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Synthesis of New Acyclic Pyrimidine Nucleoside Analogues and Preliminary Assessment of Their Cytotoxic and Genotoxic Effects *In Vitro*.

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ABSTRACT

Nucleoside analogues represent an important class of compounds of wide application range in antiviral and antitumor therapy. A regular procedure in early phases of new drugs development includes assessment of cytotoxicity and genotoxicity (mutagenicity). Here we report the synthesis of two novel C-5 hydroxypropyl pyrimidine nucleosides bearing side chains like those present in drugs already used in therapy (penciclovir and cidofovir). To confirm structures of the newly synthesized compounds FT-IR, HPLC-MS and NMR spectroscopy in solution were used. New hydroxypropyl pyrimidine nucleosides were *N*-3-(2,3-dihydroxypropyl)-5-(3-hydroxypropyl)pyrimidine-2,4-dione and *N*-3-(4-hydroxy-3-(hydroxymethyl)butyl)-5-(3-hydroxypropyl)pyrimidine-2,4-dione.Along with two new, we have also tested three previously synthesized nucleosides for genotoxicity and cytotoxicity using cytokinesis-block micronucleus (CBMN) assay on human peripheral blood lymphocytes. Not many studies so far used the same cell model and CBMN assay to determine cyto-/genotoxicity of pyrimidine nucleosides. Results of CBMN assay did not indicate statistically significant cyto-/genotoxicity towards human peripheral blood lymphocytes. Since preliminary testing did not suggest significant genotoxic or cytotoxic potency of novel hydroxypropyl pyrimidine nucleosides, they are excellent candidates for further biological evaluation, primarily as antiviral agents.

Keywords: Pyrimidine analogues; Acyclic nucleosides; CBMN assay; Genotoxicity; Cytotoxicity.

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INTRODUCTION

During the twentieth century there were many research attempts to discover the way of inhibiting malignant cell division. One of the efficient approaches to this problem was to inhibit *de novo* synthesis of DNA using various nucleoside analogues. A plethora of them were designed by modification of analogues already used in clinical practice. Modifications of new analogues led to development of new compounds efficient in inhibiting of human cancer cells proliferation. However, some of them had unacceptable toxicity profiles, provoked detrimental effects in normal cells, and also has unstable molecules. Therefore, a challenge for medicinal chemists is to synthesize new derivatives as potential drug candidates, which would not exert harmful side effects. Nucleoside analogues represent an important class of compounds of wide application range in antitumor and antiviral therapy. Structural diversity and biological importance of pyrimidines make them significant building blocks [1–3].

Antitumor and antiviral chemotherapy are often limited with unwanted side effects. Conventional cytostatic drugs cannot discriminate between normal and malignant cells, and viruses also become resistant to widely used antiviral drugs.

Among commonly used cytostatics, antibiotics, narcotics, anaesthetics and oral contraceptives, many compounds are known to be genotoxic and mutagenic. A regular procedure in early phases of new drugs development includes assessment of cytotoxicity and genotoxicity (mutagenicity) [4–6]. Although existing literature described over 200 different tests for detecting genotoxic potential, in routine analyses rapid *in vitro* tests are regularly used [7,8].

Nowadays the cytokinesis-block micronucleus (CBMN) cytome assay represents one of the most comprehensive methods, which enables the simultaneous study of chromosome damage, mitotic spindle apparatus damage, cell cycle kinetics, and cytotoxicity in treated cell cultures [9–11]. This cytogenetic method is based on the assessment of micronuclei (small nuclei-like structures located in cytoplasm) in cells that have completed one nuclear division. The assay also allows to assess other relevant biodosimetric markers: nucleoplasmic bridges (NPB), nuclear buds (NB), proportion of dividing cells (parameter of cytostasis), and cells in apoptosis and necrosis (parameters of cytotoxicity) [12–14]. Usefulness of this method for evaluation of cyto-/genotoxicity of various chemicals has been confirmed previous studies as well [15–17].

The mechanisms that drive the biological effects of acyclic nucleoside analogues, especially at the cell level have not yet been fully explained so far. All above mentioned motivated us to synthesize new acyclic nucleoside analogues, evaluate their biological activity as well as establish genotoxicity and cytotoxicity as their potential side effects.

