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Dedicated to Professor Alexandru T. Balaban on the occasion of his 80th anniversary

SYNTHESIS OF NEW OXABICYCLO[3.3.0]OCTANE NUCLEOSIDE ANALOGUES WITH PYRIMIDINE BASES

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Synthesis of new nucleosides with an oxabiciclo[3.3.0]octane fragment in the sugar moiety was performed by Vorbruggen nucleoside synthesis with silylated pyrimidine bases: 5-FU, 5-ClU, 5-BrU, C and 6-aza-U. The compounds were characterized by IR, MS, ^{1}H-NMR and ^{13}C-NMR spectra and tested for their antitumor activity comparatively with U-34 and 5IU-34. Our study points out that Cl-U-34, Br-U-34 and I-U-34 might be more efficient than U-34 against the multiplication of cancer cells, like Jurkat T lymphoblasts.

INTRODUCTION

Our program for discovering substances possessing antiviral and anticancer activity was firstly focused on nucleosides and their analogues that are for a long time a recognized class of clinically useful drugs covering these fields. Some new nucleoside analogues were synthesized having a bicyclo[2.2.1]heptane fragment instead of the sugar moiety linked to O^2, O^2,O^4- or N^1,O^4- pyrimidine atoms,[1-3] a few with increased anti-neoplastic activity.

We realized also the synthesis of new nucleoside analogues in which we introduced another fragment instead of the usual sugar moiety, a functionalized oxabicyclo[3.3.0]octane[4] one, starting from an advanced intermediate (**1**) of the total stereocontrolled synthesis of Cloprostenol prostaglandine analogue with natural optical active configuration[5] (Scheme 1). Lactol group of the intermediate (**1**) was activated by acetylation to the key intermediate (**2**) which was reacted with silylated pyrimidine bases: uracil, thymine and 5-iodouracil, by the well known Vorbruggen reaction for nucleoside synthesis, in the presence of trimethylsilyl trifluoromethanesulfonate as catalyst. We finally obtained three optically active nucleoside analogues (**3a**-**3c**) which were tested for anti-neoplastic activity on cancer cells. In the concentration range (0-30) μM thymine nucleoside (T-34, **3b**) had no significant biological activity, but uracil- (U-34, **3a**) and 5-iodouracil- (5-IU-34, **3c**) nucleosides proved to disturb uridine and thymidine metabolism of leukemia-like cells. Also, from 5-IU-34 was obtained the tritium labeled 5-^{3}HU-34 used in biological tests.[6]

Addition of iodine to the U-34 nucleoside analog (IU-34) improved the anti-cancer activity of U-34, at least by amplifying the inhibitory action on uridine and thymidine uptake by Jurkat T lymphoblasts. Therefore, we initiated the synthesis of other 5-substituted uracil nucleoside analogues and other pyrimidine nucleosides and a preliminary in vitro screening of the anti-cancer activity of U-34-type nucleoside analogues containing F, Cl, Br and I.

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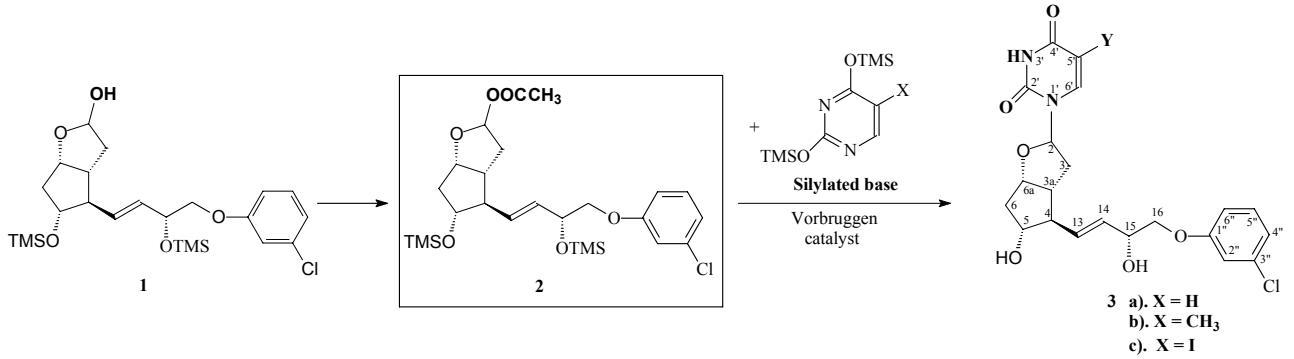


Scheme 1 – Synthesis of nucleoside analogues (**3**) with oxabicyclo[3.3.0]octane fragment as sugar moiety.

