H-6), 5.56 (t, J = 6,88 Hz, 1H, H-5′), 4.39 (d, J = 6,82 Hz, 2H, H-6′), 7.38–7.22 (m, 5H, Ph) ppm. ^{13}C NMR (DMSO-d_6): δ 164.15 (C-1′), 121.82 (C-2′), 141.87 (C-3′), 147.33 (C-4′), 102.46 (C-5′), 43.89 (C-6′), 148.72 (C-2), 161.26 (C-4), 104.13 (C-5), 143.31 (C-6), 129.53–128.88 (C–Ph), 127.43 (C-1″) ppm. MS m/z 328.2 ($M^{+\cdot}$).

4.2.1.17. 1-[5-(Phenylethynyl)uracil-1-yl]-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (17). Compound **4** (435 mg, 0.818 mmol) was treated according to a procedure that was analogous to that for the synthesis of compound **14.** The oily residue was purified by column chromatography (CH_2Cl_2/MeOH = 60:1) to yield **17** (106.3 mg, 36.7%). ^{1}H NMR (DMSO-d_6): δ 11.72 (s, 1H, NH), 7.97 (s, 1H, H-6), 5.28 (d, J = 6.90 Hz, 1H, H-5′), 4.46 (d, J = 7.08 Hz, 2H, H-6′), 7.44–7.37 (m, 5H, Ph) ppm. ^{13}C NMR (DMSO-d_6): δ 162.12 (C-1′), 122.61 (C-2′), 137.51 (C-3′), 147.08 (C-4′), 98.23 (C-5′), 42.78 (C-6′), 152.43 (C-2), 160.01 (C-4), 102.44 (C-5), 148.39 (C-6), 131.40–132.04 (C–Ph), 90.45 (C-1″), 93.48 (C-2″), 133.56 (C-3″) ppm. MS m/z 352.3 ($M^{+\cdot}$).

4.2.1.18. 1-(5-Vinyluracil-1-yl)-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (18). Compound **5** (90 mg, 0.197 mmol) was treated by a procedure analogous to that for the preparation of compound **14**. The crude oily residue was purified by column chromatography (CH_2Cl_2/MeOH = 60:1) to yield yellow oil **18** (18.2 mg, 33.3%). ^{1}H NMR (DMSO-d_6): δ 11.81 (s, 1H, NH), 8.14 (s, 1H, H-6), 5.40 (t, J = 6,68 Hz, 1H, H-5′), 4.42 (d, J = 6.75 Hz, 2H, H-6′), 6.61 (dd, J = 11.40, 6.3 Hz, 1H, H-1″), 5.77 (dd, J = 3.2, 11.54 Hz, 2H, H-2″) ppm. ^{13}C NMR (DMSO-d_6): δ 164.52 (C-1′), 122.53 (C-2′), 143.26 (C-3′), 149.12 (C-4′), 101.55 (C-5′), 41.26 (C-6′), 151.27 (C-2), 162.34 (C-4), 108.77 (C-5), 142.97 (C-6), 126.54 (C-1″), 115.22 (C-2″) ppm. MS m/z 278.2 ($M^{+\cdot}$).

4.2.1.19. 1-(5-Ethynyluracil-1-yl)-2-(2,3-dihydroxy-2-buten-4-olidylidene)ethane (19). Compound **6** (65 mg, 0.140 mmol) was treated according to a procedure that was analogous to that for the preparation of compound **14.** The crude oily residue was purified by column chromatography (CH_2Cl_2/MeOH = 60:1) to yield **19** as brown oil (21.4 mg, 55.4%). ^{1}H NMR (DMSO-d_6): δ 11.68 (s, 1H, NH), 7.89 (s, 1H, H-6), 5.45 (t, J = 6.64 Hz, 1H, H-5′), 4.45 (d, J = 6.72 Hz, 2H, H-6′), 3.87 (s, 1H, H-2″) ppm. ^{13}C NMR (DMSO-d_6): δ 164.15 (C-1′), 122.34 (C-2′), 141.17 (C-3′), 150.02 (C-4′), 98.15 (C-5′), 43.38 (C-6′), 155.58 (C-2), 161.32 (C-4), 103.56 (C-5), 147.69 (C-6), 85.76 (C-1″), 74.38 (C-2″) ppm. MS m/z 276.2 ($M^{+\cdot}$).