We report here the synthesis of two novel C-5 hydroxypropyl pyrimidine nucleosides (**3** and **5**, starting from previously synthesized precursors **2** and **4**) and their structural characterization. Using cytokinesis-block micronucleus (CBMN) assay we assessed their cyto-/genotoxicity and along with a previously synthesized and known pyrimidine nucleoside **1** (5-(3-hydroxypropyl)-*N*-1-[4-hydroxy-(3-hydroxymethyl)butyl]pyrimidin-2,4-dione) [18]. We expected that a combination of the selected methods would give more insight into the biological activity of the tested compounds as well as point to the directions in which future research should go.

EXPERIMENTAL

General

Melting points (uncorrected) were determined with BÜCHI Melting Point B-545. Precoated silica gel 60F-254 plates were used for thin layer chromatography and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0.063 – 0.2 mm); glass column was slurry-packed under gravity.

NMR spectra were measured on a Bruker AV600 spectrometer at 303 K in 5 mm NMR tubes. ^{1}H and ^{13}C NMR spectra were acquired at 600.130 and 150.903 MHz, respectively, using standard ^{1}H and ^{13}C APT techniques. Samples were measured from DMSO-d_6 and chemical shifts (in ppm) were referred to the TMS as

internal standard. Digital resolutions in ^{1}H and ^{13}C spectra were 0.37 and 0.60 Hz per point. The assignments of signals of both compounds were carried out by one and two-dimensional NMR techniques: ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC. Spectra were measured in pulsed field gradient mode (*z*-gradient).

Infrared absorption spectra were recorded using KBr pellets with an ABB Bomem FT model MB102 spectrometer, in the 4000–400 cm^{-1} region. Elemental analyses were performed on a Perkin–Elmer, series II, CHNS analyser 2400. Mass spectra were carried out with an Agilent 6410 instrument equipped with electrospray interface and triple quadrupole analyser (LC/MS/MS). High performance liquid chromatography was performed on an Agilent 1100 series system with UV detection (photodiode array detector) using Zorbax C18 reverse-phase analytical column (2.1 × 30 mm, 3.5 µm). All compounds showed > 95% purity in this HPLC system.

Procedures for the preparation of compounds

Starting compounds **2** and **4** were synthesized according to the known procedure [19]. Procedures for preparation of new derivatives **3** and **5** are described below.

*N-3-(2,3-dihydroxypropyl)-5-(3-hydroxypropyl)pyrimidine-2,4-dione (**3**)*

Compound **2** (35 mg, 1.35 mmol) was dissolved in 1 M NaOH (4 mL) and the mixture was stirred at room temperature for 20 h. Then, it was neutralized by the addition of HCl. After evaporation of the solvents and column chromatography (dichloromethane:methanol = 3:1) white crystals of compound **3** were obtained (11.76 mg; 35.5%; m.p. 140-146 ºC).

3: IR: ($\tilde{\nu}$) 3452.81, 2942.86, 1698.27 cm^{-1}. ^{1}H NMR: (δ) 11.11 (1H, s, NH), 7.33 (1H, s, H-6), 4.92 (1H, d, OH-11, *J* = 5,58 Hz), 4.64 (1H, t, OH-12, *J* = 5.79 Hz), 4.36 (1H, t, OH-9, *J* = 5.31 Hz), 3.89 (2H, t, H-10, *J* = 6.72 Hz), 3.38 (3H, m, H-11 and H-12), 3.37 (2H, m, H-9), 2.19 (2H, t, H-7, *J* = 7.29 Hz), 1.56 (2H, m, H-8) ppm. ^{13}C NMR: (δ) 164.5 (C-4), 151.4 (C-2), 143.3 (C-6), 111.9 (C-5), 69.6 (C-11), 64.1 (C-12), 60.5 (C-9), 51.3 (C-10), 31.8 (C-8), 23.2 (C-7) ppm. MS (ESI): *m/z* 245.1 ($[M+H]^{+}$). *Anal.* Calcd. (%) for $C_{10}H_{16}N_2O_5$: C, 49.17; H, 6.60; N, 11.47. Found: C, 50.10; H, 6.42; N, 11.29.