RESULTS AND DISCUSSION

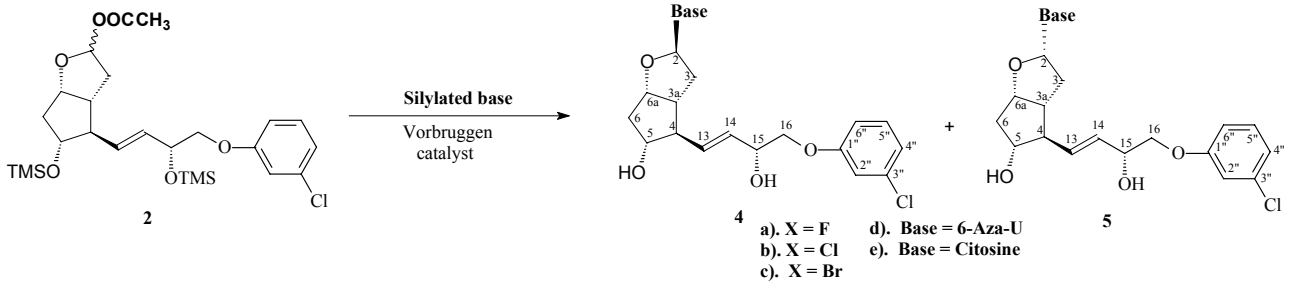
In the proceeding paper[5] we used the intermediate (**2**) with natural optical active configuration, but in this study we used this intermediate only for synthesis of optically active 6-azauracil nucleoside (**4d**) (Scheme 2). For all other compounds we started from the racemic intermediate (**1**) from the synthesis of racemic Cloprostenol prostaglandin analogue, which contains the characteristic ω-side chain and is transformed to final prostaglandin analogue by a stereocontrolled Wittig reaction for introducing the α-side chain of prostaglandins. We decided to synthesize the racemic nucleoside analogues of type (**4**) taking into account that not only natural D-series nucleosides, but also many L-enantiomers have efficient antitumor and antiviral activity. Moreover, for a preliminary *in vitro* screening it is more convenient to use the racemic form and for the specified biologically active compound to further synthesize the enantiomers.

This racemic compound was activated for the next reaction by acetylation to the lactol group like the optically active one to the key racemic intermediate (**2**) with the same IR, ^{1}H-NMR and ^{13}C-NMR spectra.

The pyrimidine bases: 5-fluorouracil (5-FU), 5-clorouracil (5-ClU), 5-bromouracil (5-BrU), 6-Azauracil (6-AzaU) and cytosine (C) were silylated to the corresponding 2,4-bis-O-trimethylsilyl derivatives with hexamethyldisilazane (HMDS) and a catalytic quantity of ammonium sulfate.[5,7] They were then used in the Vorbruggen reaction with the activated lactol (**2**) to obtain the corresponding new nucleoside analogues (**4**), which were purified by pressure chromatography. A minor quantity of anomeric nucleoside (**5**) was formed in this reaction, difficult to separate by pressure chromatography, but for 5-Cl-U and 5-Br-U the anomers (**5b**) and (**5c**) were isolated pure and characterized.

By this work we obtained **5 new nucleoside analogues** with an oxabiciclo[3.3.0]octane skeleton instead of usual sugar moiety: F-U-34, Cl-U-34, Br-U-34, 6-Aza-U-34, C-34 and two anomers (**5b**) and (**5c**) which were fully analyzed by elemental analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectra.

These 5 new nucleoside analogues were analyzed comparatively with U-34, I-U-34 for their *in vitro* biological activity on Jurkat T lymphoblasts. We investigated the effects exerted on cell viability/multiplication and on uridine uptake. Results are presented in Table 1.



Scheme 2 – Synthesis of nucleoside analogues (**4**) with oxabicyclo[3.3.0]octane fragment as sugar moiety.

