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ORIGINAL PAPER

# 2′-Deoxy-2′-azidonucleoside analogs: synthesis and evaluation of antitumor and antimicrobial activity

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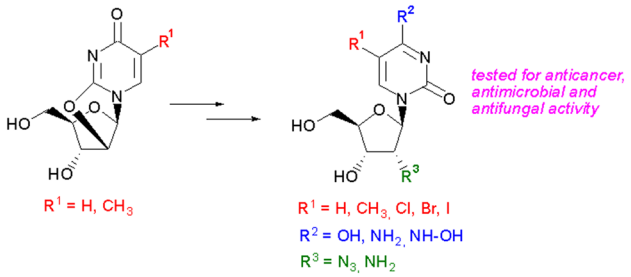
**Abstract** A series of ten pyrimidine nucleosides modified in 2′ position with azide or amine group was tested for the antibacterial, antifungal and cytotoxic activity. The cytotoxic effect was determined on three cancer (CCRF-CEM, MCF7, HeLa) and one normal (HEK293) cell lines, while antibacterial activity was evaluated on five bacterial strains. Among others, 2′-azido-2′deoxycytidine and 2′-amino-2′-deoxycytidine exhibited the strongest antiproliferative activity at 200 μM concentration, decreasing the viability of CCRF-CEM cells to 33 ± 1 and 36 ± 2%, respectively. Newly synthesized 2′-amino-2′-deoxythymidine exhibited cytotoxic effect exclusively toward HeLa cancer cell line, but not toward the normal HEK293 cells. Also, investigated compounds did not exhibit any antibacterial or antifungal activity at a concentration of 40 mM. The obtained results suggest that the presence of cytosine base is desirable for the appearance of cytotoxic effect, while the structural variations of the sugar ring play a minor role. Future modification of 2′-amino-2′-deoxythymidine could be a promising way to obtain more active anticancer substances.

**Graphical Abstract**




**Keywords** Azidonucleosides · Cytotoxicity · Antifungal · Antibacterial · CCRF-CEM · MCF7 · HeLa HEK293 cell lines

## Introduction

Nucleoside derivatives which possess the azide group (Pathak 2002) have attracted the great attention of medicinal chemists after the discovery that 3′-azido-3′-deoxythymidine (zidovudine, **1**, Scheme 1) could act as an efficient HIV reverse transcriptase inhibitor and could be applied to the treatment of acquired immune deficiency syndrome (AIDS) (Mitsuya et al. 1985) (Fig. 1). The utility and high potential of azide-modified nucleoside analogs were further supported by the discovery of 4′-azidocytidine (**2**, R1479), a relatively potent ($IC_{50}$ = 320 nM) inhibitor of RNA synthesis mediated by NS5B, the RNA polymerase encoded by HCV (Smith et al. 2007). Earlier reports have also revealed the great potential of 2-azido-2′-deoxy-3-(β-D-arabinofuranosyl) cytosine (**3**, Cytarazid) against various human tumor cell lines (Bobek et al. 1978; Cheng et al. 1981; Matsuda et al. 1991) and HSV virus strains (Cheng et al. 1981). It was

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first designed as cytidine deaminase-resistant analog of the anticancer compound—3-(β-D-arabinofuranosyl)cytosine (AraC).**Scheme 1** Synthesis of 5-halogeno-2′-deoxy-2′-azidouridine derivatives **8a–c**

In the pyrimidine 2′-azido-2′-deoxyribonucleoside series, only the unmodified 2′-azido-2′-deoxyuridine (**4**) and 2′-azido-2′-deoxycytidine (**5**) are vastly described in the literature, ignoring the remaining representatives of the class. Reported data are greatly focused on interactions of compounds **4** and **5** with different types of cellular enzymes. Because of the lack of the 2′-hydroxy group, pyrimidine 2′-azido-2′-deoxynucleosides are recognized and phosphorylated to the appropriate monophosphates by human deoxyribonucleoside salvage enzymes, such as deoxycytidine kinase (dCK) (Iwata et al. 1979; Wanf et al. 1998; Kierdaszuk et al. 1998, 1999). The diphosphate of 2′-azido-2′-deoxycytidine (**5**) exhibits strong inhibition effect on ribonucleotide reductase, a pivotal enzyme involved in the DNA synthesis, which transforms ribonucleosides into their 2′-deoxyribonucleoside analogs (Akerblom 1985; Wnuk et al. 2002; Roy et al. 2004). Additionally, in its 5′-triphosphate form, it also inhibited the action of primase in a reconstructed *Escherichia coli* enzyme system (Reichard et al. 1978). During investigation of structural requirements and determinants for nucleoside affinity to the proteins responsible for the nucleoside transport through cell membranes, 2′-azido-2′-deoxyuridine (**4**) exhibited rather high affinity to the human concentrative nucleoside transporters (hCNTs) (hCNT1 $K_i$ [μM] = 11.5 ± 0.5, hCNT3 $K_i$ [μM] = 33.3 ± 2.0, hCNT2 $K_i$ [μM] > 3000) (Zhang et al. 2003, 2005) and human equilibrative nucleoside transporters (hENTs) (hENT1 $K_i$ [μM] = 13 ± 1, hENT1 $K_i$ [μM] = 169 ± 14) (Vickers et al. 2004), investigated in the yeast expression system. 2′-Azido-2′-deoxyuridine (**4**) and 2′-azido-2′-deoxycytidine (**5**) were both tested in studies determining the effects of modifications in the pentose moiety and conformational changes on the binding of nucleoside ligands to uridine phosphorylase (UrdPase) from *Toxoplasma gondii* (el Kouni et al. 1996). While 2′-azido-2′-deoxycytidine (**5**) did not exhibit any significant binding activity, 2′-azido-2′-deoxyuridine (**4**) exhibited strong binding effect to this enzyme, with inhibitory potency of 82.1 ± 28.5 μM (3.42 times greater potency than the reference compound—β-D-uridine). Among the tested compounds, 2′-azido-2′-deoxycytidine (**5**) was also the most active one ($IC_{50}$ = 0.05 ± 0.01 μM, SI = > 2030 ± 1347) against a highly pathogenic avian H5N1 influenza A virus strain (Kumaki et al. 2011), Vietnam/1203/2004. Also, it inhibited polyoma virus DNA replication (Bjursel 1977, 1978; Eliasson et al. 1981). In another report, this compound exhibited significant inhibitory effect against leukemic L1210 cell line, rather weak antiviral activity against vaccinia virus, and lack of significant activity against HSV (Herpes Simples Virus) and VSV (vesicular Stomatitis Virus) virus strains (de Clercq et al. 1980).