*N-3-(4-hydroxy-3-(hydroxymethyl)butyl)-5-(3-hydroxypropyl)pyrimidine-2,4-dione (**5**)*

Synthesis of the compound **5** was performed in the same way as described for compound **3.** The reagents used were: compound **4** (318.6 mg, 1.17 mmol) and 1 M NaOH (25 mL). After column chromatography (dichloromethane:methanol = 5:1) compound **5** was obtained as white crystals (0.198 mg; 65.5%, m.p. 129-132 ºC).

5: IR: ($\tilde{\nu}$) 3429.09, 2943.65, 1682.87 cm^{-1}. ^{1}H NMR: (δ) 10.82 (1H, s, NH), 7.19 (1H, s, H-6), 4.36 (1H, t, OH-9, J = 5.10 Hz), 4.29 (2H, t, OH-13 and OH-14, J = 5.10 Hz), 3.81 (2H, t, H-10, J = 6.87 Hz), 3.50 (2H, m, H-9), 3.40 (2H, m, H-14), 3.37 (2H, m, H-13), 2.22 (2H, t, H-7, J = 7.89 Hz), 1.56 (2H, m, H-8), 1.46 (4H, m, H-11 and H-12) ppm. ^{13}C NMR: (δ) 163.3 (C-4), 151.0 (C-2), 136.1 (C-6), 111.3 (C-5), 61.8 (C-13 and C-14), 60.1 (C-9), 41.3 (C-12), 32.2 (C-10), 31.2 (C-8), 26.4 (C-11), 23.2 (C-7) ppm. MS (ESI): m/z 273.1 ($[M+H]^{+}$). Anal. Calcd. (%) for $C_{11}H_{18}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 52.78; H, 7.62; N, 10.1.

Assessment of genotoxic and cytotoxic activity

Potential genotoxic and cytotoxic activity of compounds **1**, **3** and **5** was evaluated using cytokinesis-block micronucleus (CBMN) assay. The lymphocyte model was chosen based on previous studies [15,17,20].

Blood sampling

Peripheral blood samples were collected from two healthy male non-smokers (aged 29 years) who were not exposed to any known genotoxic agents and did not undergo any diagnostic or therapeutic irradiations for one year before the study. An informed consent was obtained from the blood donors. A total of 20 mL of blood was collected by venepuncture under sterile conditions into blood collection tubes (BD vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) which contained lithium heparin as an anticoagulant.

Selection of the tested concentrations

The concentrations of the compounds **1**, **3** and **5** used in the CBMN assay in cultured human lymphocytes were: 1.1 mg/mL ($\mathbf{1}_{I}$, $\mathbf{3}_{I}$ and $\mathbf{5}_{I}$), 0.363 mg/mL ($\mathbf{1}_{II}$, $\mathbf{3}_{II}$ and $\mathbf{5}_{II}$) and 0.044 mg/mL ($\mathbf{1}_{III}$, $\mathbf{3}_{III}$ and $\mathbf{5}_{III}$).

These concentrations were chosen according to previously described PCV plasma concentrations [21]. Solutions of investigated concentrations were applied in cultures of peripheral blood lymphocytes in volume of 100 µL giving, with 5 mL of medium and 0.4 mL of blood, overall volume of 5.5 mL.

As a positive control, we used bleomycin (Nippon Kayaku Co. Ltd., Japan) at concentration 10 µg/mL, and *in vitro* pre-treatment lasted for three hours.

