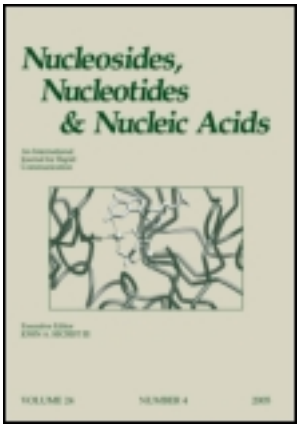

Nucleosides, Nucleotides and Nucleic Acids

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Disaccharide Pyrimidine Nucleosides and Their Derivatives: A Novel Group of Cell-Penetrating Inhibitors of Poly(ADP-Ribose) Polymerase 1

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DISACCHARIDE PYRIMIDINE NUCLEOSIDES AND THEIR DERIVATIVES: A NOVEL GROUP OF CELL-PENETRATING INHIBITORS OF POLY(ADP-RIBOSE) POLYMERASE 1

Anna S. Efremova,[1] Alexandra L. Zakharenko,[2] Stanislav I. Shram,[1] Irina V. Kulikova,[3] Mikhail S. Drenichev,[3] Maria V. Sukhanova,[2] Svetlana N. Khodyreva,[2] Nikolay F. Myasoedov,[1] Olga I. Lavrik,[2] and Sergey N. Mikhailov[3]

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□ *Nearly 30 synthetic nucleosides were tested with human recombinant poly(ADP-ribose) polymerase 1 as potential inhibitors of this enzyme. The most active compounds were some disaccharide analogues of thymidine: 3′-O-β-D-ribofuranosyl-5-iodo-dUrd (**2d**; IC_{50} = 45 μM), 3′-O-β-D-ribofuranosyl-2′-deoxythymidine (**2e**; IC_{50} = 38 μM), and 3′-O-β-D-ribofuranosyl-2′-deoxythymidine oxidized (**4**; IC_{50} = 25 μM). These compounds also reduced H_2O_2-induced synthesis of poly(ADP-ribose) in cultured human ovarian carcinoma (SKOV-3) cells in a dose-dependent manner. Furthermore, compounds **2d** or **2e** until a concentration of 1 mM did not affect growth of SKOV-3 cells, whereas dialdehyde compound **4**, as well as thymidine, exhibited a significant cytotoxicity.*

Keywords Disaccharide pyrimidine nucleosides; poly(ADP-ribose) polymerase 1 inhibitors; DNA repair; cytotoxicity; SKOV-3 human ovarian carcinoma cell line

INTRODUCTION

Nucleoside analogues may be considered as one of the most promising group of organic compounds in medicinal chemistry. Numerous drugs have been developed, which have or resemble nucleoside structure. Most of nucleosides should be phosphorylated to target polymerases and to exert

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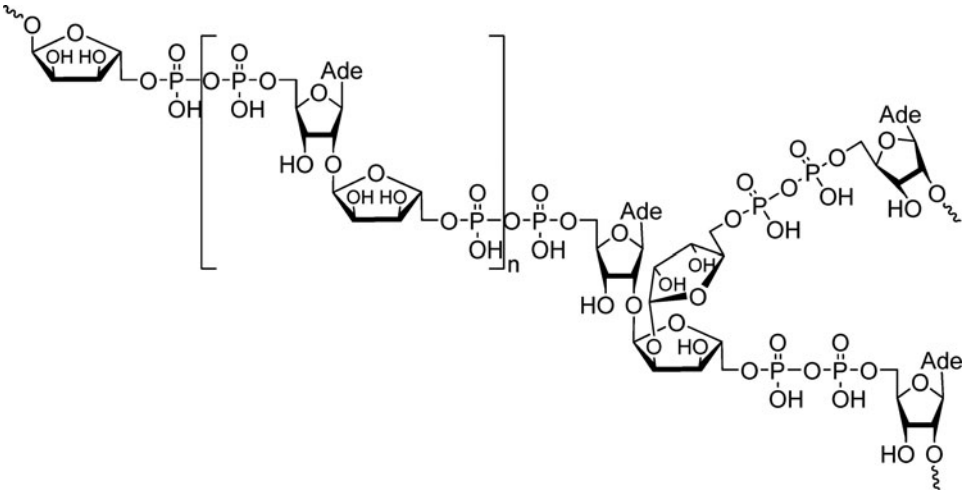


FIGURE 1 Structure of poly(ADP-ribose).

their biological effect.[1,2] In spite of great achievements in this area, there is still a room for finding new targets for nucleoside analogues. Poly(ADP-ribose) polymerase 1 (PARP-1) has been an actively pursued drug discovery target for almost three decades. Often referred to as a "guardian angel of DNA," this nuclear enzyme has been the focus of several medicinal chemistry programs in a wide range of therapeutic areas. Targeting DNA repair with PARP inhibitors has great promise as anticancer therapeutic strategy either as a single agent or as a resistance modifier in conjunction with other DNA-damaging agents including radiation therapy.[3,4]

PARP-1 converts nicotinamide adenine dinucleotide (NAD^+) into a polymer—poly(ADP-ribose) (PAR; Figure 1). It is known that PAR is involved in modulation of the chromatin structure, replication, and transcription, as well as in DNA repair and cell differentiation.[5,6] PAR molecules isolated from natural sources or synthesized in vitro with PARP contain up to 200–300 monomeric units. Linear regions of 20 to 50 units alternate with branched fragments.[7,8] The development of methods for the preparation of PAR is still a challenging problem. Only recently two syntheses of 2′-*O*-α-D-ribofuranosyladenosine, the monomeric unit of PAR, were published.[9,10]

Disaccharide nucleosides comprise an important group of natural compounds. About one hundred disaccharide nucleosides and their analogues have been isolated to date from various sources.[11–13] These compounds have a broad range of biological activities and display antibacterial, antimycotic, herbicidal, insecticidal, antitumor, and antiviral properties. Compounds of this type have an additional saccharide residue attached to one of the nucleoside hydroxyl groups via an O-glycoside bond. The presence of a disaccharide residue and a heterocyclic base makes their properties

similar to those of carbohydrates and nucleosides. We investigated 2′-*O*-α-D-ribofuranosyladenosine and some other disaccharide nucleosides as potential inhibitors of PARP-1, having in mind that thymidine (Thd) and some of its analogues may inhibit this enzyme.[14]

Most of the known competitive inhibitors of PARP-1 are heterocyclic compounds containing primary or secondary amide group.[3,15] Ability of these compounds to inhibit PARP-1 is associated with their structural homology with nicotinamide, which is known to be one of the endogenous inhibitors of PARP-1. Thd is thought to be another endogenous inhibitor of this enzyme. Among all natural nucleosides, Thd is unique to possess notable PARP-1 inhibitory activity.[16] Furthermore, Thd has a stronger inhibitory effect on PARP-1 than thymine, and some other nucleic acid components similar to Thd—such as uridine (Urd), 2′-deoxyuridine (dUrd), thymidine monophosphate, and 2′-deoxythymidine triphosphate—completely lack such activity.[16] Later structural and functional studies have shown that a number of 5-substituted derivatives of Urd and dUrd possess significantly higher activity than Thd.[14] It was found that changes in the structure of the sugar residue can also significantly affect the inhibitory potential of nucleosides.[15,17]

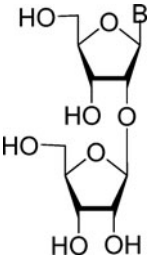
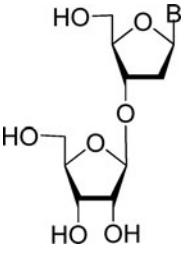
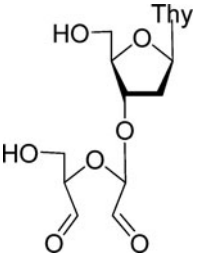
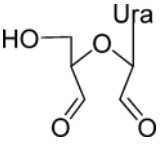
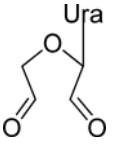
This study was aimed to investigate properties of several disaccharide nucleoside analogues as potential inhibitors of human PARP-1. To do this, experiments on the isolated recombinant enzyme and cultured human tumor cells were performed and cytotoxicity of these compounds was evaluated.