Table 1

The effect exerted *in vitro* by nucleoside analogs on MTS reduction and uridine uptake by Jurkat T lymphoblasts. Inhibitory effects were highlighted

	Effect on MTS reduction Mean (CV%)				**Effect on uridine uptake** Mean (CV%)			
Concentration (μM)	**6.25**	**12.5**	**25**	**50**	**6.25**	**12.5**	**25**	**50**
	0,91	1,08	1,05	0,90	0,97	***0,57***	***0,66***	0,98
U-34	0	-3	0	-1	-1	-3	-1	-1
	0,86	***0,87***	0,91	0,92	0,93	***0,72***	***0,87***	1,23
F-U-34	-2	-1	-2	0	-12	-5	-4	-13
	0,93	0,90	0,91	***0,79***	1,03	***0,68***	***0,63***	1,21
Cl-U-34	-1	0	-1	-1	-6	-5	-3	-7
	0,86	***0,87***	***0,85***	***0,84***	0,80	***0,72***	***0,65***	0,86
Br-U-34	-4	-1	-3	-3	-6	-4	-3	-3
	0,99	1,00	1,02	***0,62***	***0,86***	***0,58***	***0,60***	0,84
I-U-34	-1	-1	0	-3	-4	-5	-4	-2
	0,96	0,96	0,98	***0,85***	1,01	1,01	1,19	0,87
6-Aza-U-34	-1	0	-1	0	-13	-4	-7	-2

Our experimental data (Table 1) showed that all investigated nucleoside analogues, except for F-U-34, restricted cell viability/multiplication more profoundly than U-34, at 50 μM. Nevertheless, F-U-34 was active at lower concentrations (6.25, 12.5 μM), whilst Br-U-34 had a concentration-independent inhibitory action for all investigated doses.

We further showed that F-U-34, Cl-U-34, Br-U-34 and I-U-34 inhibited uridine uptake by Jurkat lymphoblasts at 6.25 μM and 12.5 μM. This effect on uridine uptake was more pronounced than the decrease of cell multiplication/viability and was observed even at non-cytotoxic concentrations of nucleoside analogues. We noticed that I-U-34 had the highest activity on Jurkat cells, by inhibiting uridine uptake at lower concentrations than U-34 (6.25 μM), whilst 6-Aza-U-34 had no real activity in this respect. C-34 had no activity on the viability/multiplication and uridine uptake by Jurkat cells (data not shown).

EXPERIMENTAL

Melting points were determined in open capillary and are uncorrected. Progress of the reaction was monitored by TLC on Merck silica gel 60 or $60F_{254}$ plates eluted with the solvent system presented for each compound. ^{1}H-NMR and ^{13}C-NMR spectra are recorded on Varian Gemini 300 BB spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively), chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra: COSY, HETCOR and trifluoroacetic acid added, were done for correct attribution of NMR signals. The numbering of the compounds is presented in the Schemes 1 and 2. Dichloromethane (DCE) was anhydrized on P_2O_5 and distilled, the other reagents were of reagent grade.

Racemic key intermediate (**2**) was obtained from racemic lactol (**1**) as we presented for optically active one in the proceeding paper[5] and presented the same IR, ^{1}H-NMR and ^{13}C-NMR spectra.

***In vitro* study, methods:**

Cells. Jurkat T lymphoblasts were maintained in culture according to ECACC (European Cell Culture Collection, UK). Cell passage was performed every 48 hrs. For the experiments, cells were seeded at 10^4 cells/ 100 μL in the absence and presence of nucleoside analogues, and were measured after 48 hrs. The solvent (DMSO) was used as control.

Cell viability/multiplication was measured by the tetrazolium salt (MTS) reduction test, using CellTiter 96^R AQ_{ueous} One Solution Cell Proliferation Assay (Technical Bulletin #TB112, Promega). Results were expressed as optical density (OD).

Uridine uptake by Jurkat cells was assessed using tritium-labeled uridine, kindly provided by Dr. Cristian Postolache from the Institute of Physics and Nuclear Engineering "Horia Hulubei", Magurele. Cells, cultivated as described above, were incubated with tritium-labeled uridine (0.5 μCi/100 μL culture) for the last 6 hrs of cultivation. Cells were harvested and β-radioactivity was recorded in scintillation cocktail, using a Canberra-Packard beta-counter. Results were expressed as pulses per minute (ppm).

Statistics. Results were expressed as mean ± standard deviation (SD) for triplicate samples. The coefficient of variation (CV%) was calculated as (mean/SD)x100. The effect of nucleoside analog was calculated against the solvent (DMSO) as (parameter value in presence of nucleoside analog)/(parameter value in presence of DMSO).

