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BIOLOGICAL ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS BASED ON POLYOL ACETALS AND THEIR DERIVATIVES

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The biological activity of cyclic acetals (penta- and dipentaerythritol, ethriol, and diglycerol) and 5-ethyl-5-hydroxymethyl-1,3-dioxane esters was studied *in vitro*. The cytotoxic properties of 5-ethyl-5-hydroxymethyl-1,3-dioxane esters were established on HEK293 and SH-SY5Y cell lines. Potential antioxidant properties of 7,11,18,21-tetraoxaspiro-5,2,2,5-heneicosane, 2-[(5-ethyl-5-hydroxymethyl)-1,3-dioxan-2-yl]phenol, 4.4-[oxydi(methylene)]-bis-2-diisopropyl-1,3-dioxolane, 4,4′-[oxydi(methylene)]-bis-1,3-dioxolane, (5-ethyl-1,3-dioxan-5-yl)methyl-2-methyl acrylate, and bis(5-ethyl-1,3-dioxan-5-yl)methyl maleate were established *in vitro* using an oxidative stress model.

Keywords: acetals, polyols, esters, cytotoxicity *in vitro*, antioxidant activity *in vitro*.

Derivatives of 2,2-dimethyl-1,3-dioxolane, 1,3-dioxolanes, and acetals of pentaerythritol and dipentaerythritol are used as additives for oils and polymers and starting materials to synthesize various plasticizers, solvents, stabilizers, alkyd and epoxide resins, corrosion inhibitors, etc. [1 – 3]. Furthermore, these compounds are attractive because of their potential applications in medicine. Compounds of this class are known to possess rather broad spectra of biological activity and have demonstrated cytotoxic, antioxidant, antimicrobial, and antiviral properties [4 – 8]. The goal of the present work was to synthesize new cyclic acetals of penta- and dipentaerythritol, ethriol, and diglycerol and esters of 5-ethyl-5-hydroxymethyl-1,3-dioxane and to study their cytotoxic and antioxidant properties *in vitro*.

EXPERIMENTAL CHEMICAL PART

Chromatographic analysis of the reaction products used an HRGC 5300 Mega Series Carlo Erba chromatograph with a flame-ionization detector (He carrier gas, flow rate 30 mL/min, column length 25 m, programmed heating from 50 to 280°C at 8°C/min). NMR spectra were recorded in $CDCl_3$ on a Bruker Avance-500 spectrometer (500.13 MHz).

Acetals **9 – 14** were prepared as before [9].

3,9-Diisopropyl-2,4,8,10-tetraoxaspiro-5,5-undecane (9). Mp 43 – 44 °C. PMR spectrum, δ, ppm: 0.82 (s, 12H, 4 CH_3), 1.96 – 2.02 (m, 2H, 2 CH), 3.54 – 3.69 (m, 8H, 4 CH_2), 4.69 (d, 2H, 2 CH, J 2.9 Hz).

7,11,18,21-Tetraoxaspiro-5,2,2,5-heneicosane (10). Mp 51 – 52 °C. PMR spectrum, δ, ppm: 1.93 – 2.01 (m, 20H, 10 CH_2), 2.98 – 3.41 (m, 8H, 4 CH_2).

Oxy-bis(methylene-1,5-dioxaspiro-5,5-undecane-3,3-diyl)dimethanol (11). Mp 58 – 59 °C. PMR spectrum, δ, ppm: 1.51 – 1.29 (m, 20H, 10 CH_2), 2.66 (d, 4H, 4 CH_a, J 3.1 Hz), 2.96 (d, 4H, 4 CH_b, J 3.0 Hz), 3.62 (s, 4H, 2 CH_2), 4.12 (s, 4H, 2 CH_2).

2-[(5-Ethyl-5-hydroxymethyl)-1, 3-dioxan-2-yl]phenol (12). Viscous colorless liquid. PMR spectrum, δ, ppm: 0.85 (s, 3H, CH_3), 1.20 (t, 2H, CH_2, J 5.0, 11.0 Hz), 3.08 (s, 2H, CH_2), 3.56 – 3.60 (m, 4H, 2 CH_2), 4.90 (d, 1H, CH, J 2.7 Hz), 6.90 – 7.12 (m, 5H, Ph).

