New Bicyclo[2.2.1]heptane Nucleoside Analogues as Antitumor Agents

CONSTANTIN TANASE[1], FLOREA G.COCU[1], MIRON T. CAPROIU[2], MONICA NEAGU[3], GINA MANDA[3]

[1]National Institute for Chemical-Pharmaceutical Research and Development, 112 Vitan Av., 74373 Bucharest, Romania

[2]Organic Chemistry Center "C.D.Nenitescu", 202 B Splaiul Independentei, Bucharest 6, Romania

[3]"Victor Babes" National Institute, 99-101 Splaiul Independentei, 050096 Bucharest, Romania

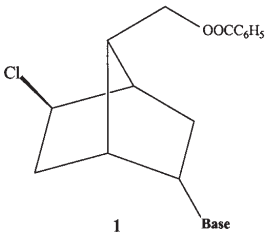
For the development of new nucleoside drugs with antitumoral activity we replaced the sugar moiety of pyrimidine nucleoside with a functionalized bicyclo[2.2.1]heptane fragment. The starting optically active compound (9) from prostaglandin analogues synthesis, was transformed in 4 steps in the key intermediate (13) in optically activity form, and coupled by Mitsunobu reaction to N^4-benzoylcytosine and 5-fluorouracil, resulting ent-27-C-Bz and ent-27-5FU-Bz nucleoside analogues. The compounds were characterized by IR, ^{1}H-NMR and ^{13}C-NMR spectra. Preliminary preclinical in vitro data shows that the ent-C-Bz exerts a biphasic cytotoxic effect on Jurkat cells at low and high concentrations, restricts viable cell multiplication and targets DNA synthesis. Meanwhile, ent-5-FU-Bz and 5-FU are cytotoxic at lower concentrations and hinder RNA synthesis in living tumor cells. We emphasize that ent-5-FU-Bz restricts Jurkat cells progenitors at earlier generations than the 5-FU standard. Our preliminary study reveals that the investigated nucleoside analogues are potential candidates for cytotoxic therapy of T lymphoblasts.

Keywords: biciclo[2.2.1]heptane pyrimidine nucleoside, carbocyclic nucleoside, Mitsunobu synthesis, NMR spectra, antitumor activity.

Carbocyclic nucleosides like nucleosides became for a long time a recognized class of clinically useful drugs possessing antitumor and antiviral activity [1].

The worldwide researches in this classs are focused to obtain new more efficient and more selective antitumor and antiviral nucleoside analogues, this being done especially by structural modification of sugar moiety, base moiety or both fragments of nucleoside.

In our previous work we replaced the sugar moiety with a functionalized oxabicyclo[3.3.0]octane fragment to obtain pyrimidine nucleoside analogues **2**, pyrimidine base being uracil and 5-iodo-uracil [2]. Deuterium and tritium labelled uracil analogues were also synthesized [3].



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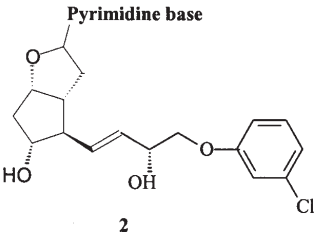

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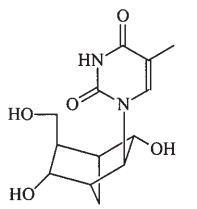
In this paper the sugar moiety is replaced with a new optically active functionalized *bicyclo[2.2.1]heptane* fragment to obtain the carbocyclic nucleosides **1**. As base, we used cytosine and 5-fluoro-uracil, the work with other bases being in progress.

Some bicyclo[2.2.1]heptane carbocyclic nucleosides derived from 3-(hydroxymethyl)bicyclo[2.2.1] heptane-2.5-diol [4] (**3**-**4**) or 7-substituted bicyclo[2.2.1]hept-5-ene-2.2-dimethanol [5] (**5**) were synthesized. Also a few analogues with 2,5-dioxabicyclo[2.2.1]heptane [6] sugar moiety (**6**) were obtained as analogues of the conformationally restricted N-type or S-type carbocyclic nucleoside analogues (**7**) and (**8**) [7]. From the last two compounds only the triphosphate N-type analogue (**7**) has antiviral activity similar to AZT-5'-triphosphate. All the other compounds proved not to have significant antiviral or antitumor activity.



