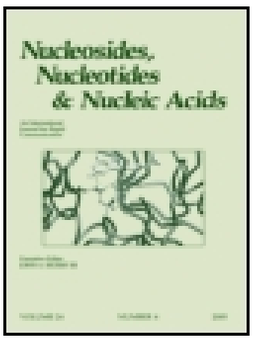

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3′-*O*- and 5′-*O*-Propargyl Derivatives of 5-Fluoro-2′-Deoxyuridine: Synthesis, Cytotoxic Evaluation and Conformational Analysis

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ABSTRACT

A series of new 3′-O- and 5′-O-propargyl derivatives of 5-fluoro-2′-deoxyuridine (**1**–**4**) was synthesized by means of propargyl reaction of properly blocked nucleosides (**2**,**4**), followed by the deprotection reaction with ammonium fluoride. The synthesized propargylated 5-fluoro-2′-deoxyuridine analogues (**1**–**4**) were evaluated for their cytotoxic activity in three human cancer cell lines: cervical (HeLa), oral (KB) and breast (MCF-7), using the sulforhodamine B (SRB) assay. The highest activity and the best SI coefficient in all of the investigated cancer cells were displayed by 3′-O-propargyl-5-fluoro-2′-deoxyuridine (**1**), and its activity was higher than that of the parent nucleoside. The other new compounds exhibited moderate activity in all of the used cell lines.

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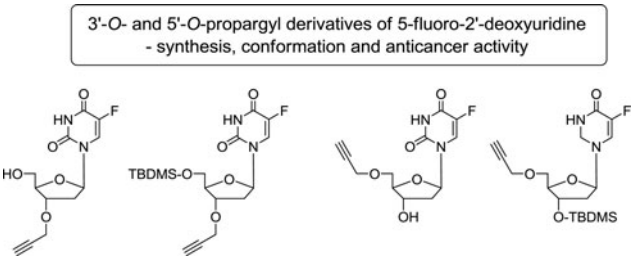
Propargylated 5-fluoro-2′-deoxyuridine derivatives; propargyl moiety; click chemistry reagents; cytotoxic activity; human cancer cell lines: HeLa; KB and MCF-7

GRAPHICAL ABSTRACT



1. Introduction

A number of fluorinated nucleoside analogues showed interesting biological properties, and among them are 5-fluorouracil (FUra, 5-FU), 5-fluoro-2′-deoxyuridine (floxuridine, 5-FdU) and 5′-deoxy-5-fluoro-N^4-pentyloxycarbonyl-cytidine (capecitabine, CAP), anticancer drugs that have broad spectrum of activity (Figure 1).[1,2]

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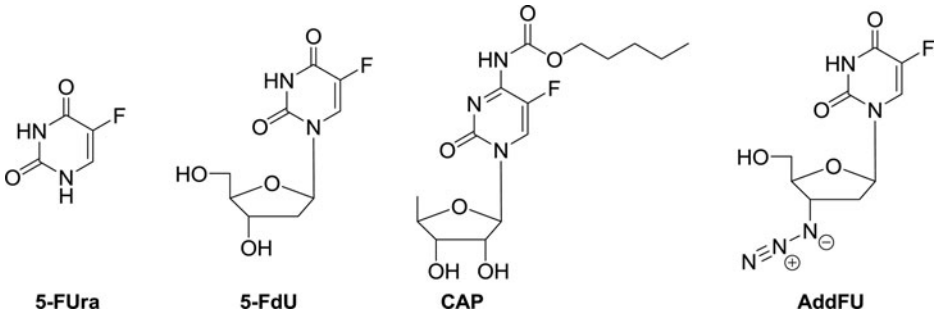


Figure 1. 5-Fluorouracil, 5-fluoropyrimidine nucleosides drugs, and AddFU

5-Fluoropyrimidine-based drugs (FUra, 5-FdU and CAP) are widely used anticancer medicines.[3,4] 5-Fluorouracil has proved to be one of the most effective chemotherapeutics for colorectal cancer, but it is also used in treatment of breast, head and neck, esophageal, gastric, and several other cancers.[5] The mechanism of anticancer action of 5-fluorouracil and 5-fluoro-2′-deoxyuridine involves their intracellular conversion to 5-fluoro-2′-deoxyuridine monophosphate (5-FdUMP), 5-fluoro-2′-deoxiuridine diphosphate (5-FdUTP) and 5-fluorouridine triphosphate (5-FdUTP).[4,5] 5-Fluoro-2′-deoxyuridine 5′-monophosphate (5-FdUMP) acts as an irreversible inhibitor of thymidylate synthase, thus interfering with DNA biosynthesis and repair. The second postulated mechanism of anticancer action of 5-fluorouracil consists of its transformation *via* 5-fluoro-2′-deoxyuridine to 5-fluoro-2′-deoxyuridine 5′-triphosphate (5-FdUTP), and its incorporation into cellular DNA, which interferes with the DNA synthesis and repair.[6,7] Moreover, 5-FdUTP is extensively incorporated into both nuclear and cytoplasmic RNA species, and this process interferes with normal RNA function and protein synthesis.[6] Capecitabine is a specially designed precursor of 5-FU which, contrary to the registered drug, can easily penetrate the cell membrane. Inside the cells, it undergoes three enzymatic transformations, which lead to 5-fluorouracil. Subsequently, it is converted to FdUMP — an irreversible inhibitor of thymidylate synthase and the common active metabolite of 5-fluorouracil, 5-fluoro-2′-deoxyuridine and capecitabine.[5,8]

It has been confirmed that the modification of pyrimidine nucleosides at the 5-position, towards obtaining fluorinated nucleoside analogues, proves to be particularly beneficial because it drives significant biological activity in those nucleosides and enables obtaining valuable drugs. Therefore, a considerable effort has been directed to the synthesis of new derivatives of 5-FdU with the propargyl moiety (Figure 2). A number of the 3′-*O*- and 5′-*O*-propargyl derivatives has been obtained,[9-12] however, the respective compounds of fluorinated pyrimidine bases has not been reported so far. 3′-*O*-Propargylated 5-fluoro-2′-deoxyuridine analogue (**1**), similarly to 5′-*O*-propargylated analogue (**3**), possesses 5-fluorouracil moiety, however, it has terminal alkyne group (propargyl group), in the sugar part, instead of 3′- or 5′-hydroxyl.

For the first time, the synthesis of 3′-*O*-propargylthymidine as a structural analogue of the potent antiretroviral agent 3′-azido-3′-deoxythymidine (AZT) was reported by Rosowsky et al.[13] The candidate was tested for cytotoxic activity

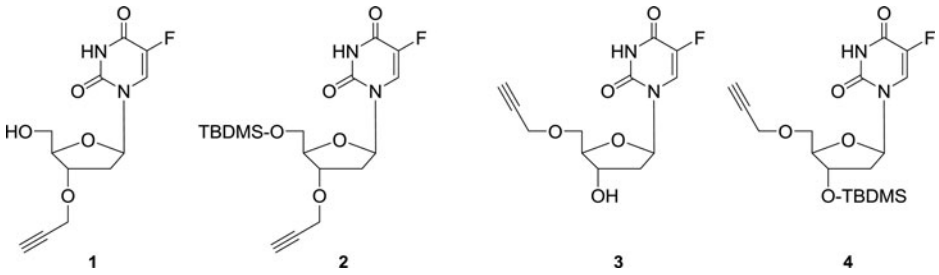


Figure 2. Propargyl derivatives of 5-fluoro-2′-deoxyuridine

against murine leukemia virus (MuLV), but no inhibition of MuLV profileration was observed. The replacement of the 3′-N=N$^+$=N$^-$ group in AZT by a 3′-OCH$_2$C≡CH group increased cytotoxicity with a simultaneous decrease in the antiretroviral activity, related to AZT.

It could be argued that in certain aspects, 3′-*O*-propargyl 5-fluoro-2′-deoxyuridine (**1**) resembles 3′-azido-2′,3′dideoxy-5-fluorouridine (AddFU), as well as its close analogue — 3′-azido-3′-deoxythymidine (AZT), both known for their anticancer and antiviral activity (AZT is used both as an anti-HIV agent and as a therapeutic in colon cancer treatment).[11] In some cases, the propargyl derivatives (**1** and **2**) can mimic biologically active molecules, constituting themselves as a new and interesting group of compounds.