4,4′-[Oxydi(methylene)]-bis-2-diisopropyl-1,3-dioxolane (13). Viscous colorless liquid. PMR spectrum, δ, ppm: 1.52 – 1.58 (m, 2H, 2 CH), 1.85 (s, 12H, 4 CH_3), 3.70 (d, 4H,

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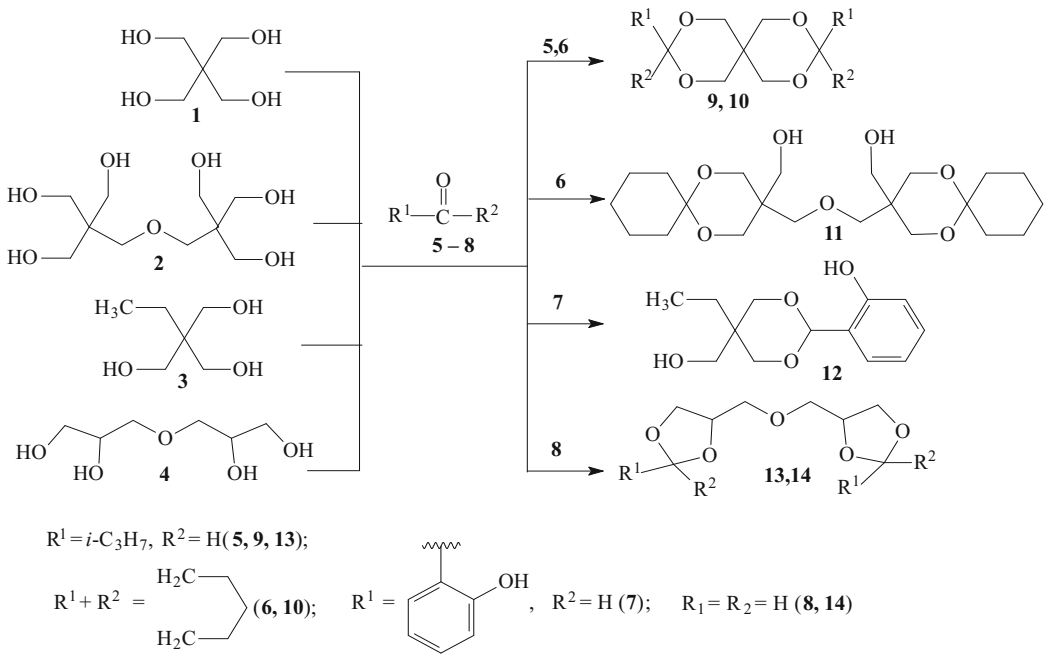

Fig. 1. Synthetic scheme for cyclic acetals of penta- and dipentaerythritol, ethriol, and diglycerol.

2 CH_2, J 3.0 Hz), 3.86 – 3.92 (s, 4H, 2 CH_2), 3.96 – 4.02 (m, 2H, 2 CH), 4.10 (d, 2H, 2 CH, J 7.0 Hz).

4,4′-[Oxydi(methylene)]-bis-1,3-dioxolane (14). Colorless liquid. The physicochemical characteristics agreed with those in the literature [9].

Esters 18 and 19. Esters of (5-ethyl-1,3-dioxan-5-yl)methyl-2-methyl acrylate (**18**) and bis(5-ethyl-1,3-dioxan-5-yl)methyl maleate (**19**) were prepared by the published method. Their physicochemical characteristics agree with those in the literature [10].

EXPERIMENTAL BIOLOGICAL PART

HEK293 (human embryonic kidney line) and SH-SY5Y cells (human neuroblastoma) were cultivated in DMEM growth medium (PanEco) containing fetal bovine serum (10%; Biowest, France), L-glutamine (2 mM; Biolot), and gentamicin sulfate (50 μg/mL; Biolot) with 5% CO_2 at 37°C. Compounds in solution at final concentrations of 1, 10, and 100 μM were added to the cells after 24 and 48 h. Viability was assayed using commercial PrestoBlue™ solution (Invitrogen) according to the manufacturer's protocol. Fluorescence was detected using a 2300 EnSpire® Multimode Plate Reader (PerkinElmer). The IC_{50} values characterizing the strength of cytotoxic properties of the compounds (concentration at which 50% inhibition of cell viability was observed) were calculated using the GraphPad Prism 4.0 program (GraphPad Software Inc.). Data are given as $M \pm SEM$, $N = 3$.