Although 2′-azido-2′-deoxycytidine (**5**) does not exhibit any significant antiretroviral activity, because of its ribonucleotide reductase-inhibition activity, it can be used as a potentiator of anti-HIV drugs, such as AZT (Giacca et al. 1994, 1996). Cytidine deaminase, an enzyme responsible for deactivation of cytidine-derived anticancer drugs, e.g., cytarabine, by limiting their bioavailability and half-life time, is also strongly inhibited by azidonucleoside **5** (Cacciamani et al. 1991). Consequently, due to its cytidine deaminase-inhibition activity, compound **5** could extend the bioavailability and half-life time and decrease therapeutic doses and side effects of several anticancer drugs. Polynucleotides possessing 2′-azido-2′-deoxyuridine (**4**) were also investigated as possible interferon inducers (Torrence et al. 1973a, b).

In our ongoing quest for the design and synthesis of new medicinally relevant nucleoside analogs and heterocyclic derivatives this reference should be: Mieczkowski et al. 2015, 2016a, b), we envisioned the possibility of synthesizing novel, 2′-azido modified pyrimidine nucleosides. Because of the lack of synthetic and biological data corresponding to the modified pyrimidine 2′-azido-2′-deoxyribonucleosides in literature, we decided to synthesize novel analogs of 2′-azido-2′-deoxyuridine (**4**) and 2′-azido-2′-deoxycytidine (**5**) and compared their biological

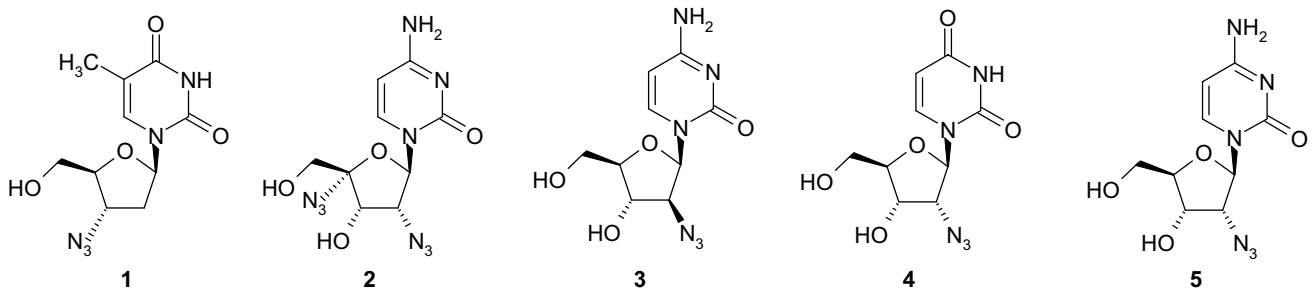



**Fig. 1** Representative, medicinally relevant pyrimidine azidonucleosides: **1** zidovudine (anti-HIV), **2** R1479 (anti-HCV), **3** Cytarazid (anticancer, anti-HSV), **4** 2′-azido-2′-deoxyuridine, **5** 2′-azido-2′-deoxycytidine (antiviral, anticancer)

activities against cancer lines, bacteria and fungi with parent compounds. For comparative studies and investigation of structure–activity relationships, we also used three 2′-amino-2′-deoxy pyrimidine nucleoside analogs.

## Experimental

Commercially available chemicals were of reagent grade purity and used as received. 2,2′-Anhydrouridine, 2,2′-anhydrothymidine (**12**), 2′-azido-2′-deoxycytidine (**3**), 2′-amino-2′-deoxyuridine (**15**) and 2′-amino-2′-deoxycytidine (**16**) were purchased from Carbosynth (UK). The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel $60F_{254}$, E. Merck). Column chromatography was performed on silica gel 60 M (0.040–0.063 mm, E. Merck). Melting points were uncorrected and measured on a Kofler apparatus. The $^{1}H$ and $^{13}C$ NMR spectra were recorded at the Department of Chemistry, Warsaw University, using Varian Unity Plus spectrometer (500 MHz) and Bruker AVANCE III HD (300 MHz) spectrometer, in $CDCl_3$ and DMSO-$d_6$, with shift values in parts per million relative to $SiMe_4$ as internal reference. The resonance assignments are based on peak integration, peak multiplicity and 2D correlation experiments. Multiplets were assigned as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dt (doublet of triplet), ddd (doublet of doublet of doublet), m (multiplet) and bs (broad singlet). High-resolution mass spectra were performed by the Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics PAS, on LTQ Orbitrap Velos, Thermo Scientific. The antibacterial and antifungal activity of compounds was assayed using standardized disc diffusion agar test. The disc diffusion method was carried out according to the standard method (Bauer et al. 1966). Clinically antimicrobial drugs, kanamycin and amphotericin B, were used as reference drugs and DMSO was used as a negative control. The results were recorded as the average diameter of growth inhibition zones, and for the reference drugs a minimum 20 mm of bacterial or fungal growth around the discs was inhibited. For the tested compounds, there were no growth inhibition zones observed.

### 2′-Azido-5-bromo-2′-deoxy-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (7a)

1.00 g (2.0 mmol, 1 eq.) of 2′-azido-2′-deoxy-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (**6**) was dissolved in 50 ml of dichloromethane and 0.88 g (4.0 mmol, 2 eq.) of silver trifluoroacetate followed by addition of 0.31 ml (6.0 mmol, 3 eq.) of bromine, and the reaction mixture was stirred at RT for 18 h. The inorganic silver salts were separated by filtration through Celite, and the organic phase was washed twice with a saturated solution of sodium thiosulfate and once with brine. The organic phase was dried over magnesium sulfate, and the volatiles were evaporated under reduced pressure. The solid residue was purified by column chromatography on silica gel using toluene:ethyl acetate, 9:1–8:2. Yield: 0.32 g (28%). Compound **7a** was obtained as white crystals, m.p.: 78.4–78.9 °C. $^{1}H$ NMR (500 MHz, $CDCl_3$): 8.96 (bs, 1H, NH), 7.99 (s, 1H, $H_6$), 6.24 (d, 1H, $^{3}J = 7.0$ Hz, 1H, $H_{1'}$), 4.35 (dd, $^{3}J = 2.0$, 5.0 Hz, 1H, $H_{3'}$), 4.10 (q, $^{3}J = 2.0$ Hz, 1H, $H_{4'}$), 3.96 (dd, $^{3}J = 2.0$ Hz, $^{2}J = 12.0$ Hz, 1H, $H_{5'b}$), 3.77 (dd, $^{3}J = 2.0$ Hz, $^{3}J = 12.0$ Hz, 1H, $H_{5'a}$), 3.51 (dd, $^{3}J = 5.0$, 7.0 Hz, 1H, $H_{2'}$), 0.95 (s, 18H, 2 × *t*-$Bu_{Si}$), 0.19 (s, 3H, $Me_{Si}$), 0.17 (s, 3H, $Me_{Si}$), 0.16 (s, 3H, $Me_{Si}$), 0.14 (s, 3H, $Me_{Si}$); $^{13}C$ NMR (125 MHz, $CDCl_3$): 158.6, 149.5, 138.5, 97.7, 86.9, 85.8, 73.5, 65.6, 62.6, 26.1, 25.7, 18.5, 18.1, − 4.7, − 5.0, − 5.2, − 5.3. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_{21}H_{39}BrN_5O_5Si_2$: 576.16676, found: 576.16672.