RESULTS

Inhibition of Human Recombinant PARP-1 by Nucleoside Analogues

For primary screening, effects of about 30 analogues of nucleosides (mainly disaccharides) on human recombinant PARP-1 have been evaluated. The most important results are summarized in Table 1 (for an overview on all compound structures also, see Table 1). It was shown that a number of disaccharide analogues of Thd (compounds **1d**, **2e**, **2f**, **2h**, and **4**), like the Thd itself, exerts a considerable inhibitory effect on PARP-1. Estimated IC_{50} values for these compounds vary from 25 to 216 μM. On the contrary, Urd disaccharide derivative **1c**, as well as Urd itself, did not show any PARP-1 inhibitory activity. However, disaccharide derivative of 5-iodo-dUrd (**2d**), but not 5-fluoro-dUrd (**2c**), was as effective in inhibiting PARP-1 ($IC_{50} = 45$ μM) as the thymidine derivatives. Interestingly, several disaccharide nucleosides (compounds **2d**, **2e**, and **4**) demonstrated a similar or even higher inhibitory activity than 3-aminobenzamide (**3-AB**), a widely used inhibitor of PARP-1. Disaccharide nucleoside derivatives in which the nitrogenous bases are Ade

TABLE 1 Inhibition of human recombinant PARP-1 by some synthetic nucleoside analogues with different carbohydrate moiety

Carbohydrate moiety	Compound	Base	Modifications	IC_{50}, μM	n_H
D-Ribose or	**Cyd**	Cyt		> 2000	
2′-Deoxy-D-ribose	**dCyd**	Cyt		> 2000	
	Urd	Ura		> 2000	
	Thd	Thy		54 ± 2[a]	0.92 ± 0.04[a]
	**1a**	Ade		> 2000	
	1b	Cyt		> 2000	
	1c	Ura		> 2000	
	1d	Thy		178 ± 6	1.03 ± 0.04
	**2a**	Ade		> 2000	
	2b	Cyt		320	1.20 ± 0.20
	2c	Ura	5-fluoro	> 2000	
	2d	Ura	5-iodo	45 ± 3	0.80 ± 0.05
	2e	Thy		38 ± 4	1.16 ± 0.14
	2f	Thy	5′-β-D-ribofuranosyl	110 ± 16	0.97 ± 0.13
	2g	Thy	5′-phosphate	> 2000	
	2h	Thy	5″-phosphate	216 ± 56	1.06 ± 0.25
3′-Rib-α-Thd	**3**	Thy		> 2000	
	**4**	Thy		25 ± 3	1.23 ± 0.17
	**5**	Ura		139 ± 22	1.47 ± 0.28
	**6**	Ura		> 2000	
	3-Aminobenzamide			57 ± 8	0.91 ± 0.09

[a] The values of IC_{50} and n_H in the table are presented as mean ± SE.

or Cyt were not active, with an exception of compound **2b** that demonstrated a weak inhibitory activity ($IC_{50} = 320\ \mu M$). It is worth noting that 2′-O-α-D-ribofuranosyladenosine,[9] a monomeric unit of PAR, was not also active towards enzyme ($IC_{50} > 2000\ \mu M$).

Oxidized Thd disaccharide derivative **4**, which can be readily prepared by treatment of **2e** with a small excess of $NaIO_4$,[18–21] exerts most pronounced inhibitory activity ($IC_{50} = 25\ \mu M$) among all nucleoside analogues tested. Its precursor, compound **2e**, demonstrated only slightly weaker inhibitory activity ($IC_{50} = 38\ \mu M$). It should be noted that α-anomer of **2e**, compound **3**, did not show any inhibitory effect at concentrations up to 1 mM. Compounds **1d**, which is the 2′-*O*-β-D-ribofuranosyl derivative of ribothymidine, demonstrated substantially lower inhibitory activity ($IC_{50} = 178\ \mu M$) than its 3′-analogue compound **2e**. Interestingly, the 3′-*O*-β-D-ribofuranosyl derivative of 2′-*O*-deoxycytidine (dCyd), compound **2b**, also showed an appreciable inhibitory activity, while dCyd, cytidine (Cyd), or its 2′-*O*-disaccharide derivative **1b** were completely inactive. In addition, it was shown that analogues of compound **2e** phosphorylated in 5′ or 5″ position (**2g** and **2h**) were significantly less active (**2h**) or not active at all (**2g**). Furthermore, it was shown that the trisaccharide derivative of Thd, which contains two ribose residues at the 2′ and 5′ positions (**2f**), markedly inhibited PARP-1 ($IC_{50} = 110\ \mu M$), although its inhibitory potential was lower than that of compound **2e**.

Finally, PARP-1 inhibitory potential of two nucleoside analogues of Urd with oxidized ribose (**5**) or erythrose (**6**) sugar residue was investigated. These compounds have structural homology; however, only compound **5** showed significant inhibitory activity ($IC_{50} = 139\ \mu M$). This could be explained by the structural features of carbohydrate moiety, because neither Urd nor its disaccharide analogue **1c** inhibited PARP-1.

Since we were dealing with the novel compounds, it was appropriate to determine the values of Hill coefficient (n_H), which not only identifies the various cooperative effects but also helps to detect nonideal inhibition. Estimated values of n_H for almost all active nucleosides tested were close to 1.00 (Table 1). The only exception was compound **5**, for which a noticeable positive cooperativity was observed ($n_H = 1.47$).

Effects of Nucleoside Analogues on H_2O_2-Induced Poly(ADP-ribose) Synthesis in Cultured Tumor Cells

The ability of some nucleoside inhibitors of PARP-1 to suppress H_2O_2-induced elevation of PAR synthesis in SKOV-3 human ovarian carcinoma cell line was assessed. It was demonstrated in control experiments that preincubation of SKOV-3 cells with the compounds tested did not stimulate basal PAR synthesis (Figure 2). After five-minute incubation with 1 mM H_2O_2, we observed a 20–25-fold increase of PAR level in cell nuclei as compared with the control (Figures 2 and 3).