In 2001, Sharpless and his co-workers formulated the "click" chemistry concept and defined the criteria for "click" reactions.[14] Sharpless[15] and Meldal[16] have independently established that Huisgen 1,3-dipolar cycloaddition reaction of organic azides and terminal alkynes is catalyzed by copper(I) ions and can be performed at room temperature, leading exclusively to 1,4-regioisomers of 1,2,3-triazole. This cycloaddition soon became the premier "click" chemistry reaction and since that time it is known as Huisgen reaction. It is not surprising that number of research groups are trying to combine the concept of click chemistry with the chemistry of nucleic acids. The propargylated 5-FdU derivatives presented in this paper constitute a powerful tool for rapid synthesis of novel, biologically active fluorinated nucleosides, used in medicinal treatment. Applying their structural potential, they can also be used to introduce fluorine to defined target molecules as mechanistic probes.[17]

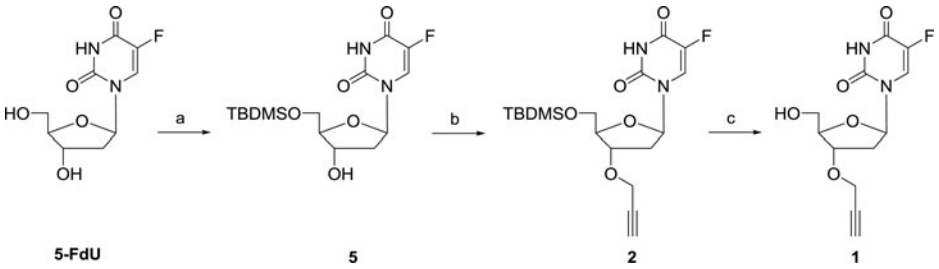
Encouraged by the aforementioned studies, we have set out to develop new propargyl derivatives of FdU with potential anticancer properties. In this paper, we present the synthesis of 3′-*O*- and 5′-*O*-propargyl derivatives of 5-fluoro-2′-deoxyuridine (**1**–**4**) and the evaluation of their cytotoxic activity in three human cancer cell lines: cervical (HeLa), oral (KB) and breast (MCF-7).

2. Results and discussion

2.1. *Chemistry*

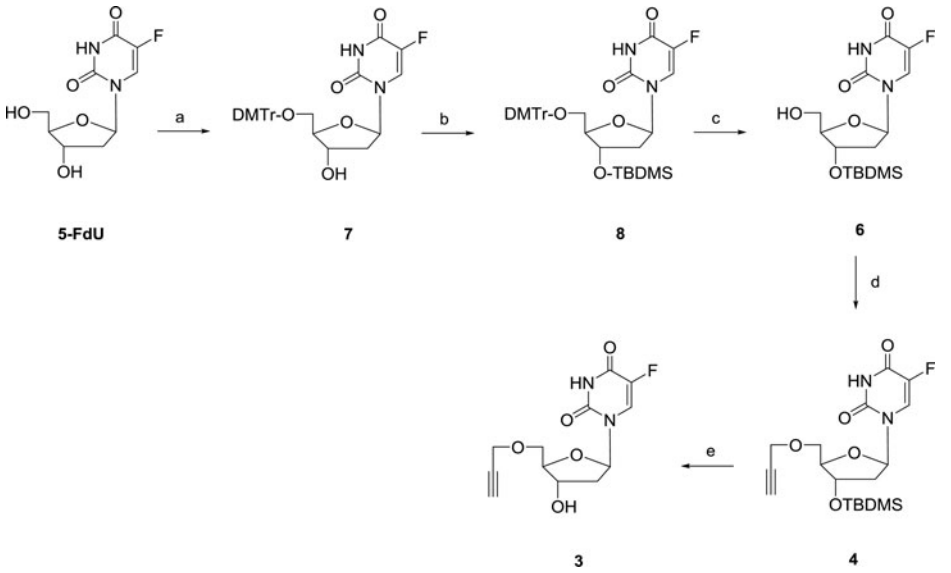
Two synthetic routes, which allow for obtaining fluorinated nucleoside analogues bearing a propargyl moiety, have been developed (containing terminal

alkyne group). Firstly, we have synthesized 3′-*O*-propargyl 5-fluoro-2′-deoxyuridine (**1**) in a four-step synthetic route. To obtain **1** we have synthesized 5′-*O*-(*tert*-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (**5**) directly from commercially available 5-fluoro-2′-deoxyuridine (5-FdU), in a simple and efficient procedure. Its further reaction with 2.5 eqs of NaH, THF as a solvent for 1 hour and then the addition of 2.5 eqs of propargyl bromide (80% solution in toluene) as alkylating agent (room temperature, 12 hours) gave the corresponding 5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-propargyl-5-fluoro-2′-deoxyuridine (**2**). The deprotection of the analog **2** with 5 eqs of NH_4F in abs. MeOH under reflux for 4 hour provided 3′-*O*-propargyl-5-fluoro-2′-deoxyuridine (**1**) (Scheme 1).



Scheme 1. Synthesis of 3′-*O*-propargyl-5-fluorouridine *via* 3′-*O*-propargyl-5′-*O*-TBDMS-5-fluoro-2′-deoxyuridine. Reagents and conditions: (a) TBDMS-Cl (1.1 eqs), imidazol (3 eqs), abs. Py, rt, Ar, 24 h; (b) *i* - NaH (5÷6 eqs), abs. THF, rt, Ar, 1 h, *ii* –$CHCCH_2Br$ (2.7 eqs), rt, Ar, 12 h; (c) NH_4F (5 eqs), abs. CH_3OH, 65°C, 4 h.

In order to further explore the aforementioned procedure, new 5′-*O*-propargyl-5-fluoro-2′-deoxyuridine derivatives **3** and **4** were designed. To prepare the target compounds, a multistep synthesis was undertaken. We have first focused on the key intermediate synthesis, i.e., 3′-*O*-(*tert*-butyldimethylsilyl) -5-fluoro-2′-deoxyuridine (**6**), using an analogous procedure for the synthesis of 3′-*O*-(*tert*-butyldimethylsilyl)thymidine, available in the literature.[18] In this synthetic route, 5-fluoro-2′-deoxyuridine (5-FdU) was transformed into 5′-*O*-(4,4′-dimethoxytrityl)-5-fluoro-2′-deoxyuridine (**7**), which subsequently reacted with TBDMS-Cl to obtain 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-5-fluoro-2′-deoxyuridine (**8**). This compound, after detritylation with *para*-toluenesulphonic acid in methylene chloride— methanol (9:1) solution gave **6** in 68–86% yield. The compound **6** could then be transformed into appropriate 5′-*O*-propargyl derivative of the type **4** by propargyl bromide (80% solution in toluene) treatment at 70°C for 3 hours. Similarly, when compound **4** was used as a substrate and treated with NH_4F, it was possible to obtain 5′-*O*-propargylated derivative **3**. All desired nucleotides were obtained in good yield (75–90%) (Scheme 2). A series of newly obtained compounds were characterized by the NMR and MS analyses.



Scheme 2. Synthesis of 5′-*O*-propargyl-5-fluorouridine *via* 3′-*O*-TBDMS-5′-*O*-propargyl-5-fluoro-2′-deoxyuridine. Reagents and conditions: (a) DMTr-Cl (1.1 eqs), abs. Py, rt, Ar, 12 h; (b) TBDMS-Cl (2.5 eqs), imidazol (3 eqs), abs. Py, rt, Ar, 24 h; (c) *para*-TsOH (1 eq), CH_2Cl_2/CH_3OH (9:1); (d) *i*–NaH (2.5 eqs), abs. THF, rt, Ar, 40 min., *ii* –$CHCCH_2Br$ (2.5 eqs), 70°C, 3 h; (e) NH_4F (5 eqs), abs. CH_3OH, 65°C, 4 h.