### 2′-azido-2′-deoxy-5-iodo-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (7b)

1.00 g (2.0 mmol, 1 eq.) of 2′-azido-2′-deoxy-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (**6**) was dissolved in 50 ml of dichloromethane and 0.88 g (4.0 mmol, 2 eq.) of silver trifluoroacetate, followed by the addition of 1.52 g (6.0 mmol, 3 eq.) of iodine, and reaction mixture was stirred at RT for 18 h. The inorganic silver salts were separated by filtration through Celite, and the organic phase was washed twice with a saturated solution of sodium thiosulfate and once with brine. The organic phase was dried over magnesium sulfate, and the volatiles were evaporated under reduced pressure. The solid residue was purified by column chromatography on silica gel using toluene:ethyl acetate, 9:1 then 8:2. Yield: 0.96 g (72%). Compound **7b** was obtained as white crystals, m.p.: 150.0–150.3 °C. $^{1}H$ NMR (500 MHz, $CDCl_3$): 9.15 (s, 1H, NH), 8.00 (s, 1H, $H_6$), 6.24 (d, 1H, $^{3}J = 7.5$ Hz, 1H, $H_{1'}$), 4.36 (dd, $^{3}J = 2.0$, 5.0 Hz, 1H, $H_{3'}$), 4.10 (q, $^{3}J = 2.0$ Hz, 1H, $H_{4'}$), 3.93 (dd, $^{3}J = 2.0$ Hz, $^{2}J = 11.5$ Hz, 1H, $H_{5'b}$), 3.76 (dd, $^{3}J = 2.0$ Hz, $^{2}J = 11.5$ Hz, 1H, $H_{5'a}$), 3.49 (dd, $^{3}J = 5.0$, 7.5 Hz, 1H, $H_{2'}$), 0.96 (s, 9H, *t*-$Bu_{Si}$), 0.95 (s, 9H, *t*-$Bu_{Si}$), 0.19 (s, 3H, $Me_{Si}$), 0.18 (s, 3H, $Me_{Si}$), 0.17 (s, 3H, $Me_{Si}$), 0.14 (s, 3H, $Me_{Si}$); $^{13}C$ NMR (125 MHz, $CDCl_3$): 159.8, 150.0, 143.5, 87.0, 85.6, 73.7, 69.5, 65.4, 62.7, 26.3, 25.8, 18.6, 18.1, − 4.7, − 4.9, − 5.1, − 5.2. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_{21}H_{39}IN_5O_5Si_2$: 624.15289, found: 624.15229.

### 2′-Azido-2′-deoxy-$O^4$-(2,4,6-triisopropylbenzenesulfonyl)-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (9)

To the solution of 0.50 g (1.0 mmol, 1 eq.) of 2′-azido-2′-deoxy-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (**6**) dissolved in 30 ml of dry dichloromethane, 0.91 g (3.0 mmol, 3 eq.) of TPSCl, 25 mg (0.2 mmol, 0.2 eq.) of DMAP and 0.41 ml

of TEA were added, and the reaction mixture was stirred at RT for 18 h. The obtained solution was then evaporated with silica gel and chromatographed using 100% toluene and then toluene:ethyl acetate 9:1. Yield: 0.56 g (73%) of colorless oil. $^1$H NMR (500 MHz, $CDCl_3$): 8.48 (d, 1H, $^3J$ = 7.5 Hz, $H_6$), 7.26 (s, 1H, $H_{Ar}$), 7.21 (s, 1H, $H_{Ar}$), 6.02 (d, 1H, $^3J$ = 7.5 Hz, $H_5$), 5.76 (d, 1H, $^3J$ = 1.5 Hz, 1H, $H_{1'}$), 4.31 (dd, $^3J$ = 5.0, 7.0 Hz, 1H, $H_{3'}$), 4.25 (septet, 2H, $^3J$ = 7.0 Hz, 2 × *i*-Pr), 4.06 (m, 2H, $H_{4'}$ + $H_{5'b}$), 3.93 (dd, $^3J$ = 1.5, 5.0 Hz, 1H, $H_{2'}$), 3.76 (m, 1H, $H_{5'a}$), 2.91 (septet, 1H, $^3J$ = 7.0 Hz, *i*-Pr), 1.33 (d, 6H, $^3J$ = 7.0 Hz, *i*-Pr), 1.27 (d, 6H, $^3J$ = 7.0 Hz, *i*-Pr), 1.26 (d, 6H, $^3J$ = 7.0 Hz, *i*-Pr), 0.94 (s, 9H, *t*-$Bu_{si}$), 0.91 (s, 9H, *t*-$Bu_{si}$), 0.13 (s, 3H, $Me_{Si}$), 0.12 (s, 3H, $Me_{Si}$), 0.11 (s, 3H, $Me_{Si}$), 0.10 (s, 3H, $Me_{Si}$); $^{13}$C NMR (125 MHz, $CDCl_3$): 167.3, 154.6, 153.8, 151.2, 145.6, 130.6, 124.1, 95.0, 89.2, 83.9, 69.1, 66.6, 60.2, 34.3, 29.7, 26.0, 25.6, 24.7, 24.4, 23.5, 23.5, 18.4, 18.0, − 4.4, − 5.0, − 5.3, − 5.6. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_{36}H_{62}N_5O_7SSi_2$: 764.39030, found: 764.38988.