4.2.1.20. 1-[5-(2-Bromovinyl)uracil-1-yl]-2-(2,3-di-*O*-benzyl-2-butene-4-olidylidene)ethane (20). The solution of anhydrous 5-(2-bromovinyl)uracil (BVU) (500 mg, 2.3 mmol) and chlorotrimethylsilane (0.05 mL) in hexamethyldisilazane (10 mL) was refluxed for 3 h under argon atmosphere. Evaporation of unreacted HMDS under reduced pressure gave oily product to which was

added 5,6-di-*O*-acetyl-2,3-di-*O*-benzyl-L-ascorbic acid (ABAA) (880 mg, 2 mmol) dissolved in anhydrous acetonitrile (10 mL). Trimethylsilyl triflate (1.7 mL) was added dropwise to the reaction mixture and it was heated at 55–70 °C for 16 h. The reaction was terminated by diluting with CH_2Cl_2 (50 mL), solvent was evaporated and oily residue purified by column chromatography (CH_2Cl_2/MeOH = 40:1) affording **20** (93.1 mg, 8.7%). ^{1}H NMR (DMSO-d_6): δ 11.06 (s, 1H, NH), 9.33 (s, 1H, H-6), 5.41 (t, J = 6.71 Hz, 1H, H-5′), 4.31 (m, 2H, H-6′), 5.16 and 5.12 (s, 2H, H-7′ and H-7″), 7.39–7.47 (m, 10H, Ph), 7.68 (d, J = 3.6 Hz, 1H, H-1″), 7.66 (d, J = 3.4 Hz, 1H, H-2″) ppm. ^{13}C NMR (DMSO-d_6): δ 175.51 (C-1′), 136.49 (C-2′), 145.87 (C-3′), 147.72 (C-4′), 75.08 (C-5′), 38.83 (C-6′), 169.45 (C-2), 172.88 (C-4), 120.41 (C-5), 130.53 (C-6), 73.52 and 72.71 (C-7′ and C-7″), 136.46 and 136.40 (C-8′ and C-8″ Ph), 127.22–131.07 (C–Ph), 134.46 (C-1″), 108.22 (C-2″) ppm. MS m/z 537.4 ($M^{+\cdot}$).

4.3. Antiviral activity assays

Antiviral activity against HSV-1, HSV-2, vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, Sindbis virus, Punta Toro virus, respiratory syncytial virus, parainfluenza-3 virus, and reovirus-1 was determined essentially as described previously.[26] 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 tested compounds. After incubation for 3 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically. Antiviral activity was expressed as the concentration required to reduce virus-induced cytopathogenicity by 50% (EC_{50}).

4.4. Cytotoxicity assays

Cytotoxicity measurements were based on the inhibition of HEL, Vero or HeLa cell growth. Cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the alteration of morphology of the cell cultures was recorded microscopically. Cytotoxicity was expressed as minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.

4.5. Antiproliferative assays

The experiments were carried out on nine human cell lines, eight of which are derived from eight cancer types and one normal fibroblast cell line. The following cell lines were used: murine leukemia (L1210), human T-lymphocytes (Molt4/C8, CEM), cervical carcinoma (HeLa), breast carcinoma (MCF-7), pancreatic carcinoma (MiaPaCa-2), laryngeal carcinoma (Hep-2), colon carcinoma (SW 620), and diploid fibroblasts (WI 38).

Murine L1210 and human Molt4/C8 and CEM cells were seeded at 50×10^3 cells/well in 96-well microtiter plates in the presence of different concentrations of the test compounds. After 2 (L1210) or 3 (Molt4/C8 and CEM) days, the cell number was determined with a Coulter counter (Coulter Electronics, England). The cytostatic concentration was determined as the compound concentration required to reduce L1210, Molt4/C8 or CEM cell growth by 50% relative to the number of cells in the untreated controls (IC_{50}). IC_{50} values were calculated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds.

The HeLa, Hep-2, MCF-7, MiaPaCa-2, SW 620, and WI 38 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. As reported previously,[17,23] the panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0 at 1×10^4 to 3×10^4 cells/mL, depending on the doubling times of specific cell line. Test agents were then added in five 10-fold dilutions (10^{-8}–10^{-4} M) and incubated for a further 72 h. Working dilutions were freshly prepared on the day of testing. The compounds were prepared as 0.04 M solutions in DMSO, and the solvent was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in the working concentrations. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay (Sigma) which detects dehydrogenase activity in viable cells. The MTT Cell Proliferation Assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. For this purpose the substance-treated medium was discarded and MTT was added to each well at a concentration of 20 μg/40 μL. After four hours of incubation, the precipitates were dissolved in 160 μL of DMSO. The absorbance (OD, optical density) was measured on a microplate reader at 570 nm. The absorbancy is directly proportional to the cell viability. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$\text{If } (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}}) \geqslant 0 \text{ then}$$

$$\text{PG} = 100 \times (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}})/(\text{mean OD}_{\text{ctrl}} - \text{mean OD}_{\text{tzero}}),$$

$$\text{If } (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}}) < 0 \text{ then}$$

$$\text{PG} = 100 \times (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}})/\text{OD}_{\text{tzero}},$$

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 two individual experiments. The results were expressed as IC_{50}, a concentration necessary for 50% inhibition, which were calculated from dose–response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the respective reference value.

Acknowledgment

Support for this study was provided by the Ministry of Science of the Republic of Croatia (Projects #0125003 and #0098093).