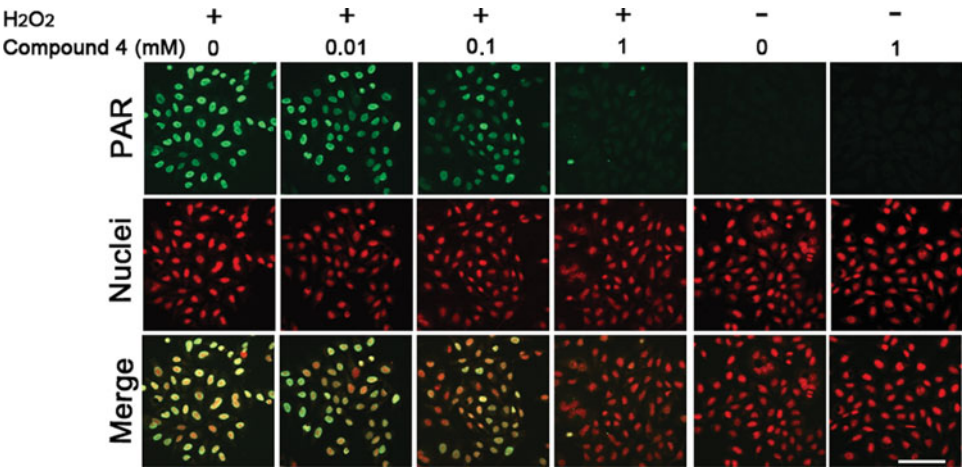


FIGURE 2 Inhibition of H_2O_2-induced synthesis of poly(ADP-ribose) (PAR) in cultured SKOV-3 human ovarian carcinoma cells by compound **4**. SKOV-3 cells were preincubated with 0.01 mM, 0.1 mM, 1 mM, or without compound **4** for one hour prior to five-minute treatment of cell cultures with 1 mM H_2O_2. The fixed cells were stained with anti-PAR antibodies (green) and propidium iodide (red) for counterstaining nuclei and then subjected to the fluorescent microscopy. The scale bar in the bottom right corner of the figure corresponds to 100 μm and refers to all images. (Color figure available online).

Preincubation of cells with compounds **2d**, **2e** (Figures 3A, 3B), or **4** (Figures 2 and 3C) for one or 24 hours resulted in dose-dependent down-regulation of PAR formation. However, the inhibitory effect of these compounds was weaker than that of Thd (Figure 3E). One-hour preincubation with 0.1 mM of Thd (Figure 3E), compound **4** (Figure 3C), or compound **2e** (Figure 3B) led to a decrease of stimulated synthesis of PAR by 72%, 50%, and 25%, respectively. It is obvious that the lower inhibitory potential of the Thd derivatives is determined by an additional ribose residue in 3′-position. However, a compound containing an oxidized second ribose residue (**4**) inhibits PAR production to a greater extent than its counterpart, compound **2e** (Figures 3B and 3C). It is of interest that one-hour preincubation of cells with all tested substances, with an exception of compound **2e**, exerted a more pronounced effect on PAR synthesis than a 24-hour exposure. The one-hour preincubation of SKOV-3 cells with 0.1 mM of oxidized Urd (**5**) slightly inhibited stimulated PAR synthesis, but at higher concentrations this nucleoside intensified the H_2O_2-induced PAR synthesis (Figure 3D). Moreover, a marked cytotoxic effect of compound **5** after 24-hour preincubation was observed as well (data not shown).

In addition, the nucleoside analogues tested also exerted their inhibitory effect on PARPs in cell culture. It is noteworthy that their inhibitory efficacy in cell culture proved to be somewhat lower than what was observed with the recombinant PARP-1. In contrast, **3-AB** demonstrated the same efficacy in culture and with the recombinant PARP-1 (Table 1, Figure 3F), whereas efficacy of Thd was even higher in culture than with the recombinant protein.

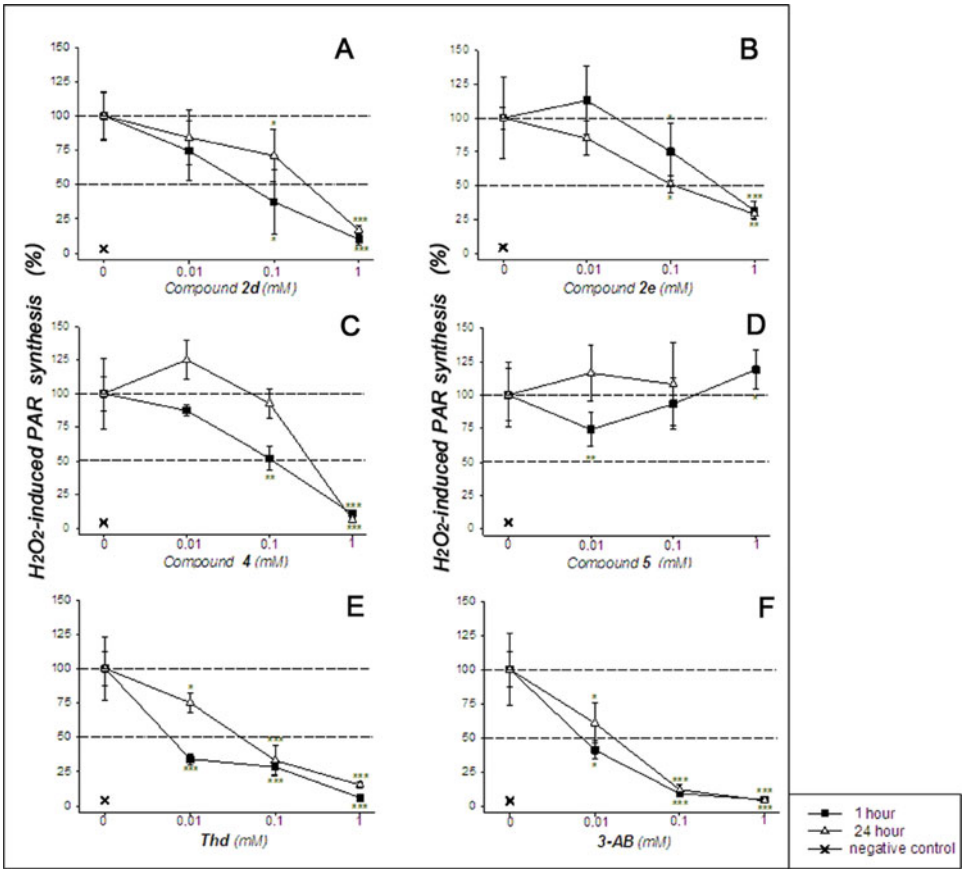


FIGURE 3 Effect of exposure of the SKOV-3 human ovarian carcinoma cells to increasing concentrations of tested nucleosides (A-E) or 3-aminobenzamide (**3-AB**; F) on H_2O_2-induced PAR synthesis. SKOV-3 cells were preincubated for one or 24 hours with compounds **2d** (A), **2e** (B), **4** (C), **5** (D), Thd (E), **3-AB** (F), or without these compounds prior to five-minute treatment of cell cultures with 1 mM H_2O_2. The level of PAR synthesis in nuclei of cells treated with 1mM H_2O_2 alone was taken as 100%. Intact cells, which were not subjected to any treatments, were considered as a negative control. The values of H_2O_2-induced PAR synthesis are presented as mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Student's *t*-test). (Color figure available online).