### 2′-Azido-2′-deoxy-4-oxime-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (10)

A solution of 0.80 g of **9** (1.05 mmol, 1 eq.) dissolved in 10 ml of THF was treated with 0.62 ml of 50% hydrazine solution in water (10.50 mmol, 10 eq.) and the reaction mixture was stirred at RT for 1 h. The volatiles were evaporated under reduced pressure, followed by co-evaporation with 20 ml of toluene (three times). The oily residue was chromatographed on silica gel using toluene:ethyl acetate 8:2. Yield: 0.43 g (80%) of colorless oil. $^1$H NMR (500 MHz, $CDCl_3$): 8.83 (bs, 1H, NH), 7.10 (d, $^3J$ = 8.5 Hz, 1H, $H_6$), 6.11 (d, 1H, $^3J$ = 5.5 Hz, 1H, $H_{1'}$), 5.65 (d, $^3J$ = 8.5 Hz, 1H, $H_5$), 4.35 (dd, $^3J$ = 3.5, 5.5 Hz, 1H, $H_{3'}$), 4.02 (m, 1H, $H_{4'}$), 3.90 (dd, $^3J$ = 2.5 Hz, $^2J$ = 11.5 Hz, 1H, $H_{5'b}$), 3.73 (dd, $^3J$ = 1.5 Hz, $^2J$ = 11.5 Hz, 1H, $H_{5'a}$), 3.59 (t, $^3J$ = 5.5 Hz, 1H, $H_{2'}$), 0.94 (s, 9H, *t*-$Bu_{Si}$), 0.92 (s, 9H, *t*-$Bu_{Si}$), 0.17 (s, 3H, $Me_{Si}$), 0.13 (s, 3H, $Me_{Si}$), 0.10 (s, 6H, 2x$Me_{Si}$); $^{13}$C NMR (125 MHz, $CDCl_3$): 149.4, 145.1, 129.9, 98.7, 85.6, 85.4, 72.7, 65.0, 62.3, 25.9, 25.7, 18.4, 18.1, − 4.7, − 5.0, − 5.5, − 5.6. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_{21}H_{41}N_6O_5Si_2$: 513.26715, found: 513.26632.

### General procedure for silyl group removal

3′,5′-*O*-di(*t*-butyldimethylsilyl)nucleosides **7a–b** and **10** (1 eq) were dissolved in THF (10 ml per 1 mmol of nucleoside). Then, the TBAF hydrate (3 eq) was added and the reaction mixture was stirred at RT for 4 h. After evaporation of the solvent and co-evaporation with toluene (20 ml, three times), the residue was dissolved in methanol, evaporated with silica gel and chromatographed using 5% methanol in chloroform.

2′-Azido-5-bromo-2′-deoxyuridine (**8a**) yield: 93%, m.p.: 116.9–117.3 °C, white crystals. $^1$H NMR (300 MHz, DMSO-$d_6$): 11.89 (s, 1H, NH), 8.44 (s, 1H, $H_6$), 5.93 (d, $^3J$ = 5.7 Hz, 1H, $OH_{3'}$), 5.77 (d, $^3J$ = 4.5 Hz, 1H, $H_{1'}$), 5.36 (t, $^3J$ = 4.8 Hz, 1H, $OH_{5'}$), 4.33 (q, $^3J$ = 5.7 Hz, 1H, $H_{3'}$), 4.17 (dd, $^3J$ = 4.5, 5.7 Hz, 1H, $H_{2'}$), 3.89 (dt, $^3J$ = 2.7, 5.7 Hz, 1H, $H_{4'}$), 3.73 (ddd, $^3J$ = 2.7, 4.8 Hz, $^2J$ = 12.3 Hz, 1H, $H_{5'b}$), 3.59 (ddd, $^3J$ = 2.7, 4.8 Hz, $^2J$ = 12.3 Hz, 1H, $H_{5'a}$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 159.2, 149.7, 139.8, 96.0, 86.4, 84.9, 69.6, 65.2, 59.4. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_9H_{11}BrN_5O_5$: 347.99381, found: 347.99561.

2′-Azido-5-iodo-2′-deoxyuridine (**8b**) yield: 64%, m.p.: 146.0–146.3 °C, white crystals. $^1$H NMR (500 MHz, DMSO-$d_6$): 11.76 (s, 1H, NH), 8.45 (s, 1H, $H_6$), 5.93 (d, $^3J$ = 5.5 Hz, 1H, $OH_{3'}$), 5.77 (d, $^3J$ = 4.5 Hz, 1H, $H_{1'}$), 5.34 (t, $^3J$ = 5.0 Hz, 1H, $OH_{5'}$), 4.33 (q, $^3J$ = 5.5 Hz, 1H, $H_{3'}$), 4.16 (dd, $^3J$ = 4.5, 5.5 Hz, 1H, $H_{2'}$), 3.90 (ddd, $^3J$ = 2.5, 3.0, 5.5 Hz, 1H, $H_{4'}$), 3.72 (ddd, $^3J$ = 3.0, 4.5 Hz, $^2J$ = 12.0 Hz, 1H, $H_{5'b}$), 3.59 (ddd, $^3J$ = 2.5, 4.5 Hz, $^2J$ = 12.0 Hz, 1H, $H_{5'a}$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 160.5, 150.1, 144.5, 86.3, 84.9, 79.2, 69.7, 69.6, 65.2, 59.5. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_9H_{11}IN_5O_5$: 395.97994, found: 395.98029.

2′-Azido-2′-deoxy-4-oxime-uridine (**11**) yield: 55%, m.p.: 192.2–192.4 °C, white crystals. $^1$H NMR (500 MHz, DMSO-$d_6$): 10.05 (s, 1H, OH), 9.66 (d, $^4J$(NH-$H_5$) = 2.0 Hz, 1H, NH), 7.03 (d, $^3J$ = 8.5 Hz, 1H, $H_6$), 5.93 (m, 1H, $OH_{3'}$ + $H_{1'}$), 5.61 (dd, $^4J$(NH-$H_5$) = 2.0 Hz, $^3J$ = 8.5 Hz, 1H, $H_5$), 5.09 (t, *J* = 5.0 Hz, 1H, $OH_{5'}$), 4.25 (dt, $^3J$ = 3.5, 5.0 Hz, 1H, $H_{3'}$), 3.88 (dd, $^3J$ = 5.0, 7.0 Hz, 1H, $H_{2'}$), 3.85 (q, $^3J$ = 3.5 1H, $H_{4'}$), 3.55 (m, 2H, $H_{5'a}$ + $H_{5'b}$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 149.3, 143.1, 129.2, 99.1, 85.3, 84.3, 71.2, 63.2, 60.9. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_9H_{13}N_6O_5$: 285.09419, found: 285.09299.