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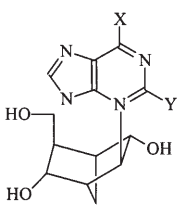

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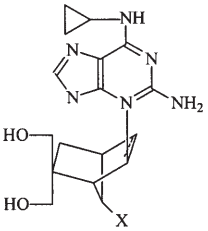

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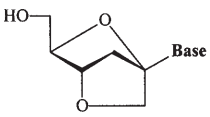

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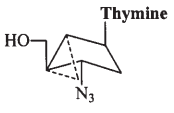

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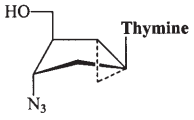

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Experimental part

Synthesis of nucleoside analogues

Melting points were determined in open capillary on a OptiMelt melting point apparatus and the values are uncorrected. Progress of the reaction was monitored by TLC on Merck silica gel 60 or $60F_{254}$ plates (Merck) eluted with the solvent system presented for each compound. IR spectra were recorded on a FT-IR- 100 Perkin Elmer spectrometer, ^{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 Schemes 1. THF and toluene were anhydrized on sodium wire, the other reagents were of reagent grade.

The starting optically active compound (9), with $[\alpha]_D^{20}$ = + 24.5°(c=1% in 0.1N KOH), m.p. 128-13°C, was transformed in the *ent*-27 alcohol (11) in the same way as the (-)- compound in the PG's synthesis. The resulted ketoalcohol (11), has $[\alpha]_D^{20}$ = - 40.5°(c=1% in MeOH), m.p. 78-79°C, ^{1}H and ^{13}C-NMR signals:

* *email: ctanase@ncpri.ro; Tel.: 0724508638*

^{1}H-RMN(DMSO-d6, δ ppm, *J* Hz): **4.63**(t, 1H, OH, deuterable, 5.2); **4.29**(dd, 1H, H-5, 4.3, 6.0); **3.78**(ddd, 1H, H-8, 5.2, 9.1, 11.2); **3.64**(dt, 1H, H-8,11.2, 5.4); **2.68**(bd, 1H, H-4, 4.9); **2.59**(m, 1H, H-1); **2.25**(dd, 1H, H-3, 17.9, 4.9); **2.17÷2.23**(m, 3H, H-6-7); **1.94**(d, 1H, H-3, 17.9). ^{13}C-RMN(DMSO-d6, δ ppm): **213.36**(C-2); **58.48**(C-5); **58.23**(C-8); **52.11**(C-1); **51.45**(C-7); **46.16**(C-4); **44.97**(C-3); **33.65**(C-6).

H, QC, Apt, COSY, HETCOR; +TFA.

Synthesis of the intermediate (12), Benzoic acid 2-chloro-5-oxo-bicyclo[2.2.1]hept-7-ylmethyl ester

100g(0.5728 moles) Ketoalcohol (**11**) were dissolved in 600 mL anh. toluene and 100 mL pyridine, the solution cooled by ice to 0°C and 78.5 mL(0.63 moles) benzoyl chloride was added dropwise under stirring. The stirring was continued overnight, monitoring the reaction by TLC (Ethyl acetate-hexane-acetic acid, 5:1:0.1, $R_{f(11)}$=0.42, $R_{f(12)}$=0.66), the mixture poured onto 1 kg crushed ice and 500 mL toluene under stirring, when there is no ice the phases were separated, the organic phase washed with sat. soln. $NaHCO_3$ (3 . 200 mL), brine (200 mL), dried (Na_2SO_4), filtered and concentrated until it began to crystallize (The aqueous phases were extracted with 300 mL toluene). After cooling on ice bath, the crystals were filtered, washed with toluene, and air dried. By concentrating the mother liquors and repeating the cristalization, there were obtained 158.3 g (99.1%) ent-27-benzoate (**12**), m.p. 121.5-122.8°C, with $[\alpha]_D^{20}$ = -37.0° (c=1% in THF), IR, ^{1}H and ^{13}C-NMR signals:

IR: 1750ms, 1720vs, 1449w, 1311mw, 1268vs, 1256vs, 1114s, 1070m, 1022m, 979w, 967w, 954w, 887w, 869w, 738mw, 711vs, 649mw, cm^{-1}.

^{1}H-NMR($CDCl_3$, δ ppm, J Hz): **8.08**(d, 2H, H-*o*, 7.6); **7.57**(t, 1H, H-*p*, 7.4); **7.44**(t, 2H, H-*m*, 7.6); **4.82**(dd, 1H, H-8, 9.3, 11.6); **4.61**(dd, 1H, H-8, 5.9, 11.6); **4.10**(dd, 1H, H-2, 4.3, 7.7); **2.95**(d, 1H, H-1, 4.9); **2.80**(d, 1H, H-4, 4.9), **2.56**(dd, 1H, H-7, 5.9, 9.3); **2.48**(dt, 1H, H-3, 4.3, 15.0); **2.41-2.26**(m, 2H, H-3-6); **1.97**(d, 1H, H-6, 18.1).^{13}C-NMR($CDCl_3$, δ ppm): **212.21**(C=O, C-5), **166.21**(COO), **133.09**(C-*p*), 129.84 (C*q*); **129.54**(C-*o*), **128.37**(C-*m*), **61.88**(C-8), **57.42**(CH-Cl), 52.65(C-4), 47.92(C-7), 46.94(C-4), **45.36**(C-6), **34.20**(C-3).