1. Synthesis of silylated pyrimidines for Vorbruggen[8,9] nucleoside synthesis

The pyrimidine bases: 5-fluorouracil (5-FU), 5-clorouracil (5-ClU), 5-bromouracil (5-BrU), 6-Azauracil (6-AzaU) and cytosine (C) were silylated to the corresponding 2,4-bis-O-trimethylsilyl derivatives with hexamethyldisilazane (HMDS) and a catalytic quantity of ammonium sulfate,[4,7] heating to reflux until the solution became clear followed by another 1-2 h of heating. The excess HMDS was removed under reduced pressure, coevaporated with anh. DCE, dissolved in anh. DCE and the solution was used in the next Vorbruggen reaction with acetyl activated lactol (**2**).

2. Synthesis of oxabicyclo[3.3.0]octane nucleoside analogues with pyrimidine bases

The new oxabicyclo[3.3.0]octane nucleosides analogues were synthesized by Vorbruggen reaction[8,9] of the previously obtained silylated pyrimidines with acetylated lactol (**2**) in anh. DCE as solvent (molar ratio silylated pyrimidine: acetylated lactol, 2:1), under anh. argon atmosphere and stirring, in the presence of trimethylsilyl trifluoromethanesulfonate as catalyst, added dropwise at -10 to -15ºC. The reaction was continued for a few hours or overnight (monitoring by TLC, dichloromethane-methanol, 9:1), stopped by adding ice and $NaHCO_3$ saturated solution and work-up as previously mentioned[5].

2.1. Synthesis of 1-{4-[4-(Chloro-phenoxy)-3-hydroxy-but-1-enyl]-5-hydroxy-hexahydro-cyclopenta[b]furan-2-yl}-5-fluoro-1H-pyrimidine-2,4-dione **(5-FU-34, 4a)**

Reaction time: overnight. TLC ($R_{f\ (4a)}$ = 0.29, $R_{f\ (5a)}$ = 0.34). Starting from 7 mmoles of acetylated lactol (**2**), 3.77 g of crude product were obtained and purified by pressure chromatography (Silica gel, eluent: dichloromethane-methanol, 9:1). 1.37 g (43.21%) Pure **5-FU-34** (**4a**) nucleoside analogue were obtained along with 0.94g of impure product impurified mainly with its anomer. The anomer (**5a**) was not obtained pure to be further characterized.

(**5a**): Elemental analysis, th. for $C_{21}H_{22}ClFN_2O_6$, C: 55.70, H:4.90, N: 6.19 found: C:55.42; H:4.63, N:6.02.

IR: 2941w, 1672s, 1594m, 1478m, 1399m, 1329m, 1260vs, 1057vs, 1022s, 964s, 902s, 847s, 776s, 683s cm^{-1}. ^{1}H-NMR(dmso-*d6*, δ ppm, *J*Hz)): **11.78**(s, 1H, NH, deuterable)**; 7.89**(d, 1H, H-6', 6.9); **7.24**(t, 1H, H-5", 8.1); **6.96**(t, 1H, H-2", 2.1); **6.94-6.87**(m, 2H, H-4", H-6"); **6.11**(t, 1H, H-2, 6.5); **5.70-5.55**(m, 2H, AB sist., H-13,14); **4.66**(dt,1H, H-6a; 3.6, 6.8); **4.30**(dt,1H, H-15; 4.6, 6.6, with TFA); **3.91**(dd, 1H, H-16, 4.6, 10.0); **3.85**(dd, 1H, H-16, 6.6, 10.0); **3.70**(q, 1H, H-5, 8.5, with TFA); **2.52-2.40**(m, 1H, H-3a); **2.27**(dt, 1H, H-6, 6.8, 13.7); **2.16-2.04**(m, 3H, 2H-3, 1H4); **1.53**(ddd, 1H, H-6, 3.6, 8.5, 13.7). ^{13}C-NMR($CDCl_3$, δ ppm): **159.75**(C-1"); **157.32**(d, J=26.0Hz, C-4'); **140.16**(d, J=229.6Hz, C-5'); **133.87**(C-3"); **132.16**(C-13 or 14); **131.10**(C-14 or 13); **125.29**(d, J=13.5Hz, C-6'); **120.68**(C-4"); **114.82**(C-2"); **113.86**(C-6"); **86.35**(C-2); **82.22**(C-6a); **76.14**(C-5); **72.48**(C-16); **69.35**(C-15); **54.07**(C-4); **45.95**(C-3a); **41.41**(C-6); **36.79**(C-3).