Cytotoxic Effects of Nucleoside Analogues on Cultured Tumor Cells

Some of the nucleoside analogues were selected for cytotoxicity study. Among them, compounds **2d**, **2e**, and **4** were taken due to their high inhibitory activity with respect to PARP-1, whereas other compounds have lower activity or none (**2a**, **5**, and **6**). Thd, dAdo, and **3-AB** were used as reference compounds. Almost two-fold decrease of the cell density was observed after 72-hour incubation of SKOV-3 cells with 1 mM dAdo or Thd as compared with the control (Figure 4). In contrast, disaccharide derivatives

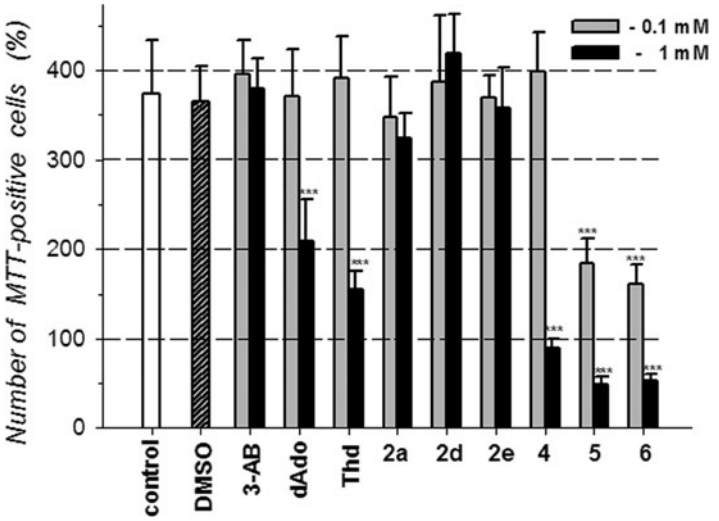


FIGURE 4 Effect of some tested nucleosides and 3-aminobenzamide (**3-AB**) on growth of the cultures of the SKOV-3 human ovarian carcinoma cells. SKOV-3 cells were cultured for 72 hours in growth medium containing 0.1 or 1 mM of **3-AB**, dAdo, Thd, or compounds **2a**, **2d**, **2e**, **4**, **5**, **6**, or contained 0.1% DMSO (the amount of DMSO equivalent to that added to growth medium of cell cultures treated with nucleoside analogues in concentration of 1 mM), or without any additives (control). Cell density in each premarked fields prior to adding the test substances was taken as 100%. The values of the number of MTT-positive cells are presented as mean ± SD. $^{***}p < 0.001$ (Student's *t*-test).

of dAdo and Thd containing a second ribose residue at the 3′-position (**2a** and **2e**) were virtually nontoxic in concentrations up to 1 mM (Figure 4). Moreover, 5-iodo-dUrd derivative **2d** with similar structure was not toxic. Thus, disaccharide derivatives of Thd, dAdo, and 5-iodo-dUrd with an additional ribose moiety at 3′-position did not display any toxicity, unlike their prototypes.

In contrast to Thd disaccharide analogue **2e**, its oxidized derivative **4** appeared to be toxic for SKOV-3 cells. It was shown that 72-hour incubation of SKOV-3 cells with 1 mM of compound **4** decreased cell density by 75% versus control, whereas this nucleoside had no effect on growth of cell culture in a concentration of 0.1 mM. It is worth noting that the oxidized Urd derivatives **5** and **6** exhibited even greater cytotoxicity. When SKOV-3 cells were incubated for 72 hours in the presence of 1 mM of these compounds, almost all cultured cells died. At a concentration of 0.1 mM, these compounds caused approximately two-fold decrease of cell density (Figure 4).

These results indicate that the cytotoxic effect of the nucleosides tested is not determined by their PARP-1 inhibitory activity. This suggestion is confirmed by an observation that a conventional PARP-1 inhibitor, **3-AB**, at a concentration of 1 mM completely inhibited PARP-1 but did not affect proliferative activity of SKOV-3 cells (Figure 4).

DISCUSSION

It is known that Thd, unlike other natural nucleosides and nitrogenous bases, is able to effectively suppress PARP activity in cells.[16] The dependence of PARP-1 inhibition efficiency on the structure of nitrogenous bases for different synthetic nucleosides has been investigated in numerous works.[14,16,22] However, the influence of carbohydrate moiety of nucleosides and its modifications on the inhibition of PARP-1 has not been thoroughly established.

Earlier, we developed approaches for the synthesis of the disaccharide nucleoside analogues (see[11–13] for details). In this study, we examined these analogues as potential inhibitors of human PARP-1. In addition, cytotoxic effects of nucleosides on cultured SKOV-3 human ovarian carcinoma cells were assessed.

Structure of the substituent at the C5-position of nitrogenous base seems to be the main factor governing inhibitory efficacy of pyrimidine nucleoside analogues.[14] As it was expected, disaccharide derivative of Thd (compounds **2e** and **4**) and 5-iodo-dUrd (**2d**) inhibit the human recombinant PARP-1 most efficiently, whereas disaccharide analogues with other heterocyclic bases were almost not active. Comparison of IC_{50} values of Thd and compound **2e** indicates that attachment of an additional ribose residue at the 3′-position of Thd molecule increases the inhibitory efficacy. The oxidized disaccharide nucleoside **4** inhibits PARP-1 more effectively than the parent **2e** or Thd. Based on these results, we suppose that dialdehyde group in the carbohydrate moiety of the nucleoside may participate in the formation of more stable protein-nucleoside complex, possibly through Schiff bases formation. However, other results obtained in experiments with oxidized Urd (**5**) suggest direct interaction of dialdehyde group with the substrate-binding site of PARP-1. It was found that oxidized nucleoside **5**, in contrast to the parent Urd or dUrd ([14,16], our data), displayed marked PARP-1 inhibitory efficacy, while dialdehyde derivative of 1-β-D-erythrofuranosyluracil (**6**) was inactive. It should be noted that from all of the tested nucleosides, considerable positive cooperativity in PARP-1 inhibition was found only for **5**. This fact may indicate that there are additional interactions of **5** with PARP-1, possibly due to the presence of the dialdehyde group, apart from the direct interaction with the active center of the enzyme through nitrogenous base. ^{1}H NMR spectra of the dialdehyde nucleoside derivatives in water are substantially more complex than those of the starting nucleosides. This is explained by the fact that dialdehyde derivatives exist in water as hydrates forming internal hemiacetals and diastereomeric dioxane derivatives,[19] which may interact with functional groups of enzyme. Natural β-configuration of nucleoside seems to be a critical factor for manifestation of the inhibitory properties of nucleosides. Indeed, α-anomers of the Thd disaccharide derivatives

(compound **3** and its oxidized form), in contrast to their β-anomers (compounds **2e** and **4**), were inactive.