References and notes

1. Abell, A. D.; Ratcliffe, M. J.; Gerrard, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1703.
2. Hakimelahi, G. H.; Shia, K.-S.; Xue, C.; Hakimelahi, S.; Moosavi-Movahedi, A. A.; Saboury, A. A.; Khalafi-Nezhad, A.; Soltani-Rad, M. N.; Osyetrov, V.; Wang, K.-P.; Liao, J.-H.; Luo, F.-T. *Bioorg. Med. Chem. Lett.* **2002**, *10*, 3489.
3. Manfredini, S.; Pavan, B.; Vertuani, S.; Scaglianti, M.; Compagnone, D.; Biondi, C.; Scatturin, A.; Tanganellli, S.; Ferraro, L.; Prasad, P.; Dalpiaz, A. *J. Med. Chem.* **2002**, *45*, 559.
4. Rahim, S. G.; Duggan, M. J. H.; Walker, R. T.; Jones, A. S.; Dyler, R. L.; Balzarini, J.; De Clercq, E. *Nucleic Acid Res.* **1982**, *10*, 5285.
5. De Clercq, E. *Pure Appl. Chem.* **1983**, *55*, 623.
6. De Clercq, E. *Biochem. Pharmacol.* **1984**, *33*, 2159.
7. Goodchild, J.; Porter, R. A.; Raper, R. H.; Sim, I. S.; Upton, R. M.; Viney, J.; Wadsworth, H. J. *J. Med. Chem.* **1983**, *26*, 1252.
8. De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947.
9. Gutierrez, A. J.; Terhorst, T. J.; Matteucci, M. D.; Froehler, B. C. *J. Am. Chem. Soc.* **1994**, *116*, 5540.
10. Ostrowski, T.; Wroblowski, B.; Busson, R.; Rozenski, J.; De Clercq, E.; Bennett, M. S.; Champness, J. N.; Summers, W. C.; Sanderson, M. R.; Herdewijn, P. *J. Med. Chem.* **1998**, *41*, 4343.
11. Kumar, R.; Wiebe, L. I.; Knaus, E. E. *Can. J. Chem.* **1996**, *74*, 1609.
12. Wigerinck, P.; Pannecouque, C.; Snoeck, R.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1991**, *34*, 2383.
13. Megati, S.; Phadtare, S.; Zemlicka, J. *J. Org. Chem.* **1992**, *57*, 2320.
14. Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 1533.
15. Haines, D. R.; Tseng, C. K. H.; Marquez, V. E. *J. Med. Chem.* **1987**, *30*, 943.
16. Larsson, A.; Stenberg, K.; Ericson, A. C.; Haglund, U.; Yisak, W. A.; Johansson, N. G.; Öberg, B.; Datema, R. *Antimicrob. Agents Chemother.* **1986**, *30*, 598.
17. Gazivoda, T.; Plevnik, M.; Plavec, J.; Kraljević, S.; Kralj, M.; Pavelić, K.; Balzarini, J.; De Clercq, E.; Mintas, M.; Raić-Malić, S. *Bioorg. Med. Chem.* **2005**, *13*, 131.
18. Raić-Malić, S.; Svedružić, D.; Gazivoda, T.; Marunović, A.; Hergold-Brundić, A.; Nagl, A.; Balzarini, J.; De Clercq, E.; Mintas, M. *J. Med. Chem.* **2000**, *43*, 4806.
19. Raić-Malić, S.; Hergold-Brundić, A.; Nagl, A.; Grdiša, M.; De Clercq, E.; Mintas, M. *J. Med. Chem.* **1999**, *42*, 2673.
20. Hakimelahi, G. H.; Moosavi-Movahedi, A. A.; Sambaiah, T.; Zhu, J. L.; Ethiraj, K. S.; Pasdar, M.; Hakimelahi, S. *Eur. J. Med. Chem.* **2002**, *37*, 207.
21. Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875.
22. Wittine, K.; Gazivoda, T.; Markuš, M.; Mrvoš-Sermek, D.; Hergold-Brundić, A.; Cetina, M.; Žiher, D.; Gabelica, V.; Mintas, M.; Raić-Malić, S. *J. Mol. Struct.* **2004**, *687*, 101.
23. Gazivoda, T.; Wittine, K.; Lovrić, I.; Makuc, D.; Plavec, J.; Cetina, M.; Mrvoš-Sermek, D.; Šuman, L.; Kralj, M.; Pavelić, K.; Mintas, M.; Raić-Malić, S. *Carbohydrate Res.* **2006**, *341*, 433.
24. Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508.
25. Stille, J. K. *Pure Appl. Chem.* **1985**, *57*, 1771.
26. De Clercq, E.; Desgranges, C.; Herdewijn, P.; Sim, I. S.; Jones, A. S.; McLean, M. J.; Walker, R. T. *J. Med. Chem.* **1986**, *29*, 213.