Modeling of oxidative stress *in vitro*. SH-SY5Y and HEK293 cells were cultivated in DMEM medium (PanEco) containing fetal bovine serum (10%; Biowest), L-glutamine (2 mM; Biolot), and gentamicin sulfate (50 mg/mL; Biolot) with 5% CO_2 at 37°C. Oxidative stress was modeled by adding H_2O_2 (50 μM, Solopharm) after 1 h. Solutions of the tested compounds at final concentrations of 1, 10, and 100 μM were added 24 h before or after incubation of the cells with H_2O_2. Viability of cells was assayed using PrestoBlue as above. Data are given as $M \pm SEM$, $N = 3$.

Statistical analysis used Statistica 6.1 standard programs (StatSoft Inc.). Significance of differences was found by the ANOVA method using the Dunnett criterion. The statistical significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Condensation of polyols **1 – 4** with ketones **5 – 8** in the presence of H_2SO_4 at 80°C produced in quantitative yields cyclic acetals **9 – 14** (Fig. 1).

Alkylation of 5-ethyl-5-hydroxymethyl-1,3-dioxane (**15**) by methacrylic (**16**) or maleic acid (**17**) in the presence of catalytic amounts of *p*-toluenesulfonic acid synthesized in 8 – 10 h in 70 – 90% yields (5-ethyl-1,3-dioxan-5-yl)methyl-2-methyl methacrylate (**18**) and bis(5-ethyl-1,3-dioxan-5-yl)methyl-2*Z*-but-2-enedionate (**19**) (Fig. 2).

The structures of heterocycles **9 – 14** and esters **18** and **19** were elucidated by PMR spectroscopy. Resonances of acetals were assigned by analyzing chemical shifts and spin—spin coupling constants (SSCC) of the heterocyclic protons [10].

The biological activity of the synthesized compounds was studied *in vitro* using HEK293 and SH-SY5Y cells. The

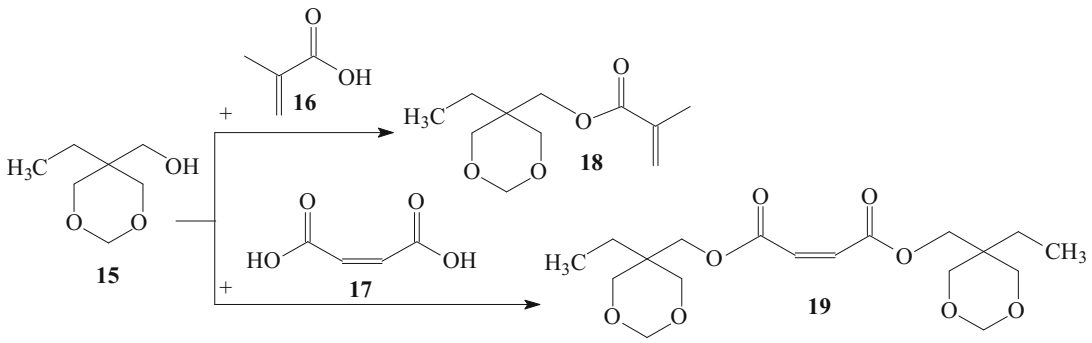

Fig. 2. Synthetic scheme for 5-ethyl-5-hydroxymethyl-1,3-dioxane esters.

study of cellular toxicity was important and a necessary initial stage of the work with the new synthesized compounds that enabled their potential antitumor properties to be revealed and nontoxic compounds to be selected to develop pharmaceutical or cosmetic compounds. The study of the influence of the compounds on cell viability found cytotoxic activity against cell line SH-SY5Y for esters of 5-ethyl-5-hydroxymethyl-1,3-dioxane **18** [(5-ethyl-1, 3-dioxan-5-yl)methyl 2-methylacrylate, the IC_{50} of which was 16.35 ± 1.43 μM], and **19** [bis(5-ethyl-1,3-dioxan-5-yl)methyl maleate, the IC_{50} of which was 39.38 ± 1.58 μM] (Table 1). It is worth noting that a similar influence was not observed against HEK293 cells. The other synthesized compounds (**9 – 14**) did not influence the viability of the used cell lines in the concentration range 1 – 100 μM.