2.2. *Biological evaluation*

The synthesized propargyl 5-fluoro-2′-deoxyuridine derivatives **1**–**4** were evaluated for their cytotoxic activity in three human cancer cell lines: cervical (HeLa), oral (KB) and breast (MCF-7) and the reference — (healthy cells) primary skin fibroblasts (HDF), employing sulforhodamine B (SRB) assay.[19] HDF cell line was maintained in a serum-free medium. The resulting cytotoxic activity data of the obtained

Table 1. In vitro cytotoxic activity of the synthesized compounds **1**–**4** in three human cancer cell lines: cervical (HeLa), oral (KB) and breast (MCF-7) and the reference — healthy cells: primary skin fibroblasts (HDF)

	Cytotoxicity (IC_{50}, μg/mL)[a] ± SD[b]							
Compound	HeLa	SI[c]	KB	SI	MCF-7	SI	HDF	log P[d]
1	1.69 ± 1.19	1.9	1.12 ± 0.29	2.9	2.01 ± 0.14	1.6	3.28 ± 0.02	−0.94
2	3.05 ± 0.09	6.4	2.63 ± 0.12	7.4	3.02 ± 0.06	6.4	19.21 ± 3.91	2.38
3	3.02 ± 3.08	2.7	3.03 ± 0.20	2.7	3.53 ± 1.03	2.3	8.19 ± 2.09	−0.94
4	3.09 ± 1.42	2.3	3.27 ± 0.24	2.2	3.71 ± 2.28	1.9	7.04 ± 0.03	2.38
5-FdU[e]	2.40 ± 0.74	2.5	2.12 ± 0.41	2.8	1.94 ± 2.12	3.0	6.06 ± 2.18	−1.72
Cytarabine (standard)	0.91 ± 0.02		0.82 ± 0.74		0.93 ± 0.09		1.81 ± 0.14	−2.32[20]

[a] IC_{50} is the compound concentration required to inhibit cell growth by 50%.
[b] SD (standard deviation) of three independent experiments.
[c] Selectivity Index (SI) was calculated for each compounds using formula: SI = IC_{50} for normal cell line (HDF)/IC_{50} for respective cancerous cell line. A beneficial SI > 1.0 indicates a drug with efficacy against tumor cells greater than toxicity against normal cells.
[d] log P (logarithm of partition coefficient) was calculated using the "miLogP" method.[21]
[e] Reference compound: 5-fluoro-2′-deoxyuridine (5-FdU)

Table 2. ^{1}H NMR spectral data for compounds **1–4** and 5-FdU[a]

	NH	H-6	H-1′	H-3′	H-4′	H-5′	H-5′′	H-2′	H-2′′
1	11.84	8.19	6.07	4.24	3.97	3.56–3.65		2.13–2.17	2.28–2.32
2	11.87	7.96	6.07	4.24	4.03	3.73–3.77	3.81–3.84	2.09–2.16	2.31–2.37
3	11.81	7.95	6.13	4.20	3.89	3.57–3.61	3.67–3.70	2.09–2.12	
4	11.84	7.96	6.11	4.39	3.89	3.59–3.62	3.67–3.70	2.20–2.25	2.07–2.12
5-FdU	11.80	8.21	6.12	4.23	3.78	3.55–3.63		2.09–2.12	

[a] Chemical shifts in DMSO-d_6. The data showing full spectra and coupling constants are provided in the "Experimental" section.

propargylated nucleosides and reference compounds are presented in Table 1. Comparison between the cancer cell lines and the corresponding normal cell line HDF was made to define the in vitro selectivity index (SI) as a measure of the therapeutic potential (Table 1). The in vitro SI of a drug is defined as the ratio of the toxic concentration to the therapeutic concentration (in vitro SI = IC_{50} non-tumor cell line/ IC_{50} tumor cell line). The highest activity in KB cancer cells was displayed by 3′-*O*-propargylated analogues **1** (IC_{50} = 1.12 μg/mL), which is more potent than the parent 5-FdU (IC_{50} = 2.12 μg/mL). This compound is also very active in HeLa cancer cells (1.69 μg/mL) and it is slightly more active than 5-FdU (2.40 μg/mL). The same compound demonstrated very similar activity in MCF-7 cancer cells, with IC_{50} = 1.94 μg/mL. Other propargyl derivatives **2–4** also exhibited relatively high activity in HeLa cancer cells (IC_{50} is about 3.00 μg/mL) whereas in MCF-7 cancer cells the activity is lower (IC_{50} about 3.5 μg/mL). In principle, all the obtained propargylated nucleosides exhibited moderate activity and were only somewhat less potent than the parent nucleoside 5-FdU. Significant increase in the SI value, higher than that of 5-FdU, was shown by propargyl compound **2** in the cancer cells. It was almost three times more selective for KB cancer cells (SI = 7.4), being also very high in other cells. Similarly, a very high SI was demonstrated by propargyl derivative **1**, which was two times more active than the parent nucleoside. The other two nucleosides — **3** and **4** showed similar SI to those of the parent nucleoside (5-FdU). These findings clearly indicate that the propargyl derivative **1**, with the hydroxyl in the 3′-position, was the most potent compound in all of the investigated cancer cell lines (HeLa, KB and MCF-7). Its activity was very close to that of 5-FdU, but the selectivity was consistently higher than that of the parent nucleoside. The propargyl nucleoside **2**, with the 5′-*tert*-butyldimethylsilyl substituent, was also found to have high activity in the three cell lines, whereas it indicated much better SI. However, nucleoside analogues **3** and **4**, with the propargyl substituent in the 5′-position, were less potent in all the cancer cells. The reasons to explain this are not clear because these propargylated nucleosides have similar structure, polarity and hydrophobicity.

Partition coefficient (log *P*) values of the compounds **1–4** were calculated[21] to determine possible correlation between the cytotoxicity data and lipophilicity (Table 1). All of the propargylated derivatives of 5-FdU were more lipophilic than 5-FdU (log *P* = −1.72), with log *P* values ranging from −0.94 to 2.38. The most active and the least active compounds, **1** and **4**, respectively, showed negative value

of log *P*, which is −0.94. This suggests hydrophilic character of these molecules, which could be well soluble in aqueous phase and not be caught by fat depots.[22] The less active compounds **2** and **4** showed moderate value of log *P* (2.38), so it can be envisaged that these compounds, due to their non-ionic character, can penetrate the cell membranes, as indicated by the encouraging values of their partition coefficients (Table 1). However, linear regression analysis did not reveal any correlation between log *P* values and the cytotoxicity data. One of the compounds tested — the propargyl derivative **1** could be metabolized to a 5′-monophosphate inside the cells, which is likely to be an inhibitor of thymidylate synthase. Moreover, the 5′-monophosphate of **1** after enzymatic phosphorylation to 5′-triphosphate (via 5′-diphosphate), can act as a competitive inhibitor of DNA polymerases and a chain terminator of the nascent DNA strand, due to the lack of a 3′-hydroxyl group.

2.3. *NMR spectroscopy and conformational analysis*

Structures of **1**–**4** were confirmed by series of ^{1}H, ^{13}C, ^{15}N, ^{19}F and ^{29}Si NMR experiments. Assignment of ^{1}H, ^{13}C, ^{15}N resonances was accomplished by the analysis of 2D spectra (^{1}H−^{1}H COSY, ^{1}H−^{13}C HSQC and HMBC, ^{1}H−^{15}N HSQC and HMBC) and compared with the data obtained for 5-FdU (see Experimental section). Detailed analysis of ^{1}H and ^{13}C chemical shift reavealed significant differences within sugar moiety and H-6 of base part, depending on the type of substituent in position of 3′-OH or 5′-OH (Tables 2 and 3). The introduction of propargyl group in position of 3′-O (**1**, **2**) caused downfield shift of resonances for C-3′, H-2′′ and H-4′ by ca 8, 0.2 and 0.2 ppm, respectively. An upfield shift by ca 3 ppm was observed for C-2′ and C-4′. In **3** and **4** the same anisotropic effect of propargyl group was observed only for C-5, i.e., downfield shift by ca 8 ppm. The observed changes may be explained as an effect of interaction of π-electrons of the triple bond with proximate protons or carbon of the sugar ring. Introduction of TBDMS group caused much smaller changes of resonances in **2** and **4**. For H-6, an upfield shift of resonance by ca 0.25 ppm was observed upon substitution with any group in **2**, **3** and **4**, compared with 5-FdU.