Introduction of a phosphate group to the 5′ or 5″ position of disaccharide nucleoside markedly reduced inhibitory potential of disaccharide nucleosides that follows from comparison of IC_{50} values for compounds **2e**, **2g**, and **2h**. Possibly, the presence of negatively charged group in the carbohydrate moiety of molecule impedes its interaction with active center of PARP-1. Previously published data confirm the lack of any significant inhibitory activity of thymidine monophosphate and 2′-deoxyuridine monophosphate.[14,16]

To assess the efficacy of PARP inhibition in living cells by the compounds studied, a test system based on the determination of PAR accumulation in cultured cells after short-term exposure to H_2O_2 has been used.[23] Compounds **2d**, **2e**, and **4** were found to be the most effective inhibitors of recombinant PARP-1 among all tested synthetic nucleosides. Besides, they suppress H_2O_2-induced synthesis of PAR in SKOV-3 cells. Differences in the nucleoside inhibitory efficacy determined with recombinant PARP-1 were also revealed in cell study. However, PARP-1-inhibitory capacity estimated on cell culture was lower than that obtained for recombinant protein for all compounds with exception of Thd. These findings agree with the data obtained by Rankin et al. in experiments on 5-BrdU.[24] Based on these results, we claim that the presence of an additional residue in the 3′ position of Thd derivatives slightly deteriorates PARP-inhibiting effect shown in the experiments on cultured cells.

The inhibitory efficacy of tested compounds depended on the duration of cell preincubation. In all cases, except for compound **2e**, one-hour preincubation resulted in a significantly stronger suppression of PAR synthesis than 24-hour preincubation. These differences could be caused by different permeability, degradation rate, and/or metabolism of these compounds. In contrast to the results obtained with the recombinant protein, compound **5** did not show substantial inhibitory effect on the cells, probably due to its high toxicity.

Thus, it is likely that the disaccharide nucleosides examined, despite their large size and hydrophilicity, are capable of easy penetration through both plasma and nuclear membranes. It is likely that transfer of these compounds through plasma membrane into the cell is carried out by nucleoside transporters and is similar to the transport of various antiviral (acyclovir, zidovudine, etc.) and antineoplastic (cytarabine, cladribine, gemcitabine, etc.) nucleoside analogues.[25–27]

Incubation of SKOV-3 cells with dAdo or Thd in concentration of 1 mM significantly suppressed culture growth and provoked cell death. At the same time, attachment of second ribose residue at the 3′-position of these nucleosides (compounds **2a** and **2e**) notably lessened their cytotoxicity. The nature

of heterocyclic base had no effect on the toxic properties of the tested compounds, since different disaccharide nucleosides were not toxic at concentrations up to 1 mM.

Alternatively, nucleosides bearing dialdehyde group in the carbohydrate part of molecule (compounds **4**, **5**, and **6**) strongly suppressed cell growth in millimolar concentrations. However, their cytotoxicity was not based on the ability to inhibit PARP-1, as their inhibitory efficacy increases in an order **6** < **5** < **4**, whereas cytotoxic activity elevates in the reverse order: **4** < **5** < **6**. In addition, the most potent PARP-1 inhibitor, **3-AB**, taken in concentration of 1 mM, in which it completely inactivates PARP-1, had no effect on culture growth in comparison with the control.

Pharmacological properties of nucleoside dialdehyde derivatives obtained by periodate oxidation of parent nucleosides were extensively studied. For example, oxidized derivatives of inosine,[28,29] 5′-deoxyinosine,[30] methylpurine,[31] and several other nucleosides[32] have been reported to display pronounced antitumor effects. Therefore, dialdehyde derivatives of disaccharide nucleosides are believed to be promising candidates for application in antitumor chemotherapy, as they combine properties of both inhibitors of PARP-1 and cytostatics.

In conclusion, we have described a lot of disaccharide analogues of pyrimidine nucleosides as prototypes for novel group of PARP-1 inhibitors. Advantageous properties of these agents are their low cytotoxicity and the ability to penetrate into cells.

EXPERIMENTAL

Chemistry

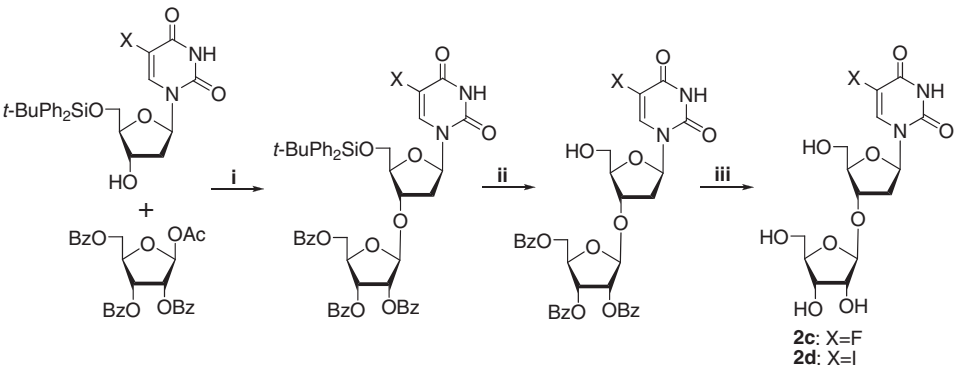
The solvents and materials of reagent grade were used without additional purification. Thin layer chromatography (TLC) was performed on Alugram SIL G/UV254 (Macherey-Nagel) with UV visualization. Column chromatography was performed on Kieselgel 0.040–0.063 mm. Reversed-phase chromatography was performed on chromatograph UVICORD-II (LKB), equipped with column (diameter 25 mm), containing modified silica gel (Polygosil C_{18}, pore diameter 60 Å, sorbent volume 30 mL). Chromatographic separation was controlled by UV-detector. ^{1}H NMR spectra were recorded on Bruker AMX 400 NMR instrument at 32°C. Chemical shifts in ppm were measured relative to the residual solvent signal as internal standard; coupling constants J are given in Hertz (Hz). The position of NH resonances was additionally confirmed by their exchange with the addition of D_2O. The UV spectra were recorded on a Cary 300UV/VIS spectrophotometer (Varian). LC-MS analysis was performed on Surveyor MSQ instrument (Thermo Finnigan, USA), operating in atmospheric pressure chemical ionization (APCI) mode with detection of positive and negative

ions and equipped with Onyx Monolithic C18 25 × 4.6 mm Part No CHO-7645 column. The eluent was a gradient of 0.1% HCOOH aqueous solution in MeCN. Chromatographic peaks were detected simultaneously with evaporative light scattering detector (ELSD), photodiode array detector (PAD), and total ion current (TIC) detector. ESI-spectroscopy was performed on Q-Tof micromass instrument (Great Britain) equipped with standard electron-spray ionization (ESI) interface.

The following compounds were prepared according to the procedures described elsewhere: 2′-(*O*-*β*-D-ribofuranosyl)-nucleosides (**1a–d**),[33,34] 3′-(*O*-*β*-D-ribofuranosyl)-2′-deoxyadenosine (**2a**),[35] 3′-(*O*-*β*-D-ribofuranosyl)-2′-deoxycytidine (**2b**),[36] 3′-(*O*-*β*-D-ribofuranosyl)-2′-deoxythymidine (**2e**),[36] *O*-*β*-D-Ribofuranosyl-(1′′-5′)-*O*-*β*-D-Ribofuranosyl-(1′′–3′)-thymidine (**2f**),[36] *O*-*β*-D-Ribofuranosyl-(1′′–3′)-thymidine-5′-*O*-phosphate (**2g**),[37] *O*-*β*-D-Ribofuranosyl-(1′′–3′)-thymidine-5′′-*O*-phosphate (**2h**).[37]

General Procedure for Preparation of 3′-*O*-modified Disaccharides 2c and 2d

Compounds **2c** and **2d** were prepared from corresponding 5′-*O*-TBDPS-deoxyuridines by their glycosylation followed by deprotection of silyl and benzoyl functionalities according to Scheme 1.