### The synthesis of 2′-azido-5-chloro-2′-deoxyuridine (8c)

The solution of 0.38 g of **4** (0.68 mmol, 1 eq.) dissolved in 5 ml of dry THF was treated with 0.1 ml of sulfuryl chloride and the reaction mixture was stirred overnight at RT. The volatiles were evaporated under reduced pressure, and the oily residue was chromatographed on the silica gel using 5% methanol in chloroform. Yield: 85 mg (41%) of colorless oil. $^1$H NMR (300 MHz, DMSO-$d_6$): 11.92 (s, 1H, NH), 8.36 (s, 1H, $H_6$), 5.93 (d, $^3J$ = 5.4 Hz, 1H, $OH_{3'}$), 5.77 (d, $^3J$ = 4.5 Hz, 1H, $H_{1'}$), 5.36 (t, $^3J$ = 4.5 Hz, 1H, $OH_{5'}$), 4.33 (q, $^3J$ = 5.4 Hz, 1H, $H_{3'}$), 4.15 (dd, $^3J$ = 4.5, 5.4 Hz, 1H, $H_{2'}$), 3.89 (dt, $^3J$ = 2.7, 5.4 Hz, 1H, $H_{4'}$), 3.73 (ddd, $^3J$ = 2.7, 4.5 Hz, $^2J$ = 12.3 Hz, 1H, $H_{5'b}$), 3.60 (ddd, $^3J$ = 2.7, 4.2 Hz, $^2J$ = 12.3 Hz, 1H, $H_{5'a}$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 159.1, 149.6, 137.4, 107.4, 86.5, 85.0, 69.6, 65.2, 59.5. HRMS (ESI): *m*/*z* $[M+H]^+$ calculated for $C_9H_{11}ClN_5O_5$: 304.04432, found: 304.04433.

### 2′-Azido-2′-deoxy-5-methyluridine (13)

The solution of 3.00 g of 2,2′-anhydrothymidine (**12**, 12.49 mmol, 1 eq) dissolved in 100 ml of dry DMF was treated with 0.58 g (22.48 mmol, 1.8 eq) of lithium fluoride, 2.98 ml of azidotrimethylsilane (22.48 mmol, 1.8 eq) and 3.37 ml of TMEDA (22.48 mmol, 1.8 eq). The reaction mixture was stirred for 48 h at 110–115 °C. The volatiles were evaporated under reduced pressure and the oily residue was chromatographed on the silica gel using 10% methanol in chloroform. Yield: 1.59 g (45%) of colorless oil. $^{1}$H NMR (500 MHz, DMSO-$d_6$): 11.40 (s, 1H, NH), 7.72 (q, $^{4}J$(Me-$H_6$) = 1.0 Hz, 1H, $H_6$), 5.95 (d, $^{3}J$ = 5.5 Hz, 1H, $OH_{3'}$), 5.90 (d, $^{3}J$ = 5.5 Hz, 1H, $H_{1'}$), 5.20 (t, $^{3}J$ = 5.0 Hz, 1H, $OH_{5'}$), 4.31 (m, 1H, $H_{3'}$), 4.04 (t, $^{3}J$ = 5.5 Hz, 1H, $H_{2'}$), 3.89 (m, 1H, $H_{4'}$), 3.68 (ddd, $^{3}J$ = 3.5, 5.0 Hz, $^{2}J$ = 12.0 Hz, 1H, $H_{5'b}$), 3.58 (ddd, $^{3}J$ = 3.5, 5.0 Hz, $^{2}J$ = 12.0 Hz, 1H, $H_{5'a}$), 1.78 (d, $^{4}J$ (Me-$H_6$) = 1.0 Hz, 1H, Me). $^{13}$C NMR (125 MHz, DMSO-$d_6$): 163.7, 150.5, 135.7, 109.7, 85.3, 85.2, 70.6, 64.3, 60.4, 12.3. HRMS (ESI): *m*/*z* [M+H]$^+$ calculated for $C_{10}H_{14}N_5O_5$: 284.09895, found: 284.09914.

### 2′-Amino-2′-deoxy-5-methyluridine (14)

1.00 g (3.53 mmol) of 2′-azido-2′-deoxy-5-methyluridine (**13**) was dissolved in 40 ml of isopropyl alcohol, and 100 mg of 10% palladium on charcoal was added. The reaction vessel was connected to a balloon filled with hydrogen and the reaction mixture was stirred at room temperature for 20 h. The resultant solution was filtered through Celite and evaporated giving 772.0 mg (85%) of **14** as a colorless oil. $^{1}$H NMR (500 MHz, DMSO-$d_6$): 7.67 (q, $^{4}J$(Me-$H_6$) = 0.5 Hz, 1H, $H_6$), 5.66 (d, $^{3}J$ = 8.0 Hz, 1H, $H_{1'}$), 3.90 (dd, $^{3}J$ = 1.5, 5.5 Hz, 1H, $H_{3'}$), 3.89 (dd, $^{3}J$ = 1.5, 3.5 Hz, 1H, $H_{4'}$), 3.56 (m, 2H, $H_{5'}$), 3.30 (dd, $^{3}J$ = 5.5, 8.0 Hz, 1H, $H_{2'}$), 1.78 (d, $^{4}J$ (Me-$H_6$) = 0.5 Hz, 1H, Me). Acidic protons: NH, OH, $NH_2$ not present in 1H-NMR spectra. $^{13}$C NMR (125 MHz, DMSO-$d_6$): 163.8, 152.2, 136.5, 109.4, 87.7, 85.9, 71.3, 61.7, 57.2, 12.3. HRMS (ESI): *m*/*z* [M+H]$^+$ calculated for $C_{10}H_{16}N_3O_5$: 258.10845, found: 258.10874.