In order to estimate the effect of substitution at 3′ and/or 5′ position with propargyl or TBDMS group on sugar puckering and syn-anti equlibrium of compounds **1**–**4**, we subjected them to a conformational analysis by means of NMR spectroscopy

Table 3. ^{13}C NMR spectral data for compounds **1**–**4** and 5-FdU[a]

	C-4	C-2	C-5	C-6	C-1′	C-4′	C-3′	C-5′	C-2′
1	156.9	149.0	140.0	124.6	84.51	84.71	78.45	61.27	36.42
2	156.9	148.8	140.0	124.0	84.67	84.20	77.90	63.07	36.50
3	157.0	149.0	140.0	124.4	84.63	85.28	70.49	69.43	39.10
4	157.0	148.9	140.0	124.5	84.42	85.09	71.76	68.75	39.10
5-FdU	157.0	149.0	139.9	124.7	84.51	87.47	70.11	60.99	39.50

[a] Chemical shifts in DMSO-d_6. The data showing full spectra and coupling constants are provided in the "Experimental" section.

and PSEUROT calculations approach.[23] The summary results of conformational preferences of **1–4** as well as of 5-FdU are presented in Table 4. In general, conformation of 2′-deoxyribose moiety in compounds **1–4** and 5-FdU showed similar preference for S-type sugar puckering, which confirms the previously published results for 2′-deoxynucleosides.[24,25] Thus, we may assume that the presence of propargyl or TBDMS substituent at 3′-OH did not diminish the gauche effect of O4′—C4′—C3′—O3′ fragment on the preferences for S-type conformers.[26] In case of **1**, propargyl group at 3′-OH significantly shifted the N↔S equilibrium towards S-type, by ca 10%, compared with 5-FdU, however this effect is contradictory to TBDMS group at 5′-OH in **2**, where the N↔S equilibrium is similar to 5-FdU. Compounds **1** and **2** preferred two different types of sugar puckering i.e. C-2′endo—C-3′exo and C-2′endo—C-1′exo, respectively. The preference for C-2′endo—C-1′exo in **2** may derive from the tendency to minimize the steric hindrance between 5′-*O*-TBDMS group and 5-fluorouracil moiety. Conformation of 2′-deoxyribose moiety and N↔S equilibrium in **3** was identical to 5-FdU and it adopted C-2′endo, but in **4**, sugar moiety preferred more twisted conformer, such as C-2′endo—C-1′exo and slightly less significant predominance of S-type population, compared with 5-FdU. Additionally, estimation of N↔S equilibrium by means of simple equations[27–29] (Table 4) confirmed significant predominance of S-type conformers, however for us, the results were less useful than those obtained with PSEUROT approach.

We have also performed NMR experiments to determine the preferred conformation around the glycosidic bond (C-1′—N-1) by observing the NOE enhancement for H-1′ and H-2′, upon irradiation of H-6 and by measuring the heteronuclear vicinal coupling constants, i.e. $^{3}J_{H1'C2}$ and $^{3}J_{H1'C6}$.[30,31] For **1–4**, the largest NOE enhancement was found in the H-2′ reasonance (2–3%), which clearly indicates pronounced preference to *anti* conformation. Additionally, the higher values of $^{3}J_{H1'C6}$ (3.8–4.0) than $^{3}J_{H1'C2}$ (1.6–1.9 Hz) confirms that *anti* orientation of base plane is predominant.

Table 4. Experimental vicinal ^{1}H-^{1}H coupling constants in Hz[a], calculated pseudorotational parameters (*P*, *ν* and *S*%) and population of *S*% conformers, derived from three different equations published in literature

	$J_{H1'-H2'}$	$J_{H1'-H2''}$	$J_{H2'-H3'}$	$J_{H2''-H3'}$	$J_{H3'-H4'}$	P, *ν*, S%	Eq 1[b]	Eq 2[c]	Eq 3[d]
1	7.90	5.95	5.80	2.35	0.55–0.75	173–187°, 30–34°, 77–82%	98.3%	68.6%	92.4%
2	7.85	5.80	5.95	2.35	2.40–2.20	133–136°, 30–40°, 66–74%	96.6%	65.2%	77.3%
3	7.10	6.30	5.75	3.60	3.00	164–167°, 32–36°, 65–70%	84.8%	64.4%	70.9%
4	6.60	6.40	6.50	3.95	3.55	146–148°, 30–33°, 59–62%	72.4%	54.2%	65.0%
5-FdU	6.85	6.35	5.95	3.45	2.80	166–167°, 30–33°, 65–69%	84.3%	57.6%	71.0%

[a] Sample temperature 298K in DMSO-d_6, sample concentration 60 mM.
[b] Equation 1 $S\% = 100(16.9\text{-}J_{2'3'}\text{-}J_{2''3'})/8.9$.[24]
[c] Equation 2 $S\% = 100(J_{1'2'}+J_{1'2''}\text{-}9.8)/5.9$.[24]
[d] Equation 3 $S\% = 100J_{1'2'}/(J_{1'2'}+J_{3'4'})$[24,26]

3. Conclusion

In conclusion, we have presented two simple and efficient procedures of synthesis of a series of propargylated 5-fluoro-2′-deoxyuridine derivatives. The obtained compounds **1**–**4** were examined for their cytotoxic activity in three human cancer cell lines: HeLa (cervical), oral (KB) and breast (MCF-7). All of them exhibit a medium cytotoxic activity, but mostly with better SI than 5-FdU. The highest activity in all of the investigated human cancer cells was displayed by propargylated nucleoside **1** and its activity was fairly as high as the parent nucleoside (5-FdU), but with several times higher SI. However, the compounds (**2** and **4**) with a silyl group (TBDMS) were less potent in all the cell lines used. Moreover, all of the novel analogs with the terminal triple bond, were synthesized as a new and interesting structural class of nucleotide analogs, which offers means to tag nucleosides with different moieties, e.g. desired aptamers or conjugates. Furthermore, all of the obtained compounds will be tested for antiviral activity. The analysis of $^{3}J_{HH}$ for **1**–**4** revealed strong preference of 2′-deoxyribofuranosyl moiety for the S-type puckering between C-2′endo−C-3′exo and C-2′endo−C-1′exo. The interpretation of $^{3}J_{H1'C2}$ and $^{3}J_{H1'C6}$ and observation of NOE enhancement for H-2′ upon H-6 irradiation, showed that *anti* orientation is predominant over *syn* in **1**–**4**.

4. Experimental part

4.1. *Chemistry*

1D and 2D ^{1}H, ^{13}C, ^{15}N and ^{29}Si NMR spectra were recorded on a Bruker Avance III 500 MHz spectrometer, equipped with 5mm broad-band multinuclear (PABBO) probe in DMSO-d_6 at 298K. 1D ^{19}F NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. Chemical shifts (δ) for ^{1}H, ^{13}C and ^{29}Si NMR were reported in ppm relative to the tetramethylsilane (TMS) peak. For ^{31}F and ^{15}N NMR δ were reported in ppm, relative to trichlorofluoromethane and liquid NH_3, respectively. Mass spectra were measured with QqToF Bruker with source of ions ESI — electrospray ionization mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated (0.2 mm) plates and vacuum flash column chromatography on silica gel 60 H (5–40 μm), purchased from Merck. Chemical reagents were purchased from Acros Organics, Alfa Aesar, Carbosynth and Sigma-Aldrich.

The analysis of conformational preferences of 2′-deoxy-β-ribofuranosyl moiety in **1**–**4** and 5-FdU was performed with the help of the PSEUROT computer program which calculates the best fit of the conformational parameters to the experimental vicinal proton-proton coupling constants ($^{3}J_{1'2'}$, $^{3}J_{1'2''}$, $^{3}J_{2'3'}$, $^{3}J_{2''3'}$, $^{3}J_{3'4'}$). In order to obtain complete view of conformational preferences, we have made 1369 calculations for **1**–**4** and 5-FdU with different initial values of pseudorotation phase angles, P_I and P_{II}, that changed independently from 0° to 350° every 10°. For statistical purposes, only the results showing rms values less than 0.1

and ϕ_{maxI}, ϕ_{maxII} between 30 and 40 were employed. The results for minor conformer were omitted in the discussion for better explanation of conformational preferences. The values for coupling constants were obtained through the simulation and iteration procedure with the DAISY module of the TOPSPIN 3.1 software package.