SCHEME 1 Synthesis of disaccharide nucleosides. Reagents and conditions: (i) $SnCl_4$, $ClCH_2CH_2Cl$, 0°C, 10 minutes; (ii) Bu_4NF/THF, r.t., 45 minutes; (iii) NH_3/MeOH, r.t., four days.

1-[5-O-tert-butyldiphenylsilyl-3-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-fluorouracil (Step i)

To a cold solution (0°C) of 1-*O*-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (484 mg, 0.96 mmol) in 1,2-dichloroethane (12 mL) under nitrogen, tin tetrachloride (0.14 mL, 1.18 mmol) was added and the solution was kept at 0°C for 10 minutes. After addition of 1-[5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-*β*-D-ribofuranosyl]-5-fluorouracil (387 mg, 0.8 mmol), the resulting solution

was kept at 0°C for 40 minutes. Then 10% aqueous solution of sodium bicarbonate (5 mL) was added and the suspension was stirred at 0°C for 20 minutes. The suspension was diluted with methylene chloride (20 mL) and filtered through Hyflo Super Cel; the organic layer was separated, washed with water (10 mL), dried, and evaporated to dryness. The residue was purified by column chromatography on silica gel (50 g). The column was washed with methylene chloride (500 mL) and then eluted with 1% ethanol in methylene chloride to give the resulting product as foam. The yield was 446 mg (60%). R_f 0.50 (methylene chloride:ethanol, 97:3). ^{1}H NMR ($CDCl_3$): 8.48 br s (1H, NH Ura^{5F}), 8.10-7.25 m (26H, Ph, Bz, H-6 Ura^{5F}), 6.21 dd (1H, $J_{1',2'a} = 6.8$, $J_{1',2'b} = 5.8$, H-1′ dUrd^{5F}), 5.82 dd (1H, $J_{3',2'} = 4.8$, $J_{3',4'} = 6.4$, H-3′ Rib), 5.62 d (1H, H-2′ Rib), 5.24 s (1H, H-1′ Rib), 4.76-4.60 m (3H, H-4′, H-5′a, H-5′b Rib), 4.55 ddd (1H, $J_{3',2'a} = 7.0$, $J_{3',2'b} = 3.3$, $J_{3',4'} = 2.7$, H-3′ dUrd^{5F}), 4.09 ddd (1H, $J_{4',5'a} = 3.0$, $J_{4',5'b} = 2.5$, H-4′ dUrd^{5F}), 3.92 dd (1H, $J_{5'a,5'b} = -11.5$, H-5′a dUrd^{5F}), 3.78 dd (1H, H-5′b dUrd^{5F}), 2.60 ddd (1H, $J_{2'a,2'b} = -13.5$, H-2′b dUrd^{5F}), 2.15 ddd (1H, H-2′a dUrd^{5F}), 1.05 s (9H, *t*-Bu).

1-[5-O-tert-butyldiphenylsilyl-3-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-iodouracil (Step i)

Analogous glycosylation of 1-[5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-ribofuranosyl]-5-iodouracil (474 mg, 0.8 mmol). The yield was 639 mg (77%) as foam. R_f 0.48 (methylene chloride:ethanol, 97:3). ^{1}H NMR ($CDCl_3$): 8.30 br s (1H, NH Ura^{5I}), 8.15-7.25 m (26H, Ph, Bz, H-6 Ura^{5I}), 6.15 dd (1H, $J_{1',2'a} = 8.2$, $J_{1',2'b} = 5.6$, H-1′ dUrd^{5I}), 5.81 dd (1H, $J_{3',2'} = 5.0$, $J_{3',4'} = 6.5$, H-3′ Rib), 5.63 d (1H, H-2′ Rib), 5.26 s (1H, H-1′ Rib), 4.78-4.68 m (2H, H-4′, H-5′a Rib), 4.61 dd (1H, $J_{5'b,5'a} = -11.7$, $J_{5'b,4'} = 5.1$, H-5′b Rib), 4.52 dt (1H, $J_{3',2'a} = 6.2$, $J_{3',2'b} = J_{3',4'} = 2.2$, H-3′ dUrd^{5I}), 4.13 ddd (1H, $J_{4',5'a} = 3.4$, $J_{4',5'b} = 3.2$, H-4′ dUrd^{5I}), 3.90 dd (1H, $J_{5'a,5'b} = -11.5$, $J_{5'b,4'} = 3.2$, H-5′a dUrd^{5I}), 3.79 dd (H-5′b dUrd^{5I}), 2.63 ddd (1H, $J_{2'a,2'b} = -13.7$, H-2′b dUrd^{5I}), 2.11 ddd (1H, H-2′a dUrd^{5I}), 1.07 s (9H, *t*-Bu).

1-[3-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-fluorouracil (Step ii)

1-[5-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-fluorouracil (446 mg, 0.48 mmol) was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (1.3 mL). The solution was kept for 45 minutes at 20°C, evaporated to dryness, co-evaporated with chloroform (10 mL), and applied onto a silica gel column (20 g). The column was washed with methylene chloride (300 mL) and with 1% ethanol in methylene chloride (200 mL) and then eluted with 2.5% ethanol in methylene chloride to give the resulting product as foam. The yield was 308 mg (93%). R_f 0.37 (methylene chloride:ethanol, 97:3). ^{1}H NMR ($CDCl_3$): 8.38 br s (1H, NH Ura^{5F}), 8.20-7.75 m (7H, Bz,

H-6 Ura^{5F}), 7.70-7.30 m (9H, Bz), 6.06 dd (1H, $J_{1',2'a} = 6.6$, $J_{1',2'b} = 6.2$, H-1′ dUrd^{5F}), 5.81 dd (1H, $J_{3',2'} = 4.9$, $J_{3',4'} = 6.9$, H-3′ Rib), 5.65 d (1H, H-2′ Rib), 5.33 s (1H, H-1′ Rib), 4.80-4.65 m (2H, H-3′ dUrd^{5F}, H-5′a Rib), 4.65-4.50 m (2H, H-4′ dUrd^{5F}, H-5′b Rib), 4.13 ddd (1H, $J_{4',5'a} = 3.2$, $J_{4',5'b} = 2.4$, $J_{4',3'} = 2.8$, H-4′ dUrd^{5F}), 3.97 dd (1H, $J_{5'a,5'b} = -11.8$, H-5′a dUrd^{5F}), 3.85 dd (1H, H-5′b dUrd^{5F}), 2.50 ddd (1H, $J_{2'b,3'} = 3.6$, $J_{2'a,2'b} = -13.9$, H-2′b dUrd^{5F}), 2.33 ddd (1H, $J_{2'a,3'} = 6.8$, H-2′a dUrd^{5F}).