### Cell culture and treatment

MCF7 adherent cells (human breast cancer cell line) were cultured in DMEM medium (Lonza) supplemented with 10% fetal bovine serum (Biowest), 2 mM L-glutamine, antibiotics (100 U/ml penicillin, 100 µg/mL streptomycin) and 10 µg/mL of human recombinant insulin (Sigma-Aldrich). CCRF-CEM suspension cells (human peripheral blood T lymphoblast cell line) were cultured in RPMI medium (Gibco) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). Cells were grown in 75 cm$^2$ cell culture flasks (Sarstedt) in a humidified atmosphere of $CO_2$/air (5/95%) at 37 °C. Cervical cancer cells HeLa and human embryonic kidney cells HEK293 were cultured in DMEM medium (Life Technology) supplemented with 10% fetal bovine serum (Life Technology) and 0.1% antibiotics (penicillin, streptomycin, Life Technology). Cells were grown in a humidified atmosphere of $CO_2$/air (5/95%) at 37 °C.

### MTT-based cytotoxicity and alamarBlue® cell viability assay

Before the treatment, MCF7 cells were trypsinized in 0.25% trypsin–EDTA solution (Sigma-Aldrich) and seeded into 96-well microplates at a density of 1.5 × 10$^4$ cells/well. Cells were treated with specific compounds dissolved in DMSO or DMSO (0.5%) at the corresponding concentrations, 18 h after plating (at 70% of confluency). CCRF-CEM were seeded at 10$^4$ cells/well and treated with the tested compounds. MTT stock solution (Sigma-Aldrich) was added to each well to a final concentration of 0.3 mg/mL. After 2 h of incubation at 37 °C, water-insoluble dark blue formazan crystals were dissolved in DMSO (200 µL) (37 °C/10 min incubation), and Sorensen's glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added (25 µL per well). Optical absorbance was measured at 570 nm using Synergy H4, BioTek microplate reader. All measurements were carried out in triplicate and the results are expressed in percentage of cell viability relative to control (cells without inhibitor in 0.5% DMSO). HeLa and HEK293 cells were seeded in 96-well microplates at a density of 7 × 10$^3$ cells/well. After 18 h, cells were treated with test compounds dissolved in DMSO at the corresponding concentrations for 24 h. Then, 10% Alamar Blue (Invitrogen) was added according to the manufacturer's protocol. After 4 h incubation, emission at 585 nm was measured with excitation at 570 nm, using a scanning multi-well spectrophotometer, DTX 880.

## Results and discussion

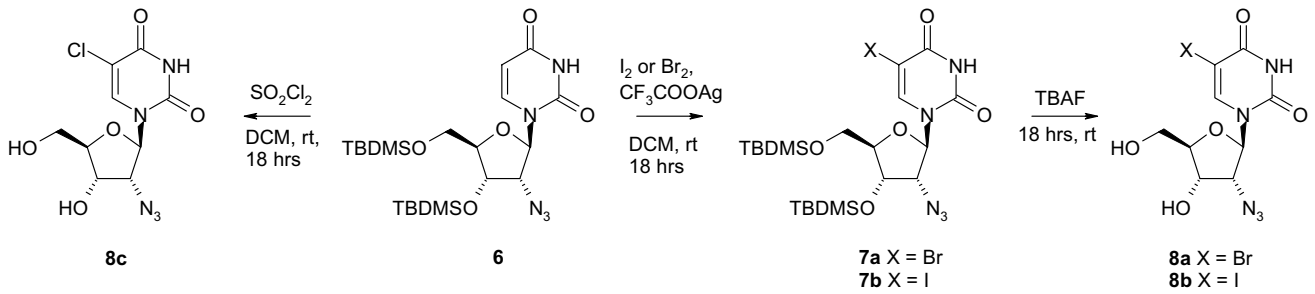
The starting point of our synthesis was the 2′-azido-2′-deoxy-3′,5′-*O*-di(*t*-butyldimethylsilyl)uridine (**6**) prepared according to the reported procedure (Gai et al. 2010). Silyl protected nucleoside **6** was then brominated in the C5 position using the $Br_2$/$CF_3COOAg$ system, which resulted in the formation of the bromo derivative **7a** with 28% yield. In a similar manner, derivative **6** was iodinated in the C5 position with $I_2$/$CF_3COOAg$ (Kobayasji et al. 1980), giving the iodo derivative **7b** with 72% yield (Scheme 1). Alternatively,

iodine monochloride ICl could be used for the synthesis of **7b** (Gold et al. 1995). Silyl intermediates **7a–b** were then deprotected with the TBAF hydrate in THF to the final nucleosides **8a** and **8b** with 93 and 64% yield, respectively. The treatment of protected nucleoside **6** with sulfuryl chloride in DCM resulted in the formation of 5-chloro derivative with concurrent cleavage of the silyl groups. The deprotected 3-chloro nucleoside **8c** was isolated as a sole product with 41% yield.

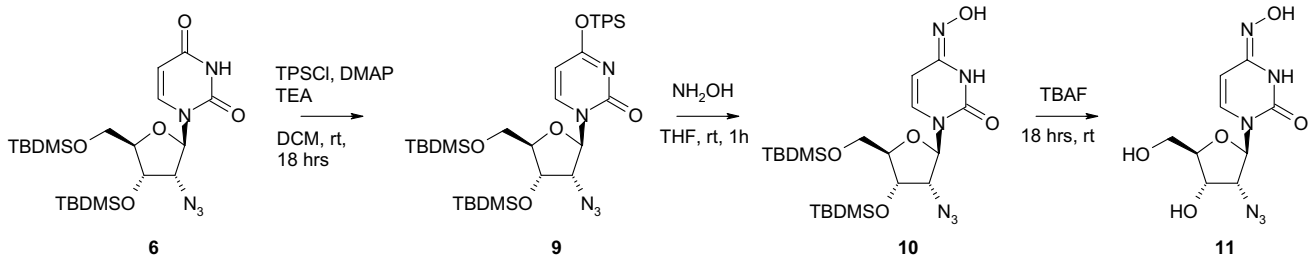
Silyl protected nucleoside **6** was also transformed into the $O^4$-TPS derivative **9** with 73% yield, using TPSCl, in the presence of triethylamine excess and catalytic amounts of 4-(dimethylamino)pyridine (Scheme 2) (Hari et al. 2011). The obtained intermediate **9** was treated then with hydroxylamine solution in THF, which resulted in the formation of oxime derivative **10** with 80% yield, and deprotected to the final product **11** with the TBAF hydrate in THF (55% yield).