4.1.1. *5′-O-(tert-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (5)*

5-Fluoro-2′-deoxyuridine (5-FdU, 0.67 g, 2.73 mmol) and imidazole (3 eq, 0.56 g, 8.19 mmol) were dissolved in anhydrous pyridine (6.72 mL), and after that *tert*-butyldimethylsilylchloride (1.1 eq, 0.45 g, 3 mmol) was added. The reaction mixture was being stirred under N_2 atmosphere overnight. The mixture was first evaporated to dryness and then evaporated twice with toluene. The organic layer was extracted with saturated aqueous sodium bicarbonate, then with methylene chloride and evaporated to dryness. The residue was purified with silica gel column chromatography, using methylene chloride–methanol (2→5→10%) as an eluent to give pure **5** (white solid, yield: 0.76 g, 76%). ^{1}H NMR (DMSO-d$_6$) δ: 0.08 (s, 6H, $(CH_3)_2Si$), 0.88 (s, 9H, $(CH_3)_3$), 2.05−2.11 (m, 1H, H-2′β, $^3J_{H2'H1'} = 6.60$ Hz, $^3J_{H2'H3'} = 6.20$ Hz, $^2J_{H2'H2''} = 13.4$ Hz), 2.13−2.18 (ddd, 1H, H-2′α, $^3J_{H2''H1'} = 5.00$ Hz, $^3J_{H2''H3'} = 3.60$ Hz, $^2J_{H2'H2''} = 13.4$ Hz), 3.73−3.76 (dd, 1H, H-5′, $^3J_{H5'H4'} = 3.00$ Hz, $^2J_{H5'H5''} = 10.5$ Hz), 3.81−3.84 (m, 2H, H-5″, H-4′, $^3J_{H5''H4'} = 2.3$ Hz, $^2J_{H5'H5''} = 10.5$ Hz), 4.19−4.21 (m, 1H, H-3′, $^3J_{H3'H2'} = 6.20$ Hz, $^3J_{H3'H2''} = 3.60$ Hz, $^3J_{H3'H4'} = 3.55$ Hz), 5.32 (d, 1H, 3′-OH, $^3J_{HH} = 4.65$ Hz), 6.10−6.13 (m, 1H, H-1′, $^3J_{H1'H2'} = 6.60$ Hz, $^3J_{H1'H2''} = 5.00$ Hz), 7.97 (d, 1H, H-6, $^3J_{HF} = 6.9$), 12.00 (brs, 1H, NH). ^{13}C NMR δ: −5.61 ($(CH_3)_2Si$), 18.01 ($C(CH_3)_3$), 25.73 ($C(CH_3)_3$), 39.10 (C-2′, signal overlapped with DMSO), 62.95 (C-5′), 69.98 (C-3′), 84.64 (C-1′), 86.98 (C-4′), 124.1 (d, C-6, $^2J_{CF} = 34.2$), 139.9 (d, C-5, $^1J_{CF} = 230.4$), 148.8 (C-2), 157.0 (d,C-4, $^2J_{CF} = 26.1$). ^{19}F NMR δ: −91.58 ($^3J_{FH} = 7.0$). ^{29}Si NMR δ: 21.56.

4.1.2. *3′-O-propargyl-5′-O-(tert-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (2)*

Sodium hydride (5 eq, 0.26 g, 6.52 mmol) was added portionwise to a stirred solution of 5′-*O*-(*tert*-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (**5**) (0.39 g, 1.1 mmol) in anhydrous THF (7.84 mL) and the mixture was stirred at room temperature, under N_2 atmosphere. After 40 min propargyl bromide (80% solution in toluene, 2.7 eq, 0.25 mL, 2.94 mmol) was added and the mixture was being stirred overnight. Then, the mixture was washed with methanol and evaporated. Analytically pure sample of compound **2** was obtained with silica gel column chromatography, using hexane–ethyl acetate (2:1) as eluent; yield: 0.30 g (white solid, 70%). ^{1}H NMR (DMSO-d$_6$) δ: 0.10 (s, 6H, $(CH_3)_2Si$), 0.89 (s, 9H, $(CH_3)_3$), 2.09−2.16 (m, 1H, H-2′β, $^3J_{H2'H1'} = 7.85$ Hz, $^3J_{H2'H3'} = 5.95$ Hz, $^2J_{H2'H2''} = 13.9$ Hz), 2.31−2.37 (ddd, 1H, H-2′α, $^3J_{H2''H1'} = 5.80$ Hz, $^3J_{H2''H3'} = 2.35$ Hz, $^2J_{H2'H2''} = 13.9$ Hz), 3.47 (t, 1H, CH propargyl, $^4J_{HH} = 2.35$ Hz), 3.73−3.77 (dd, 1H, H-5′, $^3J_{H5'H4'} = 3.31$ Hz, $^2J_{H5'H5''} = 11.4$ Hz), 3.81−3.84 (dd, 1H, H-5″, $^3J_{H5''H4'} = 3.75$ Hz, $^2J_{H5'H5''} = 11.4$ Hz), 4.04 (q, 1H, H-4′, $^3J_{H4'H5'} = 3.31$ Hz, $^3J_{H4'H5''} = 3.75$ Hz, $^3J_{H4'H3'} =$

2.40−2.20 Hz), 4.22 (dd, 2H, CH_2 propargyl, $^4J_{HH}$ = 2.38 Hz, $^2J_{HH}$ = 16.0 Hz), 4.23−4.25 (m, 1H, H-3′, $^3J_{H3'H2'}$ = 5.95 Hz, $^3J_{H3'H2''}$ = 2.30 Hz, $^3J_{H3'H4'}$ = 2.40–2.20 Hz), 6.05−6.09 (m, 1H, H-1′, $^3J_{H1'H2'}$ = 7.85 Hz, $^3J_{H1'H2''}$ = 5.80 Hz), 7.96 (d, 1H, H-6, $^3J_{HF}$ = 7.0), 11.87 (brs, 1H, NH). ^{13}C NMR δ: −5.64 $(CH_3)_2$Si, $^1J_{CH}$ = 118.8), 17.95 $(C(CH_3)_3)$, 25.72 $(C(CH_3)_3$, $^1J_{CH}$ = 124.9), 36.50 (C-2′, $^1J_{CH}$ = 136.6), 55.87 (CH_2 propargyl, $^1J_{CH}$ = 148.3), 63.07 (C-5′, $^1J_{CH}$ = 142.6), 77.41 (CH propargyl $^1J_{CH}$ = 251.4, $^3J_{CH}$ = 4.1), 77.90 (C-3′, $^1J_{CH}$ = 151.9), 79.98 (C propargyl, $^2J_{CH}$ = 50.3, $^2J_{CH}$ = 7.2), 84.20 (C-4′, $^1J_{CH}$ = 148.9), 84.67 (C-1′, $^1J_{CH}$ = 169.7), 124.0 (d, C-6, $^2J_{CF}$ = 34.1, $^1J_{CH}$ = 181.7, $^3J_{CH}$ = 3.8), 140.0 (d, C-5, $^1J_{CF}$ = 230.8, $^2J_{CH}$ = 4.7), 148.8 (C-2, $^3J_{CH}$ = 7.9, $^3J_{CH}$ = 1.7), 156.9 (d, C-4, $^2J_{CF}$ = 26.1, $^3J_{CH}$ = 7.0). ^{15}N NMR δ: 135.0 (N-1), 158.1 (N-3). ^{19}F NMR δ: −91.33 ($^3J_{FH}$ = 6.9). ^{29}Si NMR δ: 21.87. HRMS (ESI−) calcd. for $C_{18}H_{26}FN_2O_5Si$ $[M-H]^-$ 397.1595, found 397.1628.