Cytokinesis-block micronucleus (CBMN) cytome assay procedure

We applied a standard 72-h protocol for the CBMN assay as recommended by Fenech and Morley [22]. For each experimental group, duplicate cultures were set up by adding whole blood into Gibco® RPMI 1640 (1X) medium (Life Technologies, UK) supplemented with inactivated foetal calf serum (Gibco®, Invitrogen, UK), phytohemagglutinin (Remel Europe Ltd., UK), and antibiotics – penicillin and streptomycin solution (Sigma-Aldrich, Germany). Lymphocyte cultures were kept at 37 °C in sterile flasks (25 cm^2), in 5 % CO_2 humidified incubator. At the 44th hour, cytochalasine B (Sigma-Aldrich, USA) was added (6 µg/mL). After 72 h of *in vitro* growth, cultures were harvested by standard procedures including incubation in hypotonic KCl (Kemika, Croatia) solution, and several repetitions of fixation and centrifugation to obtain lymphocyte suspension. We used an ice-cold fixative made up acetic acid and methanol (Kemika, Croatia) (1:3 v/v). Lymphocyte suspension was dropped onto clean slides. Air-dried slides were stained with 5% Giemsa solution (Merck, Germany) for 10 minutes at room temperature.

Giemsa stained slides were analysed under a light microscope (Olympus, Japan) at 1000× magnification. A total of 2 × 1000 binucleated cells were examined for the frequency of MNi, nuclear buds (NB) and nucleoplasmic bridges (NPB), according to criteria proposed by Fenech *et al*.[12]. A total of 2 × 500 cells was used for assessment of mono-, bi-, and poly-nucleated lymphocytes and calculation of NDI. Finally, the amount of apoptotic and necrotic cells was used to calculate nuclear division index (NDI), following an equation:

$$NDI = \frac{M1+2(M2)+3(M3)+4(M4)}{N} \qquad (1)$$

where are:

M1–M4 – number of mono-, bi-, tri- and tetranucleated cells, N – total number of counted cells (viable) [23]. This index represents a parameter for analysis of lymphocyte mitogenic response to cytostatic effects of investigated samples [24, 25].

Determination of nuclear division cytotoxicity index (NDCI), as even more precise estimation of nuclear division status and cell kinetics, was obtained by introducing apoptotic and necrotic cells into equation:

$$NDCI = \frac{Ap+Nec+M1+2(M2)+3(M3)+4(M4)}{N} \qquad (2)$$

where is:

N – total number of counted cells (viable and non-viable) [22].

Results are expressed as the means ± S.E. (standard error).

Statistics

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The multiple comparisons of mean data among control, positive control and exposed groups was performed by one-way analysis of variance (ANOVA) and a least significant difference (LSD) was used for inter-group comparisons at $p < 0.05$.

RESULTS AND DISCUSSION

Chemistry

Synthesized compounds were C-5 substituted 3-hydroxypropylpyrimidine derivatives bearing side chains at *N*-1 and *N*-3 positions of pyrimidine moiety; side chains are like those appearing in known drugs like cidofovir and penciclovir (PCV). Compound **1**(Figure 1) was synthesized according to previously published research [18].

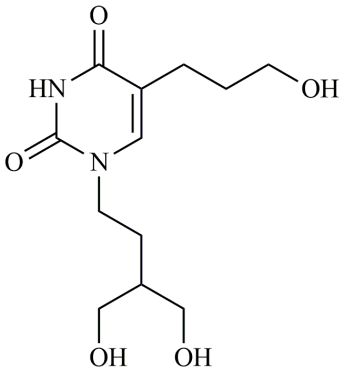

Figure 1: Structure of the compound 1

One-step synthetic route was employed in obtaining final products **3** and **5**, as outlined in Figure 2.