1-[3-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-iodouracil (Step ii)

Analogous desilylation of 1-[5-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-iodouracil (639 mg, 0.62 mmol). The yield was 409 mg (83%) as foam. R_f 0.34 (methylene chloride:ethanol, 97:3). ^{1}H NMR ($CDCl_3$): 8.37 br s (1H, NH Ura^{5I}), 8.09 s (1H, H-6 Ura^{5I}), 8.07 dd (2H, $J_{\text{ortho-meta}} = 8.0$, $J_{\text{ortho-para}} = 1.0$, *o*-Bz), 8.00 dd (2H, *o*-Bz), 7.91 dd (2H, *o*-Bz), 7.61-7.50 m (3H, *p*-Bz), 7.42 dd (4H, $J_{\text{meta-para}} = 7.6$, *m*-Bz), 7.35 dd (2H, *m*-Bz), 6.00 dd (1H, $J_{1',2'a} = J_{1',2'b} = 6.5$, H-1′ dUrd^{5I}), 5.82 dd (1H, $J_{2',3'} = 4.8$, $J_{3',4'} = 6.7$, H-3′ Rib), 5.66 d (1H, H-2′ Rib), 5.37 s (1H, H-1′ Rib), 4.79–4.71 m (1H, H-4′ Rib), 4.73 dd (1H, $J_{5'a,5'b} = -13.2$, $J_{5'a,4'} = 3.9$, H-5′a Rib), 4.63–4.54 m (2H, H-5′b Rib, H-3′ dUrd^{5I}), 4.13 q (1H, $J_{4',5'a} = J_{4',5'b} = J_{4',3'} = 2.6$, H-4′ dUrd^{5I}), 3.96 dd (1H, $J_{5'a,5'b} = -12.0$, H-5′a dUrd^{5I}), 3.83 dd (H-5′b dUrd^{5I}), 2.53 ddd (1H, $J_{2'a,2'b} = -14.0$, $J_{2'b,3'} = 3.7$, H-2′b dUrd^{5I}), 2.39 dt (1H, $J_{2'a,3'} = J_{2'a,1'} = 6.5$, H-2′a dUrd^{5I}).

1-[3-O-(β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-fluorouracil (2c, Step iii)

A solution of 1-[3-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-fluorouracil (308 mg, 0.44 mmol) in 5 M ammonia in methanol (9 mL) was kept for four days at 20°C and then concentrated in vacuo to dryness. The residue was partitioned between methylene chloride (10 mL) and water (20 mL), and the aqueous layer was washed with methylene chloride (2 × 10 mL) and evaporated to dryness; the residue was purified by reversed-phase chromatography. The column was washed with water (100 mL) and the product was eluted with 5% ethanol in water. The fractions, containing the product, were collected and evaporated. The residue was dissolved in methanol (10 mL), filtered from mechanical impurities, evaporated in vacuum, and then co-evaporated with methanol (2 × 10 mL) to give white hygroscopic foam. The yield was 118 mg (71%). R_f 0.40 (methylene chloride:ethanol, 80:20). UV (pH 1–7): λ_{max} 268 nm (ϵ 8900); (pH 13): λ_{max} 270 nm (6900). ESI: ($C_{14}H_{19}FN_2O_9$+Na^+). Calc. 401.1000. Found 401.0972. ^{1}H NMR (CD_3OD): 8.21 d (1H, $J_{H\text{-}6,F} = 6.8$, H-6 Ura^{5F}), 6.19 dd (1H, $J_{1',2'a} = 7.4$ Hz, $J_{1',2'b} = 6.0$, H-1′ dUrd^{5F}), 4.99 s (1H, H-1′

Rib), 4.42 ddd (1H, $J_{3',2'a} = 6.3$, $J_{3',2'b} = 3.2$, $J_{3',4'} = 6.0$, H-3′ dUrd^{5F}), 4.07 dd (1H, $J_{3',2'} = 4.7$, $J_{3',4'} = 6.9$, H-3′ Rib), 4.03 dd (1H, $J_{4',5'a} = 3.2$, H-4′ dUrd^{5F}), 3.95 dd (1H, $J_{4',5'b} = 3.4$, H-4′ Rib), 3.90 d (1H, H-2′ Rib), 3.80 dd (1H, $J_{5'a,5'b} = -12.0$ Hz, H-5′b dUrd^{5F}), 3.72 dd (1H, $J_{5'a,5'b} = -11.9$, H-5′b Rib), 3.67–3.53 m (2H, H-5′a dUrd^{5F}, H-5′a Rib), 2.53 ddd (1H, $J_{2'b,2'a} = -13.7$, H-2′b dUrd^{5F}), 2.19 ddd (1H, H-2′a dUrd^{5F}). ^{13}C NMR (DMSO-*d*6): 149.26 (C-4), 141.62 (C-2), 138.56 (C-6), 124.54 d ($J_{C\text{-}5,F} = 136$, C-5), 106.40 (C-1′ Rib), 84.85 (C-4′ dUrd^{5F}), 84.54 (C-1′ dUrd^{5F}), 83.67 (C-4′ Rib), 76.55 (C-3′ dUrd^{5F}), 74.46 (C-2′ Rib), 70.65 (C-3′ Rib), 62.80 (C-5′ Rib), 61.03 (C-5′ dUrd^{5F}), 37.91 (C-2′ dUrd^{5F}).

1-[3-O-(β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-iodouracil (2d, Step iii)

Analogous debenzoylation of 1-[3-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-deoxy-β-D-ribofuranosyl]-5-iodouracil (409 mg, 0,51 mmol). The yield was 178 mg (72%) as white hygroscopic foam. R_f 0.38 (methylene chloride:ethanol, 80:20). UV (pH 1–7): λ_{max} 287 nm (ϵ 8300); (pH 13): λ_{max} 280 nm (ϵ 6400). LC-MS (APCI): retention time (RT), 1.25 minutes; ($C_{14}H_{19}IN_2O_9$-H^++HCOOH). Calc. 531.01. Found 530.54. ^{1}H NMR (D_2O): 8.32 s (1H, H-6 Ura^{5I}), 6.28 t (1H, $J_{1',2'a} = J_{1',2'b} = 6.7$, H-1′ dUrd^{5I}), 5.19 s (1H, H-1′ Rib), 4.53 dt (1H, $J_{3',2'b} = J_{3',4'} = 4.0$, $J_{3',2'a} = 6.5$, H-3′ dUrd^{5I}), 4.30 dd (1H, $J_{3',2'} = 4.8$, $J_{3',4'} = 6.8$ Hz, H-3′ Rib), 4.23 dt (1H, $J_{4',3'} = J_{4',5'b} = 4.0$, $J_{4',5'a} = 4.7$, H-4′ dUrd^{5I}), 4.18 d (1H, H-2′ Rib), 4.12 td (1H, $J_{4',3'} = J_{4',5'b} = 6.8$, $J_{4',5'a} = 3.4$, H-4′ Rib), 3.96-3.91 m (2H, H-5′a dUrd^{5I}, H-5′a Rib), 3.86 dd (1H, $J_{5'b,5'a} = -12.4$, H-5′b dUrd^{5I}), 3.75 dd (1H, $J_{5'b,5'a} = -12.3$, H-5′b Rib), 2.67 ddd (1H, $J_{2'b,2'a} = -14.7$, H-2′b dUrd^{5I}), 2.45 dt (1H, H-2′a dUrd^{5I}). ^{13}C NMR (D_2O): 172.28 (C-4), 159.14 (C-2), 148.22 (C-6), 130.00 (C-5), 109.32 (C-1′ Rib), 88.56 (C-4′ dUrd^{5I}), 87.36 (C-1′ dUrd^{5I}), 85.64 (C-4′ Rib), 80.03 (C-3′ dUrd^{5I}), 77.26 (C-2′ Rib), 73.47 (C-3′ Rib), 65.54 (C-5′ Rib), 63.83 (C-5′ dUrd^{5I}), 40.54 (C-2′ dUrd^{5I}).