2.2. Synthesis of 1-{4-[4-(3-Chloro-phenoxy)-3-hydroxy-but-1-enyl]-5-hydroxy-hexahydro-cyclopenta[b]furan-2-yl}-5-Chloro-1H-pyrimidine-2,4-dione **(5-ClU-34, 4b)**

Reaction time: 3h. TLC ($R_{f\ (4b)}$ = 0.25, $R_{f\ (5b)}$ = 0.27). Starting from 7 mmoles (3.5 g) of acetylated lactol (**2**), 3.25 g of crude product were obtained which were purified by pressure chromatography (Silica gel, eluent: dichloromethane-methanol, 9:1). 1.85 g (57.05%) Pure 5-ClU-34 (**4b**) nucleoside analogue were obtained and 0.17g of impure product impurified mainly with its anomer (~3:1). A fraction of 40 mg of pure enantiomer (**5b**) was also obtained from the column.

(**4b**): Elemental analysis, th. for $C_{21}H_{22}Cl_2N_2O_6$, C: 53.74, H:4.72, N: 5.97 found: C:53.31; H:4.53, N:6.12. IR : 2824w, 1693vs, 1624m, 1595s, 1478m, 1433m, 1285m, 1265s, 1233s, 1066s, 1044s, 968m, 905m, 845m, 766m, 753m, 681s, 629m. ^{1}H-RMN (dmso-D_6; δ ppm; *J* Hz):**11.83**(s, 1H, NH, deuterable); **7.97**(s, 1H, H-6'); **7.28**(t, 1H, H-5", 8.0); **6.96**(t, 1H, H-2", 2.0); **6.92**(dd, 1H, H-4", 8.0, 2.0); **6.87**(dd, 1H, H-6", 8.0, 2.2); **6.09**(t, 1H, H-2, 6.4); **5.64**(d, 1H, H-14 or 13, 6.2); **5.62**(d, 1H, H-13 or 14, 6.2); **5.24**(d, OH-15, 4.9); **4.94**(d, OH-5, 4.9); **5.18**(d, 1H, OH-15, 4.9); **4.85**(d, 1H, OH-11, 5.6); **4.68**(td, 1H, H-6a, 6.8, 3.6); **4.30**(dt, 1H, H-15, 4.6, 6.6); **3.90**(dd, 1H, sist. AB, H-16A, 4.6, 10.0); **3.84**(dd, 1H, sist. AB, H-16B, 6.6, 10.0); **3.70**(m, 1H, H-5); **2.44**(m, 1H, H-3a); **2.27**(dt, 1H, sist. AB, H-6A, 13.7, 6.8); **2.18-2.07**(m, 3H, 2H-3, H-4); **1.52**(ddd, 1H, sist. AB, H-6B, 3.6, 8.4, 13.7). ^{13}C-RMN(dmso-D_6; δ ppm): **160.18**(C=O, C-4'); **159.76**(C-1"); **150.18**(C-2'); **138.58**(C-6'); **133.86**(C-3"); **132.62**(C-13 or C-14); **131.52**(C-14 or C-13); **131.38**(C-5"); **121.00**(C-4"); **115.18**(C-2"); **114.26**(C-6"); **107.86**(C-5'); **87.30**(C-2); **82.87**(C-6a); **76.55**(C-5); **72.82**(C-16); **69.81**(C-15); **55.48**(C-4); **46.42**(C-3a); **41.56**(C-6); **36.48**(C-3).

(**5b**): ^{1}H-NMR(dmso-*d6*, δ ppm, *J*Hz)): **11.84**(s, 1H, NH, deuterable)**; 8.22**(s, 1H, H-6'); **7.28**(t, 1H, H-5", 8.0); **7.00-6.96** (m, 2H, H-2", H-4"); **6.90**(dd, 1H, H-6", 2.2, 8.3); **5.92**(t, 1H, H-2, 7.0); **5.64**(dd, 1H, H-14 or 13, 6.6, 15.6), **5.53**(dd, 1H, H-13 or 14, 5.2, 15.6); **5.15**(br d, 2H, OH-15, OH-5); **4.44**(br dt,1H, H-6a, 6.3); **4.27**(br s,1H, H-15); **3.92-3.87**(m, 1H, H-5); **3.89**(dd, 1H, H-16, 5.0, 10.0); **3.84**(dd, 1H, H-16, 6.9, 10.0); **2.50-2.40**(m, 2H, H-3a-4); **2.11**(dt, 1H, H-6, 6.3, 14.0); **1.88-1.80**(m, 3H, 2H-3, 1H-6). ^{13}C-NMR($CDCl_3$, δ ppm): **159.60**(C-4'); 159.95(C-1"); **149.46**(C-2')**; 138.00**(C-6'); **133.69**(C-3"); **132.15**(C-13); **130.82**(C-5"); **130.13**(C-14); **120.47**(C-4"); **114.62**(C-2"); **113.73**(C-6"); **107.51**(Cq, C-5'); **85.76**(C-2); **82.01**(C-6a); **77.44**(C-5); **72.29**(C-16); **69.13**(C-15); **55.79**(C-4); **45.67**(C-3a); ***41.05(C-6);*** **37.28**(C-3).