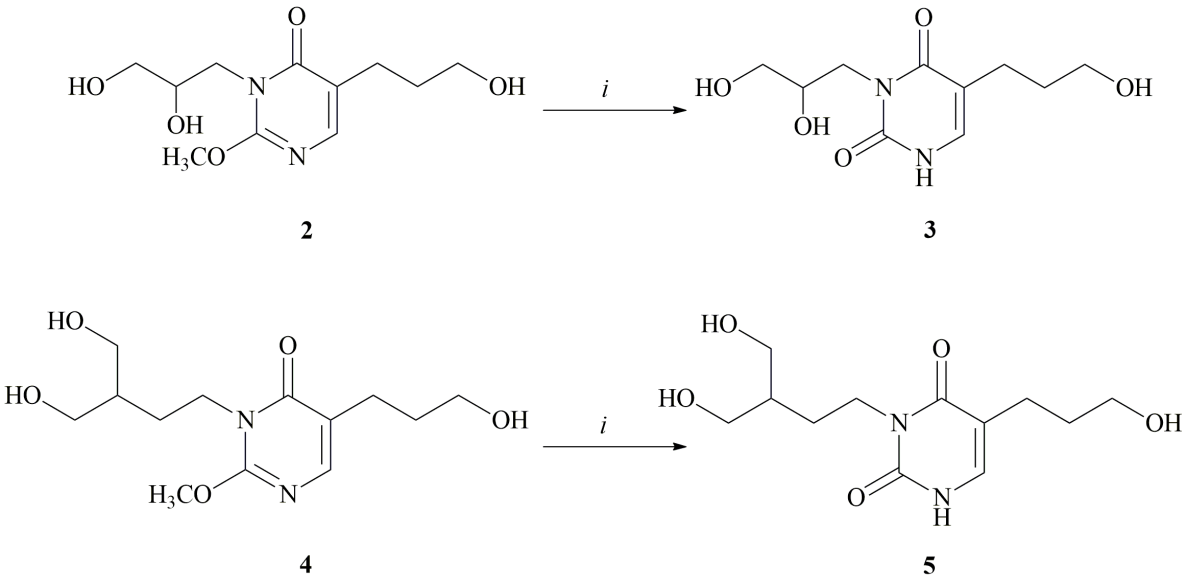

Figure 2: Synthesis of compounds 3 and 5. Reagents and conditions: *i*) 1 M NaOH, rt, 20h, room temperature

Nucleoside analogues **3** and **5** were obtained in moderate yields (35.5% and 65.5%, respectively) after base promoted hydrolysis of 5-substituted pyrimidine acyclic nucleosides **2** and **4** with sodium hydroxide.

NMR and FT-IR analyses

Molecular structure of both compounds was characterized by 1D and 2D NMR techniques. Spin

systems H-7 – H-8 – H-9 – OH-9 (**3** and **5**), H-10 – H-11 – (OH-11) – H-12 – OH-12 (**3**) and H-10 – H-11 – H-12 – H-13 – OH-13 (**5**) of the substituted side chains were determined through ^{1}H-^{1}H COSY experiments. Related chemical shifts are given in the Experimental part. The signal at7.33 ppm (**3**) and 7.19 ppm (**5**) relates to H-6 nucleus. NH proton had resonance at 11.11 ppm (**3**) and 10.82 ppm (**5**). ^{13}C signals throughout the molecule were assigned by means of ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC. Cross peaks in HMBC spectra enabled the identification of the C-2 (164 ppm) and C-4 (151 ppm) signals. C-4 showed H7/C4 and H10/C4 cross peaks. For C-2 only H6/C2 cross peak was found. The resonance of C-5 was found at 119 ppm.

The chemical identities and structures of **3** and **5** were also characterized by FTIR spectroscopy. Along with the evident bands for the common structure groups (uracil moiety, hydroxyl), a particularly important one was noticed. FT-IR spectrum of compound **3** showed a lactam's carbonyl C=O stretching band at 1682.65 cm^{-1} and compound **5** at 1698.27 cm^{-1}.

Cytokinesis-block micronucleus cytome assay

The results of the cytokinesis-block micronucleus cytome assay in human peripheral blood lymphocytes treated with compounds **1**, **3** and **5** are summarized in Table 1.

Results of the CBMN cytome assay suggest a low potential of all three compounds to induce MN, NB, and NPB at the tested concentrations.

There were no statistically significant increases in the number of apoptotic and necrotic cells with respect to negative control. As anticipated, positive control (bleomycin) had the highest value for all parameters of the CBMN cytome assay, which confirms the sensitivity and specificity of the method used (Table 1).