4.1.3. *3′-O-propargyl-5-fluoro-2′-deoxyuridine (1)—desilylation of compound 2*

Ammonium fluoride (5 eq, 0.18 mg) was added to a stirred solution of **2** (1 mmol, 0.40 mg) in abs. MeOH (4 mL) and the reactants were heated at reflux for 4 h. After that time, the silica gel was added to the reaction mixture and it was evaporated under reduced pressure. The residue was purified with silica gel column chromatography, using the mixture chloroform–methanol (5→10%) as an eluent, to afford product **1** (white solid, yield 75–87%). ^{1}H NMR (DMSO-d_6) δ: 2.13−2.17 (m,1H, H-2′β, $^3J_{H2'H1'}$ = 7.90 Hz, $^3J_{H2'H3'}$ = 5.80, $^2J_{H2'H2''}$ = 13.9 Hz), 2.28−2.32 (ddd, 1H, H-2′α, $^3J_{H2''H1'}$ = 5.95 Hz, $^3J_{H2''H3'}$ = 2.35, $^2J_{H2'H2''}$ = 13.9 Hz), 3.47 (t, 1H, CH propargyl, $^4J_{HH}$ = 2.4 Hz), 3.56−3.65 (m, 2H, H-5′/5″, $^3J_{H5'H4'}$ = 4.20 Hz, $^3J_{H5''H4'}$ = 3.60, $^2J_{H5'H5''}$ = 11.9 Hz), 3.96−3.98 (m, 1H, H-4′, $^3J_{H4'H5'}$ = 4.20 Hz, $^3J_{H4'H5''}$ = 3.60, $^3J_{H4'H3'}$ = 0.55−0.75 Hz), 4.21 (dd, 2H, CH_2 propargyl, $^4J_{HH}$ = 2.4 Hz, $^2J_{HH}$ = 16.0 Hz), 4.23−4.25 (m, 1H, H-3′, $^3J_{H3'H2'}$ = 5.80, $^3J_{H3'H2''}$ = 2.35, $^3J_{H3'H4'}$ = 0.55−0.75 Hz), 5.24 (t, 1H, 5′-OH, $^3J_{HH}$ = 5.00 Hz), 6.06−6.09 (m, 1H, H-1′, $^3J_{H2'H1'}$ = 7.90 Hz, $^3J_{H2''H1'}$ = 5.95 Hz), 8.19 (d, 1H, H-6, $^3J_{HF}$ = 7.2), 11.84 (brs, 1H, NH). ^{13}C NMR δ: 36.42 (C-2′, $^1J_{CH}$ = 136.0), 55.90 (CH_2 propargyl $^1J_{CH}$ = 148.0), 61.27 (C-5′, $^1J_{CH}$ = 141.0), 77.29 (CH propargyl $^1J_{CH}$ = 251.0, $^3J_{CH}$ = 4.1), 78.45 (C-3′, $^1J_{CH}$ = 150.9), 80.19 (C propargyl, $^2J_{CH}$ = 50.0, $^2J_{CH}$ = 7.3), 84.51 (C-1′, $^1J_{CH}$ = 169.6), 84.71 (C-4′, $^1J_{CH}$ = 148.4), 124.6 (d, C-6, $^2J_{CF}$ = 34.4, $^1J_{CH}$ = 182.2, $^3J_{CH}$ = 4.0), 140.0 (d, C-5, $^1J_{CF}$ = 230.2, $^2J_{CH}$ = 4.7), 149.0 (C-2, $^3J_{CH}$ = 8.1, $^3J_{CH}$ = 1.7), 156.9 (d, C-4, $^2J_{CF}$ = 26.2, $^3J_{CH}$ = 7.0). ^{15}N NMR δ: 135.1 (N-1), 157.7 (N-3). ^{19}F NMR δ: − 91.23 ($^3J_{FH}$ = 7.1). HRMS (ESI−) calcd for $C_{12}H_{12}FN_2O_5$ $[M-H]^-$ 283.0730, found 283.0725.

4.1.4. *5′-O-(4,4′-dimethoxytrityl)-5-fluoro-2′-deoxyuridine (7)*

5-Fluoro-2′-deoxyuridine (5-FdU, 2.19 g, 9.0 mmol) was coevaporated two times with anhydrous pyridine and next dissolved in pyridine (20 mL). The resulting solution was chilled, followed by addition of the 4,4′-dimethoxytrityl chloride (1.05 eq, 3.16 g, 9.34 mmol). The mixture was being stirred at room temperature for 3 h under N_2 atmosphere and left at low temperature overnight.

After that methanol was added and the mixture was evaporated to dryness. The residue oil was purified with silica gel column chromatography, using chloroform–methanol (2→5%) as an eluent to obtain pure **7** (white solid, yield: 4.48 g, 92%). ^{1}H NMR (DMSO-d$_6$) δ: 2.14−2.18 (ddd, 1H, H-2′α, $^3J_{H2''H1'}$ = 5.30 Hz, $^3J_{H2''H3'}$ = 4.20 Hz, $^2J_{H2'H2''}$ = 13.9 Hz), 2.22−2.27 (m, 1H, H-2′β, $^3J_{H2'H1'}$ = 6.50 Hz, $^3J_{H2'H3'}$ = 6.70 Hz, $^2J_{H2'H2''}$ = 13.6 Hz), 3.12−3.15 (dd, 1H, H-5′, $^3J_{H5'H4'}$ = 5.40 Hz, $^2J_{H5'H5''}$ = 10.6 Hz), 3.24−3.27 (dd, 1H, H5″, $^3J_{H5''H4'}$ = 2.90 Hz, $^2J_{H5'H5''}$ = 10.6 Hz), 3.73 (s, 6H, 2× CH_3O), 3.87−3.89 (m, 1H, H-4′, $^3J_{H4'H5'}$ = 5.40 Hz, $^3J_{H4'H5''}$ = 2.90, $^3J_{H4'H3'}$ = 2.00 Hz), 4.25−4.29 (m, 1H, H-3′, $^3J_{H3'H2'}$ = 6.70, $^3J_{H3'H2''}$ = 4.20, $^3J_{H3'H4'}$ = 2.00 Hz), 5.33 (d, 1H, 3′-OH, $^3J_{HH}$ = 4.65 Hz), 6.12−6.15 (m, 1H, H-1′, $^3J_{H1'H2'}$ = 6.50 Hz, $^3J_{H1'H2''}$ = 5.30 Hz), 6.87−6.89 (dd, 4H, *meta* in 4-MeOC_6H_4-), 7.22−7.32 (m, 7H, DMTr), 7.38, 7.39 (2× brs, 2H, DMTr), 7.88 (d, 1H, H-6, $^3J_{HF}$ = 6.8), 11.85 (brs, 1H, NH). ^{13}C NMR δ: 39.30 (C-2′), 55.04, 55.02 (2× $CH_3OC_5H_4$), 63.65 (C-5′), 70.05 (C-3′), 84.53 (C-1′), 85.58 (C-4′), 85.82 (CPh_3), 113.2 (C *meta* in 4-MeOC_6H_4-), 124.5 (d, C-6, $^2J_{CF}$ = 33.8), 127.6, 127.9 (C *ortho* in 4-MeOC_6H_4-), 135.3, 135.5 (C-1 in 4-MeOC_6H_4-), 140.0 (d, C-5, $^1J_{CF}$ = 231.5), 144.8 (C in C_6H_5), 148.9 (C-2), 157.1 (d, C-4, $^2J_{CF}$ = 26.0), 158.11, 158.13 (C *para* in 4-MeOC_6H_4-). ^{15}N NMR δ: 136.0 (N-1), 157.9 (N-3). ^{19}F NMR δ: −91.41 ($^3J_{FH}$ = 6.8).

4.1.5. *3′-O-(tert-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-5-fluoro-2′-deoxy uridine (8)*