2.3. Synthesis of 1-{4-[4-(3-chloro-phenoxy)-3-hydroxy-but-1-enyl]-5-hydroxy-hexahydro-cyclopenta[b]furan-2-yl}-5-bromo-1H-pyrimidine-2,4-dione **(5-BrU-34, 4c)**

Reaction time: 3h. TLC (($R_{f\ (4c)}$ = 0.24, $R_{f\ (5c)}$ = 0.29). dichloromethane-methanol, 10:0.5, $R_{f\ (4c)}$ = 0.15, $R_{f\ (5c)}$ = 0.21). Starting from 7 mmoles (3.5 g) of acetylated lactol (**2**), 2.98 g of crude product were obtained which were purified by pressure chromatography (Silica gel, eluent: dichloromethane-methanol, 9:1). Resulted 1.62 g slightly impure product which after two recristalizations from methanol-dichloromethane gave 1.15 g (32.4%) pure 5-BrU-34 (**4c**) nucleoside analogue, m.p. 160.9-162ºC and 0.37g of impure product impurified with its anomer in a ratio of about 3:1. From the column a fraction of 50 mg of pure enantiomer (**5c**) was also obtained.

(**4c**): Elemental analysis, th. for $C_{21}H_{22}ClBrN_2O_6$, C: 49.09, H:4.31, N: 5.45 found: C:49.38; H:4.48, N:5.61. IR: 1679vs, 1593m, 1447m, 1268s, 1069vs, 1033vs, 970m, 907m, 771s, 681s, 717s. ^{1}H-NMR(dmso-d_6, δ ppm, *J*Hz)): **11.79**(s, 1H, NH, deuterable)**; 8.01**(s, 1H, H-6'); **7.28**(t, 1H, H-5", 8.1); **6.98-6.94**(m, 2H, H-2"-4"); **6.90**(dd, 1H, H-6", 2.0, 8.2); **6.09**(t, 1H, H-2, 6.5); **5.65**(1H, H-14 or 13, 6.0), **5.62**(d, 1H, H-13 or 14, 6.0); **4.68**(dt,1H, H-6a; 3.6, 6.7); **4.30**(dt,1H, H-15; 4.7, 6.6); **3.91**(dd, 1H, H-16A, 4.7, 10.1); **3.85**(dd, 1H, H-16B, 6.6, 10.1); **3.71**(q, 1H, H-5, 8.0, with TFA); **2.47**(m, 1H,

H-3a); **2.28**(dt, 1H, H-6, 6.7, 13.7); **2.18-2.07**(m, 3H, 2H-3, 1H-4); **1.53**(ddd, 1H, H-6, 3.6, 8.0, 13.7). ^{13}C-NMR(dmso-d_6, δ ppm): **159.66**(C-4'); **159.18**(C-1"); **149.71**(C-2'); **140.49**(C-6'); **133.69**(C-3"); **132.01**(C-13); **130.89**(C-14 or 5"); **130.78**(CH, C-5" or C-14); **120.51**(C-4"); **114.81**(C-2"); **113.74**(C-6"); **95.65**(Cq, C-5'); **87.05**(C-2); **82.49**(C-6a); **76.04**(C-5); **72.46**(C-16); **69.23**(C-15); **54.92**(C-4); **45.89**(C-3a); **41.05**(C-6); **36.11**(C-3).