Synthesis of the intermediate (13), Benzoic acid 2-chloro-5-hydroxy-bicyclo[2.2.1]hept-7-ylmethyl ester

55.7g (0.2 moles) intermediate (**12**) were dissolved in 0.5 L methanol and 0.5 L tetrahydrofurane, the solution was cooled to -16°C (salt-ice bath) and a solution of 8.5g (0.22 moles) $NaBH_4$ in 150 mL water was added dropwise in 1.5 h under stirring. The stirring was continued for 1.5 h, monitoring the end of reaction by TLC (ethyl acetate-hexane - acetic acid, 5:4:0.1, $R_{f(12)}$=0.68, $R_{f(13)}$=0.53, $R_{f(14)}$=0.46), then carefully 20 mL acetic acid added and stirred 0.5 h. The reaction mixture was concentrated under reduced pressure, the residue distributed between 250 mL CH_2Cl_2 and 250 mL water (water extracted with 250 mL CH_2Cl_2) the organic phases washed with 250 mL sat. soln. $NaHCO_3$, 200 mL brine, dried (Na_2SO_4), filtered, concentrated to an oil. Resulted in quantitative yield 57g, $[\alpha]_D^{20}$ =+2.12° (c=1% in THF), IR, ^{1}H and ^{13}C-NMR signals:

IR: 3428br, 2963m, 1716vs, 1698vs, 1451ms, 1315ms, 1271vs, 1176m, 1112s, 1083s, 1070s, 1026ms, 1002s, 942m, 904m, 709vs, 685s cm^{-1}.

^{1}H-NMR($CDCl_3$, δ ppm, J Hz): **8.04**(d, 2H, H-*o*, 7.4); **7.56**(t, 1H, H-*p*, 7.4); **7.43**(t, 2H, H-*m*, 7.4); **4.74**(dd, 1H, H-8, 9.0, 11.4); **4.57**(dd, 1H, H-8, 6.4, 11.4); **4.23**(dt, 1H, H-5, 3.3, 11.0); **4.04**(dd, 1H, H-2, 3.8, 8.0); **2.90**(bs, 1H, OH); **2.84**(dd, 1H, H-3, 8.0, 13.7); **2.50**(m, 2H, H-1-4); **2.18-2.10**(m, 1H, H-7); **2.06**(dt, 1H, H-3, 3.8, 14.7); **0.94**(dd, 1H, H-6, 3.2, 13.7). ^{13}C-NMR($CDCl_3$, δ ppm): **166.50**(COO),**132.92**(C-*p*), **129.56**(C-*o*), **128.34**(C-*m*), **70.00**(C-5), **63.05**(C-8); **60.24**(CH-Cl), **48.36**(CH, C-1), **48.05**(CH, C-7), **45.40**(CH, C-4), **39.42**(CH_2, C-6), **32.52**(CH_2, C-3).

General procedure for the Mitsunobu reaction of bicyclo[2.2.1]heptane alcohols with ^{4}N-Benzoylcytosine and 5-fluoro-uracil

To a solution of bicyclo[2.2.1]heptane alcohols (**13**) + (**14**) (5 mmoles; 1.4 g) and triphenylphosphine (10 mmoles, 2.62 g) in 34 mL anh. THF was added 10 mmoles pyrimidine base (1.30g 5-fluorouracil and 2.15 g N^4-benzoylcytosine). To this suspension we added dropwise10 mmoles 40% toluene solution of DEAD (5 mL) diluted with 25 mL THF, in 2.5 hrs. The reaction mixture was stirred 6 days at r.t., monitoring the progress of the reactions by TLC (I, hexane-Ethyl acetate, 2:1). The solvent was removed under reduced pressure and the residue purified by multiple column chromatography (hexane-ethyl acetate, 3:1) to obtain the pure nucleoside analogue.