Potential antioxidant properties of the new compounds were determined using an oxidative stress model *in vitro* with H_2O_2 (50 μM, 1 h). Oxidative stress is known to have a negative effect on mitochondrial functioning and, as a result, cell viability, the change of which reflects the antioxidant properties of the compounds [11]. The compounds were added to cells 24 h before or after induction of oxidative stress. The used concentrations of the esters of 5-ethyl-5-hydroxymethyl-1,3-dioxane (**18** and **19**) were based on toxicity test results (Table 1). Incubation with **10** (7,11,18,21-tetraoxaspiro-5,2,2,5-heneicosane), **12** {2-[(5-ethyl-5-hydroxymethyl)-1, 3-dioxan-2-yl]phenol}, **18**, and **19** increased the viability of HEK293 cells under oxidative stress (Table 2). It is noteworthy that this increase reached values comparable to those in the control group (native cells without stress). Furthermore, the positive effect of these compounds did not depend on the time of administration to the cells (before or after H_2O_2).

Nerve tissue possesses extremely high susceptibility to oxidative stress [12]. According to the results in Table 2, compounds **10** and **12** increased the survival of SH-SY5Y cells, which were a model of neuronal cells, under stress conditions induced by H_2O_2. Compounds **13** {4,4′-[(oxydi(methylene)]-bis-2-diisopropyl-1,3-dioxolane} and **14** {4,4′-[oxydi(methylene)]-bis-1,3-dioxolane} exhibited positive effects on the analyzed parameter only with preincubation of the cells with these compounds before induction of stress. The

TABLE 1. Influence of Compounds on Cell Viability (*M* ± *SEM*)

Compound	MM, g/mol	IC_{50}, μM			
		24 h		48 h	
		HEK293	SH-SY5Y	HEK293	SH-SY5Y
9	431.00	> 100	> 100	> 100	> 100
10	486.00	> 100	> 100	> 100	> 100
11	414.00	> 100	> 100	> 100	> 100
12	238.00	> 100	> 100	> 100	> 100
13	274.00	> 100	> 100	> 100	> 100
14	190.00	> 100	> 100	> 100	> 100
18	214.00	> 100	16.35 ± 1.43*	> 100	11.25 ± 1.31*
19	372.00	> 100	57.78 ± 7.58*	62.83 ± 1.19	39.38 ± 1.58*

* $p < 0.05$ vs. IC_{50} for HEK293 cells.

TABLE 2. Influence of Compounds on Cell Viability in Oxidative Stress Model ($M \pm SEM$)