(**5c**): ^{1}H-NMR(dmso-*d6*, *δ* ppm, *J*Hz)): **11.82**(s, 1H, NH, deuterable); **8.29**(s, 1H, H-6'); **7.28**(t, 1H, H-5", 8.0); **7.00-6.96**(m, 2H, H-2"-4"); **6.90**(dd,1H, H-6", 2.3, 8.3); **5.92**(t, 1H, H-2, 7.0); **5.64**(dd, 1H, H-13, 6.5, 15.6), **5.53**(dd, 1H, H-14, 5.4, 15.6); **5,16**(d, 1H, OH, 4.0); **5,15**(d, 1H, OH, 5.2); **4.44**(dt,1H, H-6a; 2.1, 6.4); **4.27**(m, 1H, H-15); **3.90**(m, 1H, H-5); **3.90**(dd, 1H, H-16A, 4.7, 10.0); **3.84**(dd, 1H, H-16B, 6.6, 10.0); **2.50-2.40**(m, 2H, H-3a, H-4); **2.10**(dt, 1H, H-6, 6.5, 14.1); **1.89-1.78**(m, 3H, 2H-3, 1H6). ^{13}C-NMR($CDCl_3$, δ ppm): **159.57**(C-4'); **159.07**(C-1"); **149.66**(C-2'), **140.48**(C-6'); **133.67**(C-3"); **132.15**(C-13); **130.81**(C-5"); **130.10**(C-14); **120.46**(C-4"); **114.61**(C-2"); **113.73**(C-6"); **95.07**(Cq, C-5'); **85.72**(C-2); **82.00**(C-6a); **77.42**(C-5); **72.28**(C-16); **69.11**(C-15); ***55.77(C-4);*** **45.66**(C-3a); **39.79**(C-6); **37.32**(C-3).

2.4. Synthesis of 2-{4-[4-(3-Chloro-phenoxy)-3-hydroxy-but-1-enyl]-5-hydroxy-hexahydro-cyclopenta[b]furan-2-yl}-2H-[1,2,4]triazine-3,5-dione **(6-Aza-U-34, 4d)**

Reaction time: overnight. TLC ($R_{f\ (4d)}$ = 0.26). Starting from 7 mmoles (3.5 g) of optically active acetylated lactol (**2**), 2.62 g of crude product were obtained which were purified by pressure chromatography (Silica gel, eluent: dichloromethane-methanol, 9:1). Resulted 1.57 g (51.4%) pure **6-AzaU-34** (**4d**) nucleoside analogue as foam, $[\alpha]_D^{20}$= -80.4º(1% in methanol). The anomer (**5d**) was not isolated pure to be further characterized.

(**4d**): Elemental analysis, th. for $C_{20}H_{22}ClN_3O_6$, C: 55.11, H:5.09, N: 9.64 found: C:55.34; H:4.83, N:9.74. IR: 3386w, 2964m, 1687vs, 1593m, 1479m, 1404m, 1329m, 1284m, 1256s, 1231m, 1060s, 1021s, 970m, 871m, 772m, 681m. ^{1}H-NMR(dmso-d_6, δ ppm, JHz)`: **9.53**(s, 1H, NH, with TFA); **8.69**(s, 1H, H-5'); **7.28**(t, 1H, H-5", 8.1); **6.98**(t, 1H, H-2", 2.0); **6.97-6.88**(m, 2H, H-4", H-6"); **6.11**(t, 1H, H-2, 6.5); **5.73**(d, 1H, H-14 or 13, 6.1); **5.62**(d, 1H, H-13 or 14, 6.1); **5.25**(br s, 1H, OH-15, deuterable); **4.94**(br s, OH-5, deuterable); **4.61**(dt,1H, H-6a; 3.6, 6.7); **4.30**(dt,1H, H-15; 4.6, 6.6); **3.91**(dd, 1H, H-16A, 4.6, 10.0); **3.84**(dd, 1H, H-16B, 6.6, 10.0); **3.71**(m, 1H, H-5); **2.47**(m, 1H, H-3a); **2.28**(dt, 1H, H-6, 6.7, 13.7); **2.18-2.06**(m, 3H, 2H-3, 1H-4); **1.52**(ddd, 1H, H-6, 3.6, 8.3, 13.7). ^{13}C-NMR($CDCl_3$, δ ppm): **165.73**(C-4'); **159.79**(C-1"); **155.38**(C-2'); **141.37**(C-5'); **133.89**(C-3"); **132.25**(C-13 or 14); **130.95**(C-14 or 13); **131.01**(C-5"); **120.71**(C-4"); **114. 83**(C-2"); **113.91**(C-6"); **94.14**(C-5'); **86.57**(C-2); **81.51**(C-6a); **76.03**(C-5); **72.52**(C-16); **69.40**(C-15); **55.08**(C-4); **46.00**(C-3a); **41.41**(C-6); **36.69**(C-3).