***ent*-27-C-Dibenzoate,** (**15**), *Benzoic acid 2-(4-benzoylamino-2-oxo-2H-pyrimidin-1-yl)-5-chloro-bicyclo[2.2.1]hept-7-ylmethyl ester*: The reaction mixture was then refluxed 5 days. Resulted: 270 mg, a less polar product (R_f = 0.52) which has no cytosine signals in ^{1}H-NMR and ^{13}C-NMR spectra, with $[\alpha]_D^{20}$ = -20,78°(c=1% in THF) and 570 mg of pure nucleoside compound (**15**) (R_f = 0.20), crystallized from hexane-ethyl acetate, m.p. 137.3-138.9°C, with $[\alpha]_D^{20}$ = + 27,95°(c=1% in THF) which was characterized by IR, ^{1}H and ^{13}C-NMR:

IR: 1703s, 1688s, 1579s, 1505m, 1488s, 1429ms, 1393s, 1347s, 1283vs, 1248vs, 1120m, 1095m, 1075m, 1058m, 1025w, 790w, 709vs, 686s cm^{-1}.

^{1}H-NMR($CDCl_3$, δ ppm, J Hz): **8.69**(br singlet, 1H, NH); **8.36**(d, 1H, H-6', 5.6); **8.04**(dd, 2H, H-*o*, 1.9, 7.2); **7.90**(d, 1H, H-5', 5.6); **7.81**(dd, 2H, H-*o*, 1.9, 7.2); **7.52-7.32**(m, 6H, H-*p*, H-*m*); **4.76**(dd, 1H, H-5, 2.6, 7.0); **4.70**(dd, 1H, H-8, 8.8, 11.4); **4.55**(dd, 1H, H-8, 6.7, 11.4), **3.85**(dd, 1H, H-2, 3.8, 8.0); **2.66-2.61**(m, 2H, H-4-7), **2.60**(d, 1H, H-1, 4.5); **2.21**(dt., 1H, H-3, 4.3,13.4), **2.09**(dd, 1H, H-3, 8.0,13.4), **1.88**(dd, 1H, H-6, 7.0, 14.3), **1.69**(br dt, 3.3, 15.4) or **1.73-1.65**(m, 1H, H-6). ^{13}C-NMR($CDCl_3$, δ ppm): **166.38**(C-4'), **165.89**(COO), **160.17**(CH, C-), **159.31**(C-2'), **132.78; 132.58**(C-*p*), **129.50; 128.83**(C-*o*), **128.12, 127.22**(C-*m*), **104.34**(C-5'), **78.05**(C-5), **62.64**(C-8); **59.07**(CH-Cl), **46.70**(C-1), **46.34**(C-7), **44.28**(C-4), **38.98**(C-6), **36.21**(C-3).

ent-27-5FU-Benzoat, (17), *Benzoic acid 2-chloro-5-(5-fluoro-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-bicyclo[2.2.1]hept-7-ylmethyl ester*. Resulted 420 mg (21.4%) pure nucleoside as oil (R_f = 0.36), with $[\alpha]_D^{20}$ = + 10.23°(c=1% in THF) which was characterized prin ^{1}H and ^{13}C-NMR:

IR: 2975m, 1772s, 1715vs, 1602m, 1585s, 1466s, 1451msh, 1415s, 1354s, 1315m, 1268vs, 1202m, 1165s, 1114s, 1069ms, 1035s, 990m, 908m, 780m, 712vs cm^{-1}.

^{1}H-NMR($CDCl_3$, δ ppm, J Hz): **8.00**(m, 2H, H-*o*); **7.96**(dd, 1H, H-6', 2.3, 8.0); **7.48** (m, 1H, H-*p*, 7.3); **7.34**(tt, 2H, H-*m*,1.5, 7.3); **4.85**(dd, ~0.5H, H-5, 2.8, 6.9); **4.71**(ddd, 1H, H-8, 1.5, 8.6, 11.8); **4.59**(dd,~0.51H, H-5, 3.3, 6.8); **4.57**(dd, 1H, H-8, 7.1, 11.8); **3.84**(dt, 1H, H-2, 3.9, 7.8); **2.63-2.56**(m, 3H, H-4-7-1), **2.21**(dt., 1H, H-3, 4.4, 14.8), **2.09**(dd, 1H, H-3, 8.0,13.4); **1.89**(dd, 1H, H-3, 7.1, 14.4), **1.83**(dd, 1H, H-6, 7.1, 14.1), **1.74-1.67**(m, 1H, H-6, 3.9). ^{13}C-NMR($CDCl_3$, δ ppm): **166.46**(C-1"); **158.90**(C_q-); **144.65**(C_q); **143.44,**

143.165(C J=20.5), **141.285,137.821**[139.55(d, C-5, J=226.5)]; **132.91**(C-*p*), **129.61**(C-*o*), **129.00; 125.25**(C-6'), **128.34**(C-*m*), **78.73, 78.20**(d,C-5), **62.67**(C-8); **59.12, 58.76**(C-Cl), **46.55**(C-1), **46.31**(C-7), **44.20**(C-4), **38.83**(C-6), **36.38; 36.10**(C-3).