Compound **7** (3.6 g, 6.56 mmol) and imidazole (5 eq, 2.2 g, 33.0 mmol) were dissolved in anhydrous pyridine (36 mL) and after that *tert*-butyldimethylsilyl chloride (1.2 eq, 1.2 g, 7.9 mmol) was added. The reaction mixture was being stirred at room temperature under Ar atmosphere overnight. The mixture was evaporated to dryness and then evaporated twice with toluene. The organic layer was extracted with saturated aqueous sodium bicarbonate, then with methylene chloride and evaporated to dryness. The residue was purified with silica gel column chromatography, using methylene chloride–methanol (5%) as an eluent to give pure **8** (white solid, yield: 2.40 g, 55%). ^{1}H NMR (DMSO-d$_6$) δ: −0.06, −0.01 (s, 6H, $(CH_3)_2$Si), 0.77 (s, 9H, $(CH_3)_3$), 2.12−2.17 (m, 1H, H-2′α, $^3J_{H2''H1'}$ = 5.50 Hz, $^3J_{H2''H3'}$ = 5.40 Hz, $^2J_{H2'H2''}$ = 12.8 Hz), 2.29−2.34 (ddd, 1H, H-2′β, $^3J_{H2'H1'}$ = 6.70 Hz, $^3J_{H2'H3'}$ = 6.50 Hz, $^2J_{H2'H2''}$ = 12.8 Hz), 3.17−3.21 (dd, 1H, H-5′, $^3J_{H5'H4'}$ = 5.00 Hz, $^2J_{H5'H5''}$ = 10.8 Hz), 3.22−3.25 (dd, 1H, H-5″, $^3J_{H5''H4'}$ = 3.20 Hz, $^2J_{H5'H5''}$ = 10.8 Hz), 3.73 (s, 6H, 2×CH_3O), 3.78−3.80 (m, 1H, H-4′, $^3J_{H4'H5'}$ = 5.00 Hz, $^3J_{H4'H5''}$ = 3.20, $^3J_{H4'H3'}$ = 4.00 Hz), 4.39−4.43 (m, 1H, H-3′, $^3J_{H3'H2'}$ = 6.50, $^3J_{H3'H2''}$ = 5.40, $^3J_{H3'H4'}$ = 4.00 Hz), 6.10−6.12 (m, 1H, H-1′, $^3J_{H1'H2'}$ = 6.70 Hz, $^3J_{H1'H2''}$ = 5.50 Hz), 6.87−6.89 (dd, 4H, *meta* in 4-MeOC_6H_4-), 7.23−7.31 (m, 7H, DMTr), 7.37−7.40 (m, 2H, DMTr), 7.95 (d, 1H, H-6, $^3J_{HF}$ = 6.8), 11.86 (brs, 1H, NH). ^{13}C NMR δ: −5.18, −4.81 ($(CH_3)_2$Si), 17.54 ($C(CH_3)_3$), 25.56 ($C(CH_3)_3$), 39.30 (C-2′, signal overlapped with DMSO), 55.01 (2× $CH_3OC_5H_4$), 62.83 (C-5′), 70.86 (C-3′), 84.26 (C-1′), 85.00 (C-4′), 85.89 (CPh_3), 113.2 (C *meta* in 4-MeOC_6H_4-), 124.7 (d, C-6, $^2J_{CF}$ = 33.8), 127.6, 127.8 (C *ortho* in 4-MeOC_6H_4-), 135.2, 135.3 (C1 in 4-MeOC_6H_4-), 139.9 (d, C-5,

$^{1}J_{CF} = 231.2$), 144.6 (C in C_6H_5), 148.9 (C-2), 157.0 (d, C-4, $^{2}J_{CF} = 26.1$), 158.1 (C *para* in 4-$MeOC_6H_4$-). ^{19}F NMR δ: -91.50 ($^{3}J_{FH} = 7.0$). ^{29}Si NMR δ: 20.25.

4.1.6. 3′-O-(tert-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (6)

Detritylation of 3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-dimethoxytrityl-5-fluoro-2′-deoxyuridine (**8**) (1,70 g, 2.26 mmol) was carried out using 5% *para*-toluenesulphonic acid in methylene chloride–methanol (9:1) solution (41 mL). The reaction was being stirred for 15 min at room temperature. Next saturated aqueous sodium bicarbonate and methylene chloride were added. After the extraction, the crude product **6** was purified with silica gel column chromatography, using methylene chloride–methanol (5→10%) as an eluent to give pure **6** (white solid, yield: 1.07 g, 70%). ^{1}H NMR (DMSO-d_6) δ: 0.08 (s, 6H, $(CH_3)_2$Si), 0.87 (s, 9H, $(CH_3)_3$), 2.06−2.11 (ddd, 1H, H2′α, $^{3}J_{H2''H1'} = 6.20$ Hz, $^{3}J_{H2''H3'} = 3.70$ Hz, $^{2}J_{H2'H2''} = 13.4$ Hz), 2.18−2.23 (m, 1H, H2′β, $^{3}J_{H2'H1'} = 6.90$ Hz, $^{3}J_{H2'H3'} = 6.20$ Hz, $^{2}J_{H2'H2''} = 13.4$ Hz), 3.53−3.57 (ddd, 1H, H-5′, $^{3}J_{H5'H4'} = 3.60$ Hz, $^{2}J_{H5'H5''} = 11.9$ Hz), 3.60−3.64 (ddd, 1H, H-5″, $^{3}J_{H5''H4'} = 3.80$ Hz, $^{2}J_{H5'H5''} = 11.9$ Hz), 3.77 (q, 1H, H-4′, $^{3}J_{H4'H5'} = 3.60$ Hz, $^{3}J_{H4'H5''} = 3.80$, $^{3}J_{H4'H3'} = 3.40$ Hz), 4.40−4.43 (m, 1H, H-3′, $^{3}J_{H3'H2'} = 6.20$, $^{3}J_{H3'H2''} = 3.70$, $^{3}J_{H3'H4'} = 3.40$ Hz), 5.19 (t, 1H, 5′-OH, $^{3}J_{HH} = 4.95$ Hz), 6.09−6.12 (m, 1H, H-1′, $^{3}J_{H1'H2'} = 6.90$ Hz, $^{3}J_{H1'H2''} = 6.20$ Hz), 8.18 (d, 1H, H-6, $^{3}J_{HF} = 7.2$), 11.82 (brs, 1H, NH). ^{13}C NMR δ: −4.93, −4.85 ($(CH_3)_2$Si), 17.66 ($C(CH_3)_3$), 25.65 ($C(CH_3)_3$), 39.10 (C-2′, signal overlapped with DMSO), 60.54 (C-5′), 71.58 (C-3′), 84.34 (C-1′), 87.44 (C-4′), 124.7 (d, C-6, $^{2}J_{CF} = 34.4$), 140.0 (d, C-5, $^{1}J_{CF} = 230.0$), 149.0 (C-2), 157.0 (d, C-4, $^{2}J_{CF} = 26.2$). ^{15}N NMR δ: 136.3 (N-1), 157.8 (N-3). ^{19}F NMR δ: −91.49 ($^{3}J_{FH} = 6.9$). ^{29}Si NMR δ: 19.85.

4.1.7. 3′-O-(tert-butyldimethylsilyl)-5′-O-propargyl-5-fluoro-2′-deoxyuridine (4)

Sodium hydride (2.5 eq, 0.04 g, 1.67 mmol) under N_2 atmosphere was added portionwise to a stirred solution of 3′-*O*-(*tert*-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (**6**) (0.14 g, 0.38 mmol) in anhydrous THF (3.0 mL). After stirring for 40 min, the mixture was treated with propargyl bromide (80% solution in toluene, 2.5 eq, 0.18 mL, 1.9 mmol) and heated at 70°C for 3h. Then the mixture was washed with methanol and evaporated to dryness under reduced pressure. Analytically pure sample of compound **4** was obtained with silica gel column chromatography, using hexane–ethyl acetate (2:1) as an eluent; yield: 0.13 g (white solid, 85%). ^{1}H NMR (DMSO-d_6) δ: 0.08 (s, 6H, $(CH_3)_2$Si), 0.87 (s, 9H, $(CH_3)_3$), 2.07−2.12 (ddd, 1H, H-2′α, $^{3}J_{H2''H1'} = 6.40$ Hz, $^{3}J_{H2''H3'} = 3.95$ Hz, $^{2}J_{H2'H2''} = 13.4$ Hz), 2.20−2.25 (m, 1H, H-2′β, $^{3}J_{H2'H1'} = 6.60$ Hz, $^{3}J_{H2'H3'} = 6.50$ Hz, $^{2}J_{H2'H2''} = 13.4$ Hz), 3.49 (t, 1H, CH propargyl, $^{4}J_{HH} = 2.35$ Hz), 3.59−3.62 (dd, 1H, H-5′, $^{3}J_{H5'H4'} = 3.55$ Hz, $^{2}J_{H5'H5''} = 10.6$ Hz), 3.67−3.70 (dd, 1H, H-5″, $^{3}J_{H5''H4'} = 4.25$ Hz, $^{2}J_{H5'H5''} = 10.6$ Hz), 3.89 (q, 1H, H-4′, $^{3}J_{H4'H5'} = 3.55$ Hz, $^{3}J_{H4'H5''} = 4.25$ Hz, $^{3}J_{H4'H3'} = 3.55$ Hz), 4.21 (dd, 2H, CH_2 propargyl, $^{4}J_{HH} = 2.38$ Hz, $^{2}J_{HH} = 15.9$ Hz), 4.37−4.40 (m, 1H, H-3′, $^{3}J_{H3'H2'} = 6.50$ Hz, $^{3}J_{H3'H2''} = 3.95$ Hz, $^{3}J_{H3'H4'} = 3.55$ Hz), 6.09−6.12 (m, 1H, H-1′, $^{3}J_{H1'H2'} = 6.60$ Hz, $^{3}J_{H1'H2''} = 6.40$ Hz), 7.96 (d, 1H, H-6, $^{3}J_{HF} = 7.1$), 11.84