Dialdehyde Derivative 4

To 0.3 mL of aqueous solution of compound **2e** (112 mg, 0.3 mmol), 0.7 mL of 0.5 M sodium periodate was added dropwise at 20°C. The reaction mixture was kept for 20 minutes at 20°C and then diluted with ethanol (10 mL). The precipitate of $NaIO_3$ was filtered-off and washed with ethanol (10 mL). The filtrates were evaporated in vacuum to dryness. The residue was dissolved in methanol (2 mL), filtered from traces of $NaIO_3$, and evaporated in vacuum to dryness. The residue was co-evaporated with methanol (2 × 5 mL) and dried over P_2O_5 in vacuum dessicator during several days. The yield was 110 mg (98%) as white hygroscopic foam. R_f 0.65 (extending spot) in methylene chloride:ethanol, 80:20.

Dialdehyde Derivative 5

To 1.0 mL of aqueous solution of Urd (244 mg, 1.0 mmol), 2.4 mL of 0.5 M sodium periodate was added dropwise at 20°C. The reaction mixture was kept for 20 minutes at 20°C and then diluted with ethanol (30 mL). The precipitate of $NaIO_3$ was filtered-off and washed with ethanol (10 mL) and the filtrate was evaporated in vacuum to dryness. The residue was dissolved in methanol (7 mL), filtered from inorganic salts, and the filtrate was evaporated in vacuum to dryness. The residue was co-evaporated with methanol (2 × 10 mL) and dried over P_2O_5 during several days. The yield was 235 mg (97%) as white hygroscopic foam. R_f 0.71 (extending spot) in methylene chloride:ethanol, 80:20. ^{1}H NMR Spectrum in D_2O is very complex due to the presence of hydrated species of dialdehyde that may form diastereomeric internal hemiacetals and dioxane derivatives and it is very similar to the published one.[18]

Dialdehyde Derivative 6

Analogous oxidation of 1-(β-D-erythrofuranosyl)uracil[38] (65 mg, 0.3 mmol) with sodium periodate yielded 62 mg (96%) of compound **6**. R_f 0.76 (extending spot) in methylene chloride:ethanol, 80:20. ^{1}H NMR (D_2O): 7.70 d (1H, $J_{6,5} = 7.8$, H-6 Ura), 5.86 d (1H, H-5 Ura), 5.56 d (1H, $J_{2',1'} = 4.0$, H-1′ oxidized erythrose), 5.15-5.00 m (2H, H-2′, H-4′ oxidized erythrose), 3.57 dd (1H, $J_{5'a,5'b} = -10.6$, $J_{5'a,4'} = 4.4$, H-5′a oxidized erythrose), 3.50 dd (1H, $J_{5'a,5'b} = -10.6$, $J_{5'b,4'} = 4.7$, H-5′b oxidized erythrose).

PARP-1 Activity Assay

Purified human recombinant PARP-1 and [^{3}H] NAD^+ were prepared as described elsewhere.[39–41] The PARP-1 inhibiting activities of nucleosides were assayed as described previously.[42] Briefly, reaction mixtures (15 μL) containing 2 optic units (A_{260})/mL DNAse I-activated calf thymus DNA, 300 μM NAD+, 0.4 μCi [^{3}H] NAD^+, 10% DMSO, and tested compounds in the various concentrations if required in the buffer (50 mM Tris, pH 8.0, 20 mM MgCl2, 150 mM NaCl, 7 mM β-mercaptoethanol) were preincubated for five minutes at 37°C, and then recombinant human PARP-1 was added to the final concentration of 200 nM. After one minute, the reaction was stopped by dropping the 12 μL aliquote on the Whatman 1 paper filters moistened in 5% trichloroacetic acid. Filters were washed four times with 150 mL of 5% trichloroacetic acid, once with 90% ethanol, and then air-dried. [^{3}H] ADP-ribose incorporation into the acid insoluble material was quantified using a scintillation counter Tri-Carb 2800 (Perkin Elmer) in the toluene scintillator.

Cell Culture

SKOV-3 human ovarian carcinoma cell line was kindly provided by Dr. Galina A. Posypanova (Moscow Research Institute of Medical Ecology, Russia). The cell line was cultured in RPMI 1640 (BioloT), containing 10% fetal bovine serum (PAA Laboratories), 2 mM *L*-glutamine, and 50 μg/mL gentamicin at 37°C in a humidified atmosphere with 95% air and 5% CO_2.

Cytotoxicity Analysis

Cytotoxicity of all tested compounds was determined by the change in the proliferative status of cultured cells using MTT test. Exponentially growing SKOV-3 cells were seeded in 96-well plates at a density of 2.5×10^3 cells/well (200 μL per well); 24 hours later, the number of attached cells in premarked fields were counted and nucleosides or **3-AB** were added to the growth medium. The cells were cultured for 72 hours and then the medium in each well was replaced with the medium containing 0.5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). After 3-hour incubation at 37°C, the number of the living (i.e., accumulating formazan) cells was counted in each premarked field. Finally, the density of living cells in the selected fields after 72-hour incubation with the tested compound was calculated and expressed as a percentage of live cells relative to their original number 24 hours after seeding.

Determination of Poly(ADP-Ribose) in Cultured Cells

Exponentially growing SKOV-3 cells were seeded in 4-well plates on coverslips at a density of 30×10^3 cells/well in 1 mL growing medium. After 48 hours, nucleosides or **3-AB** were added and the cultures were further incubated either for one or 24 hours. Then the cells were treated with hydrogen peroxide (1 mM, 5 minutes) in the presence of tested compounds and fixed by methanol. The fixed cells were incubated with mouse monoclonal antibody to PAR (10H; Santa Cruz Biotechnology) at 1:300 dilution, followed by incubation with goat antimouse antibody conjugated with Alexa-488 (Molecular Probes) at 1:1000 dilution. Nuclei were counterstained with propidium iodide (PI) and photomicrographs were obtained using confocal microscope LSM-510 (Carl Zeiss). The photomicrographs were analyzed by AxioVision 4.8 (Carl Zeiss) and ImageJ 1.42q (National Institutes of Health, USA) programs.

Data Analysis

All data were analyzed using the Sigma Plot 11.0 (Systat Software Inc.) or GraphPad Prism 5.00 (GraphPad Software Inc.) software. The IC_{50} and n_H

values were calculated using the four parameter logistic fit equation. Statistical analysis of experimental data was performed by the Student's unpaired *t*-test. The difference between the values was considered significant when $p < 0.05$.

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