2.5. *Synthesis of 4-Amino-1-{4-[4-(3-chloro-phenoxy)-3-hydroxy-but-1-enyl]-5-hydroxy-hexahydro-cyclopenta[b]furan-2-yl}-3,4-dihydro-1H-pyrimidin-2-one* **(C-34, 4e)**

Reaction time: overnight. The crude product was dissolved in 50 mL anh. methanol, 5 mL of a stock solution of 0.2M MeONa in methanol added and stirred overnight monitoring the reaction by TLC (Ethyl acetate-hexane-acetic acid, $R_{f\ BzC-34}$ = 0.41; $R_{f\ (4e)}$ = 0.04). The reaction mixture was neutralized with 1 mL acetic acid, 5 g silicagel were added, concentrated, coevaporated with benzene and purified by pressure chromatography (eluent: dichloromethane-methanol, 9:1). Starting from 7 mmoles (3.5 g) of acetylated lactol (**2**), 1.35 g (44.4%) pure C-34 (**4e**) nucleoside analogue resulted as foam. The anomer (**5e**) was not obtained pure to be further characterized.

(**4e**): Elemental analysis, th. for $C_{21}H_{22}ClFN_2O_6$, C: 58.13, H: 5.56, N: 9.68 found: C: 57.81; H: 5.39, N: 9.87. IR: 3330s, 3197s, 2931m, 1640vs, 1591vs, 1525m, 1479vs, 1408m, 1363m, 1281m, 1229m, 1060s, 1027s, 968m, 867w, 777m, 678w. ^{1}H-NMR(dmso-*d6*, *δ* ppm, *J*Hz): **7.46**(d, 1H, H-6', 7.4); **7.29**(t, 1H, H-5", 8.1); **7.14**(br d, 2H, deuterable, -NH_2); **7.01**(t, 1H, H-2", 2.2); **6.97**(dd, 1H, H-4", 8.1, 2.2); **6.91**(dd, 1H, H-6", 8.1, 2.2); **6.13**(t, 1H, H-2, 6.3); **5.73**(d, 1H, H-5', 7.4); **5.73-5.65**(m, 2H, AB sist., H-13,14); **5.20**(br s, 1H, HO-15, deuterable); **4.88**(br s, 1H, HO-5, deuterable); **4.62**(dt,1H, H-6a; 3.7, 6.8); **4.31**(m, 1H, H-15), **3.92**(dd, 1H, H-16, 4.6, 9.9); **3.86**(dd, 1H, H-16, 7.0, 9.9); **3.70**(m, 1H, H-5); **2.41**(m_q, 1H, H-3a); **2.30**(dt, 1H, H-6, 7.1, 13.6); **2.20-2.07**(m, 2H, 2H-3); **1.91**(m, 1H, H-4), **1.54**(ddd, 1H, H-6, 3.7, 8.6, 13.6).

^{13}C-NMR($CDCl_3$, δ ppm): **165.62**(C-4'); **159.64**(C-1"); **155.18**(C-2'); **141.12**(C-6'); **133.71**(C-3"); **132.03**(C-13 or 14); **130.95**(C-14 or 13); **130.85**(C-5"); **120.50**(C-4"); **114.68**(C-2"); **113.75**(C-6"); **93.98**(C-5'); **86.39**(C-2); **81.51**(C-6a); **76.01**(C-5); **72.42**(C-16); **69.21**(C-15); **54.93**(C-4); **45.85**(C-3a); **41.26**(C-6); **36.57**(C-3).

CONCLUSIONS

Starting from an advanced intermediate, (±)- and (+)-(**1**), from the synthesis of Cloprostenol prostaglandin analogue we obtained **5 new nucleoside analogues** which contain this oxabiciclo[3.3.0]octane prostaglandin skeleton instead of the usual sugar moiety of nucleosides: F-U-34, Cl-U-34, Br-U-34, C-34, 6-Aza-U-34, (the first 4 compouns as racemic, whilst the last one as optilally active compound). Though difficult to separate by pressure chromatography, we obtained also two pure anomers (**5b**) and (**5c**). The new nucleoside analogues and anomers were characterized by elemental analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectra.

Nucleosides analogues were tested *in vitro* for their anticancer activity, comparatively with U-34 and I-U-34. Our study points out that Cl-U-34, Br-U-34 and I-U-34 might be more efficient than U-34 against the multiplication of cancer cells, like Jurkat T lymphoblasts, and that I-U-34 proved to be the most promising nucleoside analogue from this series.

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