Preclinical in vitro testing protocol

Reagents

RPMI 1640 medium, fetal bovine serum, antibiotic-antimycotic solution, POPOP, PPO, toluene and ethanol were purchased from Sigma-Aldrich. CellTiter 96R AQueous One Solution Cell Proliferation Assay and CytoTox 96 Non-Radioactive Cytotoxicity Assay were obtained from Promega Corporation. Vybrant DyeCycle Orange stain and CFDA-SE were purchased from Invitrogen-Molecular Probes. Tritium-labeled uridine and thymidine were obtained from the Institute of Physics and Nuclear Engineering "Horia Hulubei", Magurele, Romania.

Nucleoside analogues: ent-27-Citosine-dibenzoate (ent-C-Bz), ent-27-5-FUracil-Benzoate (ent-5-FU-Bz), and pyrimidine analogue 5-F-Uracil (5-FU).

Cells

The standardized Jurkat cell line with human leukemia T cell lymphoblast characteristics was purchased from the European Collection of Cell Cultures (ECACC) and maintained in culture according to the protocol provided by the cell line depositor.

Cellular parameters

Cell viability/multiplication was measured by the MTS reduction test, using CellTiter 96^{R} AQ$_{ueous}$ One Solution Cell Proliferation Assay. Concomitantly, cell death was evaluated as disruption of membrane integrity and measured by the LDH release test, using Cytotox96 Non-Radioactive Cytotoxicity Assay. Both tests were performed according to the manufacturer instructions.

The cell cycle was examined by flow cytometry, using the Vybrant DyeCycle Orange stain in living cell suspensions, according to the manufacturer's instructions. Briefly, at the end of cell cultivation in the absence and presence of nucleoside analogues (experimental systems containing at culture initiation 0.3 . 10^6 cell/mL), Jurkat cells (1 . 10^6 cells/mL) were labeled with 2µL of a 5mM Vybrant DyeCycle Orange stain. Cultures were incubated 30 min at 37^{0}C, and further investigated by flow cytometry. Data acquisition was performed with a FACSCalibur flow cytometer (Becton Dickinson), using the CellQuest software. The cell cycle was analyzed at the level of living cells (gated in the forward versus side scatter diagram), using the ModFit software.

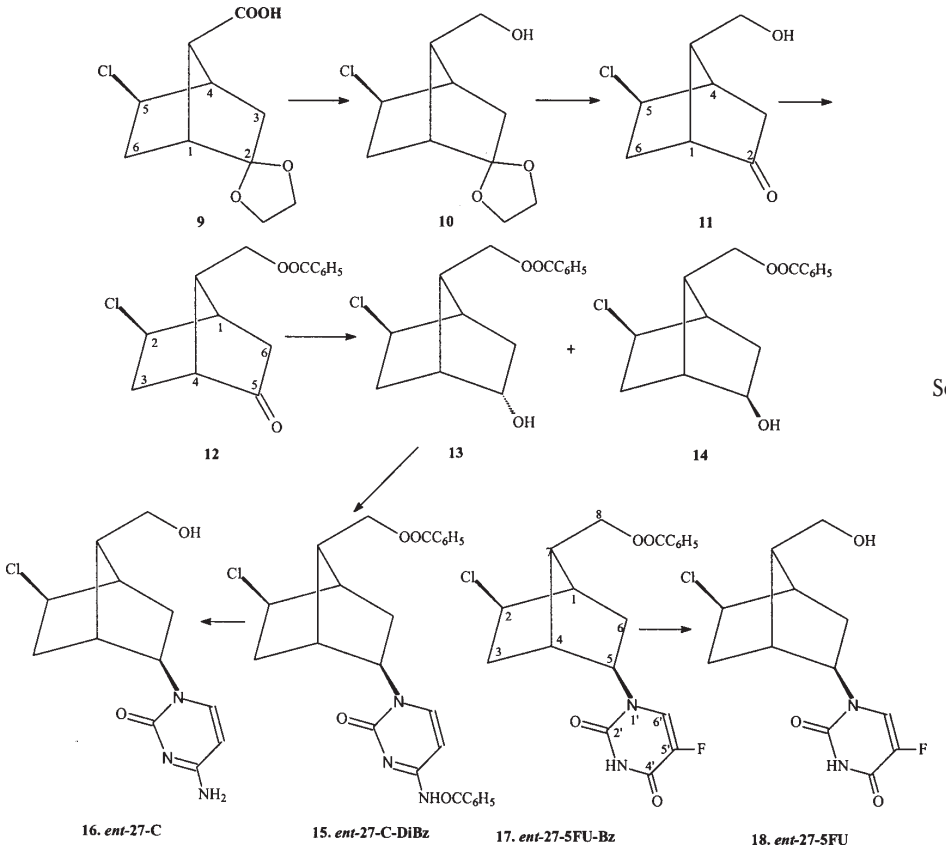
Cell progenitors of proliferative Jurkat cells were assessed by flow cytometry, using carboxyfluorescein diacetate succinimidyl ester (CFDA-SE), according to manufacturer instructions. Briefly, cells were loaded with 2µM CFDA-SE at a cell density of 50 . 10^6 cells/mL and, after thorough washing and equilibration, were cultivated in the absence and presence of nucleoside analogues (0,3 . 10^6 cell/mL). Data acquisition was performed with a FACSCalibur flow cytometer (Becton Dickinson), using the CellQuest software. Cell progenitors were identified at the level of living cells (gated in the forward versus side scatter diagram), using the ModFit software.