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Table 1: Results of the cytokinesis-block micronucleus cytome assay in human peripheral blood lymphocytes treated with compounds 1, 3 and 5 at three different concentrations, as well as in the negative and positive control samples

Parameter	Negative control	1_I	1_{II}	1_{III}	3_I	3_{II}	3_{III}	5_I	5_{II}	5_{II}	Positive control
Distribution of BN cells according to number of micronuclei (MN)											
0	1996	1994	1997	1998	1996	1995	1994	1991	1996	1998	1955
1	4	6	3	2	4	5	5	9	4	2	36
2	0	0	0	0	0	0	1	0	0	0	4
3	0	0	0	0	0	0	0	0	0	0	4
4	0	0	0	0	0	0	0	0	0	0	1
BN_{MN} cells (‰ ± SD)	2.0±0.0	3.0±0.0	1.5±0.7	1.0±0.0	2.0±0.0	2.5±0.7	3.0±1.4	4.5±0.7	2.0±0.0	1.0±0.0	22.5±0.7*
No. of MN (Mean ± SD)	2.0±0.0	3.0±0.0	1.5±0.7	1.0±0.0	2.0±0.0	2.5±0.7	3.5±2.1	4.5±0.7	2.0±0.0	1.0±0.0	30.5±1.4*
Distribution of BN cells according to number of NBs											
0	1997	1998	1996	1999	2000	1999	1999	1999	1999	1999	1983
1	3	2	4	1	0	1	1	4	1	2	17
BN_{NB} cells (‰ ± SD)	1.5±0.7	1.0±0.0	2.0±1.4	0.5±0.7	0	0.5±0.7	0.5±0.7	2.0±1.4	0.5±0.7	1.0±0.0	8.5±0.7
No. of NB (Mean ± SD)	1.5±0.7	1.0±0.0	2.0±1.4	0.5±0.7	0	0.5±0.7	0.5±0.7	2.0±1.4	0.5±0.7	1.0±0.0	8.5±0.7
Distribution of BN cells according to number of NPBs											
0	2000	1998	1998	1999	1997	1997	1996	1998	1997	1998	1990
1	0	2	2	1	3	3	4	2	3	2	10
BN_{NPB} cells (‰ ± SD)	0	1.0±0.0	1.0±0.0	0.5±0.7	1.5±0.7	1.5±0.7	2.0±0.0	1.0±0.0	1.5±0.7	1.0±0.0	5.5±1.4
No. of NPB (Mean ± SD)	0	1.0±0.0	1.0±0.0	0.5±0.7	1.5±0.7	1.5±0.7	2.0±0.0	1.0±0.0	1.5±0.7	1.0±0.0	5.5±1.4
Apoptotic cells (‰ ± SD)	1.5±0.7	2.0±0.0	2.5±0.7	2.0±0.0	4.0±1.4	5.0±1.4	1.0±1.4	2.0±0.0	1.0±0.0	1.0±0.0	21.5±3.5*
Necrotic cells (‰ ± SD)	0	0.5±0.7	0.5±0.7	0.5±0.7	0.5±0.7	0.5±0.7	0.5±0.7	0.5±0.7	0	0	14.0±1.4*

BN – binucleated cells; BN_{MN} cells – binucleated cells with micronuclei BN_{NB} cells – binucleated cells with nuclear buds; BN_{NPB} cells – binucleated cells with nucleoplasmic bridges; Positive control – lymphocytes treated for 3 h in vitro with bleomycin at 10 µg/ml;1_I: acyclic nucleoside analogue 1 concentration 1.1 mg/mL; 1_{II}: acyclic nucleoside analogue 1 concentration 0.363 mg/mL; 1_{III}: acyclic nucleoside analogue 1 concentration 0.044 mg/mL; 3_I: acyclic nucleoside analogue 3 concentration 1.1 mg/mL; 3_{II}: acyclic nucleoside analogue 3 concentration 0.363 mg/mL; 3_{III}: acyclic nucleoside analogue 3 concentration 0.044 mg/mL; 5_I: acyclic nucleoside analogue 5 concentration 1.1 mg/mL; 5_{II}: acyclic nucleoside analogue 5 concentration 0.363 mg/mL; 5_{III}: acyclic nucleoside analogue 5 concentration 0.044 mg/mL. Microscopic evaluation was performed using a light microscope at 1000× magnification (oil immersion); To establish the MN frequencies, 2×1000 cells were scored. Data are expressed as mean±SD of two independent evaluations; *- significantly different from negative control

Lymphocyte proliferation analysis

Results regarding lymphocyte proliferation in lymphocyte cultures treated *in vitro* with tested compounds **1**, **3** and **5**and in the corresponding negative and positive controls are reported in Table 2. At the concentrations tested, none of the selected compounds significantly impaired lymphocyte proliferation or significantly affected the nuclear division index (NDI) and nuclear division cytotoxicity index (NDCI) values compared to negative control. Only treatment with bleomycin (positive control) showed significant cytostatic and cytotoxic effects, which were expected, since the compound is an effective antitumor agent.