(brs, 1H, NH). ^{13}C NMR δ: −4.98, −4.87 $(CH_3)_2Si$, $^1J_{CH}$ = 118.6), 17.63 $(C(CH_3)_3)$, 25.64 $(C(CH_3)_3$, $^1J_{CH}$ = 126.0), 39.10 (C-2′, $^1J_{CH}$ = signal overlapped with DMSO), 57.87 (CH_2 propargyl, $^1J_{CH}$ = 148.4), 68.75 (C-5′, $^1J_{CH}$ = 141.5), 71.76 (C-3′, $^1J_{CH}$ = 150.9), 77.59 (CH propargyl, $^1J_{CH}$ = 251.1, $^3J_{CH}$ = 4.1), 79.66 (C propargyl, $^2J_{CH}$ = 49.9, $^2J_{CH}$ = 7.1), 84.42 (C-1′, $^1J_{CH}$ = 169.6), 85.09 (C-4′, $^1J_{CH}$ = 150.2), 124.5 (d, C-6, $^2J_{CF}$ = 34.3, $^1J_{CH}$ = 181.9, $^3J_{CH}$ = 4.0), 140.0 (d, C-5, $^1J_{CF}$ = 230.8, $^2J_{CH}$ = 4.6), 148.9 (C-2, $^3J_{CH}$ = 7.9, $^3J_{CH}$ = 1.9), 157.0 (d, C-4, $^2J_{CF}$ = 26.1, $^3J_{CH}$ = 7.0). ^{15}N NMR δ: 135.7 (N-1), 157.9 (N-3). ^{19}F NMR δ: −91.25 ($^3J_{FH}$ = 7.0). ^{29}Si NMR δ: 20.23. HRMS (ESI−) calcd for $C_{18}H_{26}FN_2O_5Si$ $[M-H]^-$ 397.1595, found 397.1583.

4.1.8. *5′-O-propargyl-5-fluoro-2′-deoxyuridine (3)–desilylation of compound 4*

Ammonium fluoride (5 eq, 0.18 mg) was added to a stirred solution of **4** (1 mmol, 0.40 mg) in abs. MeOH (4 mL) and the reactants were heated at reflux for 4 h. After that time, the silica gel was added to the reaction mixture and it was evaporated under reduced pressure. The residue was purified with silica gel column chromatography, using the mixture chloroform–methanol (5→10%) as an eluent, to afford product **3** (white solid, yield 75–87%). ^{1}H NMR (DMSO-d_6) δ: 2.09−2.12 (dd, 2H, H-2′β, H-2′α, $^3J_{H2'H1'}$ = 7.10 Hz, $^3J_{H2''H1'}$ = 6.30 Hz, $^3J_{H2'H3'}$ = 5.75 Hz, $^3J_{H2''H3'}$ = 3.60 Hz, $^2J_{H2'H2''}$ = 14.0 Hz), 3.48 (t, 1H, CH propargyl, $^4J_{HH}$ = 2.35 Hz), 3.57−3.61 (dd, 1H, H-5′, $^3J_{H5'H4'}$ = 4.36 Hz, $^2J_{H5'H5''}$ = 10.6 Hz), 3.67−3.70 (dd, 1H, H-5″, $^3J_{H5''H4'}$ = 3.35 Hz, $^2J_{H5'H5''}$ = 10.6 Hz), 3.89 (q, 1H, H-4′, $^3J_{H4'H5'}$ = 4.36 Hz, $^3J_{H4'H5''}$ = 3.35 Hz, $^3J_{H4'H3'}$ = 3.00 Hz), 4.16−4.26 (dd, 3H, H-3′, CH_2 propargyl, $^3J_{H3'H2'}$ = 5.75 Hz, $^3J_{H3'H2''}$ = 3.60 Hz, $^3J_{H3'H4'}$ = 3.00 Hz, $^4J_{HH}$ = 2.35 Hz, $^2J_{HH}$ = 15.9 Hz), 5.35 (t, 1H, 3′-OH, $^3J_{HH}$ = 4.05 Hz), 6.11−6.15 (m, 1H, H-1′, $^3J_{H1'H2'}$ = 7.10 Hz, $^3J_{H1'H2''}$ = 6.30 Hz), 7.95 (d, 1H, H-6, $^3J_{HF}$ = 7.1), 11.81 (brs, 1H, NH). ^{13}C NMR δ: 39.10 (C-2′, $^1J_{CH}$ = signal overlapped with DMSO), 57.85 (CH_2 propargyl $^1J_{CH}$ = 148.4), 69.43 (C-5′, $^1J_{CH}$ = 142.4), 70.49 (C-3′ $^1J_{CH}$ = 150.2), 77.55 (CH propargyl $^1J_{CH}$ = 251.1, $^3J_{CH}$ = 4.2), 79.77 (C propargyl, $^2J_{CH}$ = 50.0, $^2J_{CH}$ = 7.1), 84.63 (C-1′, $^1J_{CH}$ = 170.6), 85.28 (C-4′, $^1J_{CH}$ = 148.6), 124.4 (d, C-6, $^2J_{CF}$ = 34.3, $^1J_{CH}$ = 181.8, $^3J_{CH}$ = 4.0), 140.0 (d, C-5, $^1J_{CF}$ = 231.0, $^2J_{CH}$ = 4.5), 149.0 (C-2, $^3J_{CH}$ = 7.9, $^3J_{CH}$ = 1.8), 157.0 (d,C-4, $^2J_{CF}$ = 26.1, $^3J_{CH}$ = 7.0). ^{15}N NMR δ: 135.1 (N-1), 157.8 (N-3). ^{19}F NMR δ: −91.21 ($^3J_{FH}$ = 7.0). HRMS (ESI−) calcd for $C_{12}H_{12}FN_2O_5$ $[M-H]^-$ 283.0730, found 283.0727.

4.2. *Biology*

4.2.1. *Cell cultures*

When it comes to biological experiments, human cancer cells HeLa (cervical cancer cell line) and KB (*Carcinoma nasopharynx*) were cultured in RPMI 1640 medium and human cancer cells MCF-7 (breast cancer cell line) were cultured in D-MEM medium. Both media were supplemented with 10% fetal bovine serum, 1% l-glutamine and 1% penicillin/streptomycin solution. The cell lines were stored at

37°C, in an incubator. The 5×10^4 cells was determined as the optimal plating density of the cell lines. All the cell lines were obtained from The European Collection of Cell Cultures (ECACC) supplied by Sigma-Aldrich.

4.2.2. *In vitro cytotoxicity assay*

The protein-staining sulforhodamine B (SRB, Sigma-Aldrich) microculture colorimetric assay, developed by the National Cancer Institute (USA) for in vitro antitumor screening, was used in this study to estimate the cell number by providing a sensitive index of total cellular protein content, linear to cell density. The monolayer cell culture was trypsinized and the cell count was adjusted to 5×10^4 cells. The diluted cell suspension (0.1 mL; approximately 10.000 cells) was added to each well of the 96 well microtiter plate. After 24 hours, when a partial monolayer was formed, the supernatant was washed out and 100 μL of six different compound concentrations (0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 μM) were added to the cells in microtitre plates. The tested compounds were dissolved in DMSO (20 μM) and the content of DMSO did not exceed 0.1%; this concentration was found to be nontoxic to the cell lines. The cells were exposed to compounds for 72 hours. After this time, 25 μL of 50% trichloroacetic acid was added to the wells and the plates were incubated for 1 hour at 4°C. Then the plates were washed out with the distilled water to remove traces of medium and next dried by the air. The air-dried plates were stained with 100 μL SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing it out with 1% acetic acid and then drying with air overnight. The optical density was measured at 490 nm. All cytotoxicity experiments were performed three times and the values presented in Table 1, which are the mean values. Cell survival was measured by comparing the percentage of absorbance with the control (non-treated cells). Cytarabine (Sigma-Aldrich) was used as the internal standard.

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