Nucleotide uptake *via* the salvage pathway was evaluated using the tritium-labeled uridine or thymidine incorporation method, reflecting RNA and DNA synthesis, respectively (9,10).

Results and discussions

Synthesis of the compounds

To realize the synthesis of the new bicyclo[2.2.1]heptane carbocyclic nucleoside we started from the enantiomer compound (**9**), resulted as by-product in the total stereocontrolled synthesis of prostaglandin analogues of natural serie D. This compound has two functional groups to be used in the synthesis of carbocyclic nucleosides: the carboxyl group, which must be reduced to hydroxymethyl group of nucleosides, and the 5-keto group also to be



Scheme 1. Synthesis of ent-27 pyrimidine carbocyclic nucleoside analogues

reduced to the secondary alcohol which is used to couple the bicyclo[2.2.1]heptane fragment to pyrimidine or purine base by Mitsunobu reaction with inversion of configuration.

Reduction of the carboxyl group with borane and deprotection of the ethyleneketal group by acid catalyzed transketalization with acetone gave ent-27 ketoalcohol (**11**). All the compounds (**9**) to (**11**) are crystallized, easily purified compounds from PG's synthesis.

The coupling of nucleobase must be realized on the 5th position of the bicyclo[2.2.1]heptane skeleton, on a functional group created from the 5-keto group, a hydroxyl group or an activated group derived from this. Therefore we found it useful to protect first the primary hydroxyl group as benzoate (benzoyl chloride, pyridine, almost quantitative) which does not react in further sequence'reactions and then to reduce 5-ketone group to a secondary alcohol group (scheme 1).

The reduction was realized with sodium borohydride in methanol to the alcohols (**13**) and (**14**) in a ratio of ~10:1, difficult to separate by column chromatography. Reduction with 2 equivalents of a more selective reagent 9-BBN resulted in a slower selectivity to the alcohol (**13**). The alcohol (**13**) is the key intermediate for use in the synthesis of new carbocyclic nucleosides with bicyclo[2.2.1]heptane fragment in the sugar moiety. However, in the present experiments we used the mixture of alcohols (**13**) and (**14**) taking into account that, during the purification of the nucleoside, the secondary product from the alcohol (**14**) will be separate at this stage.

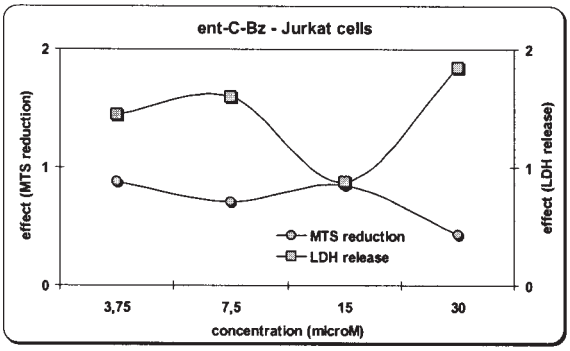
So, the mixture of alcohols was reacted in the Mitsunobu conditions (Ph_3P, DEAD,THF) with N^4-benzoyl-cytosine and 5-fluoro-uracil and the resulted benzoylated carbocyclic nucleosides (**15**) and (**17**) were purified by pressure chromatography. ent-27-C-DiBenzoate (**15**) was obtained crystalized, m.p. 137.3-138.9°C in 23.8% yield and ent-27-5FU-Benzoat (**17**) as oil 21.4% yield. In both reactions a major less polar by-product was also formed which doesn't present the signals of pyrimidine ring in ^{1}H and ^{13}C-NMR. Analytical data confirmed the proposed structure of the compounds, in ^{1}H and ^{13}C-NMR spectra being present the specific signals of the pyrimidines and bicyclo[2.2.1] heptane fragments of the molecule, but there is a difference between these two compounds. If the cytosine derivative is a single compound, the 5-FU-nucleoside appears to be a mixture of two isomers (in ^{1}H-NMR the protons at δ 4.85 and 4.59 ppm have each ~0.5H intensity on integration; in ^{13}C-NMR, the C-2, C-3, C-5 appears as doublet). This fact will be followed in the reaction of the same bicyclo[2.2.1]heptane intermediate with the N^3-acylated - 5-FU in which the N^3-linkage is blocked [8].