Table 2: Effect of tested compounds 1, 3 and 5 on cell proliferation kinetics and nuclear division index (NDI) in treated lymphocytes, as well as in the negative and positive control samples

Experimental group	Distribution of cells according to number of nuclei				NDI	NCDI
	M1	M2	M3	M4		
Negative control	428	1262	72	238	2.060	2.061
1_I	462	1345	75	118	1.925	1.926
1_{II}	400	1194	213	193	2.100	2.101
1_{III}	397	1211	172	220	2.108	2.109
3_I	423	1376	77	124	1.951	1.952
3_{II}	330	1421	107	142	2.031	2.031
3_{III}	232	1501	129	138	2.087	2.087
5_I	391	1290	192	127	2.028	2.030
5_{II}	514	1176	138	172	1.984	1.987
5_{III}	492	1110	203	195	2.051	2.051
Positive control	1140	791	34	35	1.482*	1.500*

Positive control – lymphocytes treated for 3 h *in vitro* with bleomycin at 10 µg/ml;
NDI – nuclear division index, NCDI – nuclear division cytotoxicity index;
Microscopic evaluation was performed using a light microscope at 1000× magnification (oil immersion); NDI and NCDI were determined by examining 2×1000 cells per sample; *- significantly different from negative control.

Obtained results are particularly important having in mind that registered drug cidofovir showed genotoxicity in micronucleus test on human peripheral blood lymphocytes [26].
The previous results of genotoxicity tests of nucleoside analogues have shown that *in vitro* and *in vivo* genotoxicity profiles are qualitatively similar. Bacterial mutation assay (Ames test) results are negative, but results for the mouse lymphoma assay at the $TK^{-/+}$ locus and *in vitro* and *in vivo* results for chromosomal effects are all positive [27–30].

Not many studies so far used the same cell model and CBMN assay to determine cyto-/genotoxicity of pyrimidine nucleosides. Most of them focused on 5-fluorouracil, which is known, and widely used antineoplastic drug. The association between occupational exposure to 5-FU and incidence of MNi in peripheral blood lymphocytes of nurses was reported by Cavallo *et al*. [31]. In their *in vitro* study, Kang *et al*. [32] also found that 5-fluorouracil (5-FU) effectively induced MNi in human peripheral blood lymphocytes. In a recent study by Gajski *et al*. [16] association between 5-FU and MNi induction was reported for human lymphocytes and HepG2 cells.

The lack of data regarding the outcomes of CBMN assay on related compounds with possible antiviral properties complicated the interpretation of the results obtained in our study. We have to stress that this study was the first one which evaluated the incidence of MNi and other types of cytogenetic damage covered by the "cytome" assay, after treatment with selected acyclic 5-substituted pyrimidine nucleosides. Therefore, we

added original and novel information regarding their cyto-/genotoxicity at cellular level.

This study is to a certain extent limited by the fact that human lymphocytes represent a population of resting cells that do not possess intrinsic metabolic activation. Therefore, to prove the significance of our findings, further studies using other appropriate cell lines are needed. Nevertheless, our findings provide valuable information regarding toxicity of novel nucleoside analogues and represent a solid base for our future investigations.

CONCLUSION

Overall, five nucleoside analogues have been synthesized and have been used for biological evaluation, as these compounds represent important class drugs of wide application range in antitumor and antiviral therapy. Obtained parameters of *in vitro* testes did not indicate any statistically significant genotoxicity and cytotoxicity of synthesized compounds. Having in mind that these new nucleoside analogues are not genotoxic or cytotoxic, they are excellent candidates for further biological evaluation, primarily as antiviral agents.

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