2′-Azido-2′-deoxyuridine (**4**) was synthesized according to a literature procedure (Kirschenheuterer al. 1994) and the reported conditions were then applied to the synthesis of 2′-azido-2′-deoxy-5-methyluridine (2′-azidothymidine, **13**). The treatment of 2,2′-anhydrothymidine (**12**) with lithium fluoride and azidotrimethylsilane in DMF, in the presence of TMEDA at 110–115 °C, led to the opening of the anhydro bridge and formation of 2′-azido derivative **13** with 45% yield (Scheme 3). The opening of anhydronucleoside **12** could be also performed with lithium azide/HMPA instead of azidotrimethylsilane (Pokrovskii et al. 2005). 2′-Azidothymidine (**13**) was then dissolved in isopropyl alcohol and hydrogenated in the presence of catalytic amount of 5% palladium on activated charcoal, giving 2′-aminothymidine (**14**) with 85% yield. The reduction of azide to the amine group could also be done with triphenylphosphine in ammonium hydroxide (Pokrovskii et al. 2005).

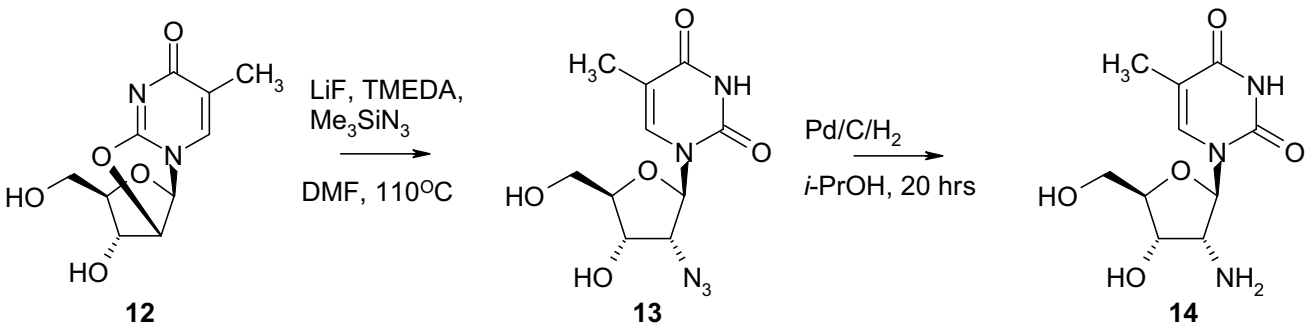
In the initial experiments, the synthesized compounds **4**, **8a–c**, **11**, **13**, **14** and commercially available reference substances **5**, **15** and **16** were tested for their antiproliferative activity on MCF7 (human breast adenocarcinoma) and CCRF-CEM (T cell lymphoblastic leukemia) cell lines (Table 1). The investigated compounds possessed substituent variations on sugar ($N_3$ versus $NH_2$ in $C_{2'}$ position) and/or pyrimidine ring (H, Cl, Br, I in $C_5$ position, C=O and C=N–OH in $C_4$ position), giving an attractive pool for the investigation of structure–activity relationships. The most active compound in the 2′-azido-2′-deoxy series, the 2′-azido-2′-deoxycytidine (**5**), decreased cell viability at 200 μM concentration to 33 ± 1% for the CCRF-CEM cell line. The structural modifications of 2′-azido-2′-deoxycytidine (**5**) led to the $N^4$-hydroxy-2′-azido-2′-deoxycytidine (**11**) (introduction of the –OH group in the $N^4$ position of the cytidine ring) and 2′-azido-2′-deoxyuridine (**4**) (exchange of cytosine into the uracil ring)—compounds deprived of antiproliferative activity. 5-Chloro- (**8c**) 5-bromo- (**8a**), 5-iodo (**8b**) and 5-methyl derivative (**13**) also did not exhibit any significant




**Scheme 1** Synthesis of 5-halogeno-2′-deoxy-2′-azidouridine derivatives **8a–c**




**Scheme 2** Synthesis of 4-oxime-uridine derivative **11**




**Scheme 3** Synthesis of 2′-azidothymidine (**13**) and 2′-aminothymidine (**14**)

**Table 1** Antiproliferative activity against MCF7 and CCRF-CEM cell lines of 2′-amino and 2′-azidonucleoside analogs

| Compound | Concentration 200 μM (cell viability % ± SD, after 24 h incubation) | |
|---|---|---|
| | MCF7 | CCRF-CEM |
| **4** | 101 ± 2 | 102 ± 5 |
| **5** | 82 ± 5 | 33 ± 1 |
| **8a** | 98 ± 3 | 101 ± 1 |
| **8b** | 100 ± 2 | 103 ± 5 |
| **8c** | 97 ± 4 | 99 ± 6 |
| **11** | 95 ± 3 | 92 ± 7 |
| **13** | 96 ± 3 | 95 ± 7 |
| **14** | 94 ± 2 | 101 ± 6 |
| **15** | 94 ± 2 | 70 ± 6 |
| **16** | 89 ± 4 | 36 ± 2 |

antiproliferative activity against both lines of cancer cells. In the 2-amino-2′-deoxy- series, 2′-amino-2′-deoxycytidine (**16**) showed the highest cytotoxic activity, decreasing cell viability at 200 μM concentration to 36 ± 2% for CCRF-CEM, with an effect similar to the one observed for 2′-azido-2′-deoxycytidine (**5**). The exchange of the azido group into the amino group in compound (**5**) had almost no influence on the cytotoxic effect on the investigated cell lines. In contrast, uridine analogs of compound **16**: 2′-amino-2′-deoxuridine (**15**) and its 5-methyl derivative (**14**) did not decrease cell viability at 200 μM concentration.

In further experiments, the investigated compounds **4**, **5**, **8a–c**, **13**, **14, 15** and **16** were tested for their antiproliferative activity with the use of HeLa (cervical cancer) and Hek293 (human embryonic kidney) cell lines (Fig. 2).

The obtained results showed that compounds **8a** and **14** exhibited the strongest antiproliferative effect on HeLa and Hek293 cells. Compound **8a** showed similar cytotoxic effect on both HEK293 and HeLa cell lines ($IC_{50}$ approximately 3.5 mM), whereas molecule **14** exhibited toxic effect only on HeLa cells ($IC_{50}$ about 1.7 mM). Other compounds did not demonstrate any significant effect on cell growth. The above results suggest that in the case of CCRF-CEM and MCF-7cell lines, only the cytidine derivatives **5** and **16** exhibit noticeable cytotoxic effect, and apparently the presence of cytosine ring is required for the antiproliferative effect. The modifications of the sugar ring did not alter cell viability, as both 2′-azide- and 2′-amino derivatives showed similar antiproliferative effect. As for the cell lines, CCRF-CEM cells were more susceptible to cytidine derivatives, while MCF7 cells did not exhibit significant susceptibility. In comparison to CCRF-CEM and MCF7, HeLa and HEK293 cell lines were less susceptible to the tested compounds. Because of the minor effect on HeLa and HEK293 cell survival, most of the examined 2′-modified nucleosides seem to be unsuitable scaffolds for novel anticancer drugs. However, the finding that newly synthesized compound **14** showed cytotoxic effect exclusively on cancer cell line HeLa and not on normal HEK293 is extremely interesting. Future modification of this particular compound could be a promising way to obtain more powerful anticancer substances.