Though the compounds obtained were afterwards hydrolized to the nucleoside analogues (**16**) and (**18**) [8]we used the prodrug benzoate protected nucleosides (**15**) and (**17**) in biological testing of their toxicity and antitumoral activity, taking into account that ester groups could be hydrolized by enzimes to the target (unesterified) nucleosides.

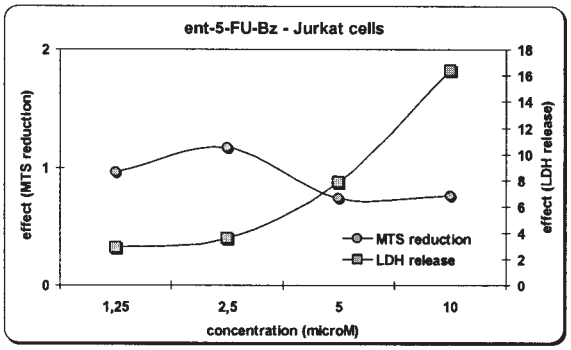
Preclinical in vitro testing for anti-tumoral activity

We performed a preliminary study regarding the antitumor action of the nucleoside analogues ent-C-Bz and ent-5-FU-Bz, in comparison with the conventional therapeutic nucleoside analogue 5-FU. Therefore we investigated the effects exerted *in vitro* by the above mentioned analogues on the standardized human tumor cell line Jurkat, presenting characteristics of human lymphoblastic T cell leukemia. We studied the following cellular parameters: cell viability and multiplication (measured by the MTS reduction test which estimates the relative amount of living cells in culture), membrane integrity (assessed by the LDH release test), cell cycle (evaluated in living cells by flow cytometry with the fluorescent Vybrant Orange stain), proliferative cells progenitors (measured by flow cytometry using CFDA-SE as fluorescent marker), nucleotide metabolism *via* the salvage pathway (evaluated as tritium-labeled uridine or thymidine incorporation). Tumor cells were cultivated for 48h in the presence of nucleoside analogues, and thereafter cellular parameters were measured.

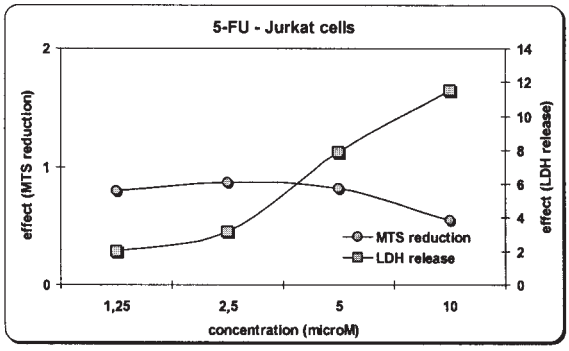
We first analyzed the effects of the above mentioned nucleoside analogues on Jurkat cells, by following-up cell viability and multiplication, evaluated as MTS reduction, in



a).



b).



c).

Fig. 1. Dose-effect curves for ent-C-Bz, ent-5-FU-Bz and 5-FU on Jurkat tumor cells cultured with nucleoside analogues for 48h

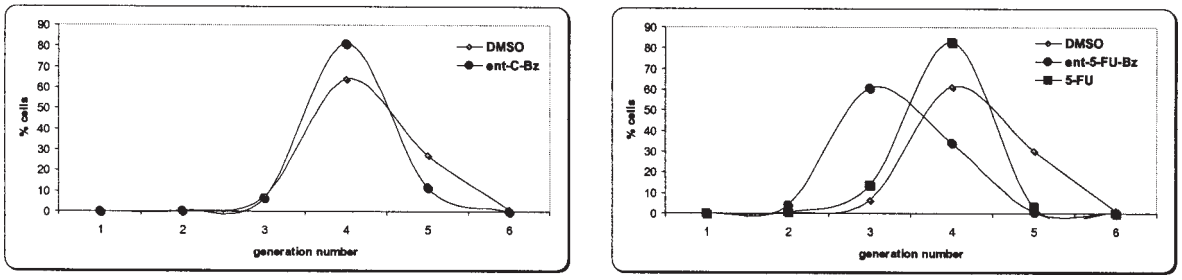


Fig. 2. The percentage of Jurkat cells in successive generations after 48h-treatment with 30ìM ent-C-Bz, 10µM ent-5-FU-Bz or 10µM 5-FU