Compound, concentration, μM		Survival, %			
		HEK293		SH-SY5Y	
		before H_2O_2	after H_2O_2	before H_2O_2	after H_2O_2
Control		100.00 ± 5.03	100.00 ± 3.78	100.00 ± 5.03	100.00 ± 3.78
H_2O_2, 50 μM		83.19 ± 2.54*	80.13 ± 2.68*	76.22 ± 2.46*	73.81 ± 3.51*
9	1	82.19 ± 3.54	76.65 ± 7.99	77.18 ± 7.89	79.57 ± 0.89
	10	85.01 ± 7.35	79.58 ± 3.99	76.42 ± 3.76	76.85 ± 1.20
	100	84.02 ± 7.67	78.01 ± 7.08	72.34 ± 5.82	79.57 ± 0.89
10	1	85.42 ± 1.65	81.06 ± 0,.63	79.57 ± 1.89	78.25 ± 1.45
	10	93.76 ± 1.14#	86.19 ± 3.61	76.85 ± 1.20	86.47 ± 1.52#
	100	88.36 ± 2.45	91.55 ± 0.64#	80.53 ± 2.83#	90.71 ± 3.89#
11	1	81.45 ± 7.76	75.29 ± 4.87	76.22 ± 4.46	73.39 ± 2.12
	10	82.33 ± 8.45	75.02 ± 3.16	87.20 ± 7.52	66.06 ± 5,.10
	100	84.32 ± 2.07	78.01 ± 6.08	73.14 ± 5.80	64.93 ± 3.94
12	1	87.07 ± 0.98	82.55 ± 0.16	76.22 ± 3.94	74.69 ± 3.53
	10	94.87 ± 0.69#	95.43 ± 4.83#	79.52 ± 1.86	85.04 ± 5.57
	100	98.17 ± 0,.97#	97.11 ± 7.41#	88.76 ± 0.70#	80.70 ± 4.88#
13	1	84.32 ± 2.07	83.85 ± 7.22	88.81 ± 1.75#	73.69 ± 2.35
	10	91.74 ± 7.71	83.37 ± 6.55	90.80 ± 3.10#	73.81 ± 4.07
	100	85.43 ± 8.90	73.16 ± 3.46	91.10 ± 2.67#	76.22 ± 3.57
14	1	84.10 ± 6.15	76.90 ± 3.71	76.22 ± 1.75	74.74 ± 2.12
	10	86.10 ± 5.61	79.97 ± 2.01	81.12 ± 0.25	72.98 ± 2.22
	100	70.22 ± 6.45	68.99 ± 5.70	86.70 ± 2.12#	73.81 ± 5.69
18	1	85.61 ± 1.60	80.56 ± 0.56	…	…
	0.1	…	…	85.61 ± 1.60	80.56 ± 0.56
	10	89.55 ± 1.77#	85.18 ± 0.64#	…	…
	1	…	…	89.55 ± 1.77#	85.18 ± 0.64#
	100	94.87 ± 2.73#	100.27 ± 1.47#	…	…
	10	…	…	94.87 ± 2.73#	100.27 ± 1.47#
19	1	88.65 ± 1.19	83.76 ± 0.85	88.65 ± 1.19	83.76 ± 0.85
	10	94.27 ± 1.52#	86.67 ± 0.99#	94.27 ± 1.52#	86.67 ± 0.99#
	50	104.39 ± 1.21#	93.69 ± 2.46#	104.39 ± 1.21#	93.69 ± 2.46#
	100	91.10 ± 1.24#	84.92 ± 4.16	…	…

* $p < 0.05$ vs. corresponding control; # $p < 0.05$ vs. corresponding group "H_2O_2, 50 μM".

reduced viability found for esters of 5-ethyl-5-hydroxymethyl-1,3-dioxane (**18** and **19**, Table 2) was probably caused by their cytotoxic activities (Table 1). It is noteworthy that **18** and **19** showed activity only against HEK293 cells; **13** and **14**, against SH-SY5Y cells, which suggested a certain tissue specificity for their action (Table 2). The activity of **10** and **12** against HEK293 cells was comparable to that against SH-SY5Y cells.

Structural features of chemical compounds are known to play a significant role in the manifestation of their pharmacological effects [13]. According to the literature, compounds with an aromatic ring bonded to one or several hydroxyls or with unsaturated bonds in their structures effectively accept singlet oxygen and hydroxyl radical, thereby exhibiting antioxidant activity [14]. OH groups located at various positions can alter biological activity. Therefore, the combination of

such pharmacophores with functional structures determined the antioxidant properties of synthesized **9 – 14**. Let us presume that the simultaneous presence of the structural fragments in **9** and **11** could not decrease or fully suppress the negative influence of free-radical oxidation on the cells. However, the presence of a 1,3-dioxolane or cyclohexane group in **13**, **14**, and **10** or a phenol in **12** may have been responsible for the antioxidant properties observed for them [15].

Thus, compounds exhibiting cytotoxic and potential antioxidant activity were observed among the new cyclic ketals of penta-and dipentaerythritol, ethriol, diglycerol, and esters of 5-ethyl-5-hydroxymethyl-1,3-dioxane. The results were indicative of the potential for studying these and related compounds and helped to expand possible applications of them as biologically active agents.

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