2′-Azido-2′-deoxy- and 2′-amino-2′-deoxy- nucleoside derivatives were evaluated for their antibacterial and antifungal (against *Candida albicans* ATCC 10231) activities. Antibacterial studies were carried out against two Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633) and three Gram-negative bacterial strains (*E. coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 9027). The results showed that all the synthesized compounds did not exhibit antibacterial or antifungal activity at 40 mM concentration.

## Conclusions

A series of ten pyrimidine nucleosides modified at the 2′ position with azide or amine group was subjected to the investigation of biological activity. The tested compounds exhibited a rather weak cytotoxic effect.

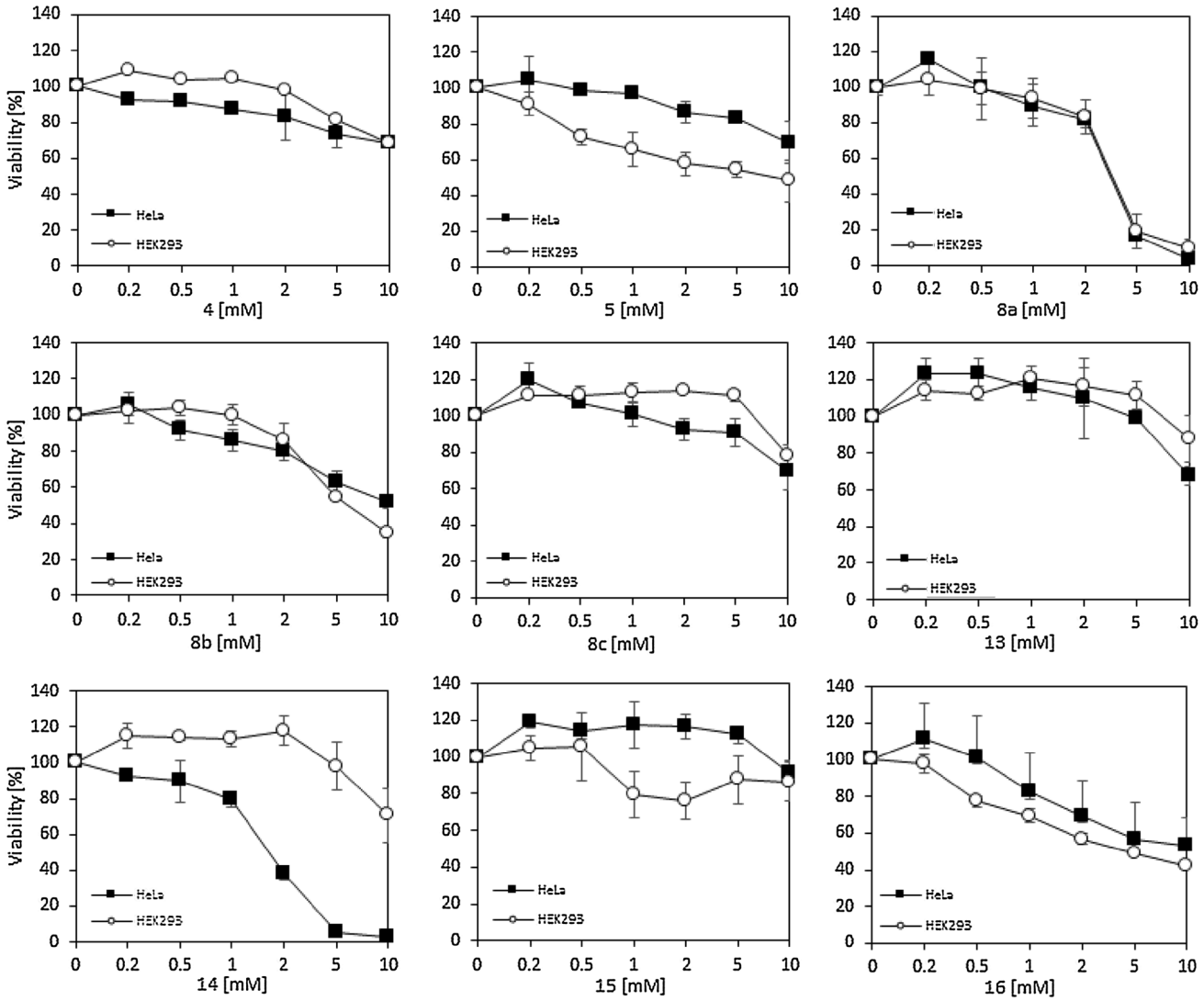



**Fig. 2** Cytotoxic properties of 2′-amino and 2′-azidonucleosides against HeLa and HEK293 cell lines

2′-Azido-2′-deoxycytidine and 2′-amino-2′-deoxycytidine showed antiproliferative activity against CCRF-CEM cells, while newly synthesized 2′-amino-2′-deoxythymidine exhibited cytotoxic effect exclusively toward HeLa cancer cell line, but not toward the normal HEK293 cells. The lack of inhibition of cell growth by 2′-azido-2′-deoxyuridine analogs could be attributed to the poor phosphorylation by TK1 and TK2 enzymes to appropriate monophosphates, a key determinant responsible for exhibiting the cytotoxic effect. Although in rare cases the nucleoside derivatives did not require transformation to appropriate monophosphates, the observed biological effect is related to the non-nucleosidic mode of action (McGuigan et al. 2004). Therefore, to increase the bioavailability of synthesized compounds and to overcome the problem with the first phosphorylation step, by thymidine kinases TK1 and TK2, we are currently working on the synthesis of appropriate monophosphate pro-drugs of the obtained 2′-azido-2′-deoxynucleoside analogs, followed by investigation and comparison of the cytotoxic effects of nucleosides and their 5′-monophosphate esters. These results will be published in due course.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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