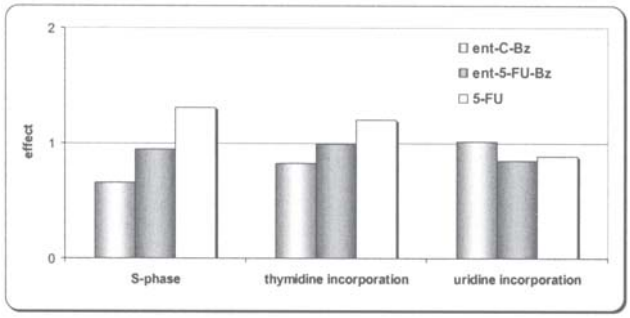


Fig. 3. Correlation between the percentage of cells in the S-phase of cell cycle and thymidine/uridine uptake by Jurkat cells treated for 48h with 30ìM ent-C-Bz, 10ìµM ent-5-FU-Bz or 10µM 5-FU

correlation with LDH release. Cytotoxicity is reflected by simultaneous inhibition of MTS reduction and enhancement of LDH release by the investigated nucleoside analogues. The dose-effect curves for ent-C-Bz, ent-5-FU-Bz and 5-FU on Jurkat cells are described in figure 1. A biphasic cytotoxic effect of ent-C-Bz at lower (7.5µM) and higher (30µM) concentrations was highlighted. Meanwhile, ent-5-FU-Bz shows a more pronounced cytotoxic effect starting with 5µM; it is worth noticing that a disruption of the membrane integrity at lower concentrations is recorded, without a significant decrease of viable cell number. An almost similar effect is registered for the conventional chemotherapeutic 5-FU.

We further investigated the proliferation characteristics of the remaining viable Jurkat cells, after 48hrs of treatment with cytotoxic concentrations of nucleoside analogues (30µM ent-C-Bz, 10µM ent-5-FU-Bz and 10µM 5-FU). As shown in figure 2, ent-5-FU-Bz impedes cell proliferation at the level of the 4th generation, while 5-FU at the level of the 5th one, compared with the corresponding solvent. ent-C-Bz moderately restrains tumor cell proliferation at the 5th generation. The experimental data presented in figure 3 reveal that ent-C-Bz determines a decrease of the percentage of living cells in the S-phase of cell cycle, accompanied by the inhibition of thymidine uptake. 5-FU-based nucleoside analogue moderately decrease uridine uptake, while not significantly affecting the living cells in the S-phase, including thymidine uptake.

Conclusions

Starting from the enantiomer compound (**9**), resulted as by-product in the total stereocontrolled synthesis of prostaglandin analogues of natural serie D, by a 4 reactions sequence, we obtained in high yield the optically active key intermediate (**13**) for bicyclo[2.2.1]heptane carbocyclic nucleoside synthesis.

Nucleoside synthesis was realized by a Mitsunobu reaction using Ph_3P, DEAD and N^4-benzoyl-cytosine and 5-fluoro-uracil as base. *ent*-C-Bz was obtained as a single compound and ent-FU-Bz as a mixture of two isomers, these nucleoside analogs being used as ester prodrugs in the following biological tests.

All the compounds were analysed by optical rotation, IR and ^{1}H and ^{13}C-NMR spectra.

Our preliminary preclinical *in vitro* data shows that ent-C-Bz is cytotoxic for Jurkat cells at high concentrations (30µM); simultaneously, the nucleoside analogue moderately restricts cell multiplication and targets DNA synthesis. ent-5-FU-Bz and 5-FU are cytotoxic at lower concentrations (≥ 5µM) and tends to hinder RNA synthesis. We emphasize that ent-5-FU-Bz restricts Jurkat cells progenitors at earlier generations than the 5-FU standard. Our preliminary study reveals that the investigated nucleoside analogues are potential candidates for cytotoxic therapy of T lymphoblasts. Further studies have to be performed for establishing the anti-neoplastic action of the investigated nucleosides on other types of standardized tumor cell lines.

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