Synthesis and cytotoxic properties of some cyclic acetals of diols and their dichlorocyclopropyl derivatives*

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Cyclic acetals of ethylene glycol, *cis*-but-2-ene-1,4-diol and their dichlorocyclopropyl derivatives were synthesized. Compounds simultaneously containing the *gem*-dichlorocyclopropane and 1,3-dioxacycloalkane cycles are shown to exhibit cytotoxic activity against the HEK293, SH-SY5Y, HepG2, MCF-7, A549, and Jurkat cell lines. The obtained results open up prospects for further studies of antitumor activity of 2-(2,2-dichlorocyclopropyl)-2-ethyl-1,3-dioxolane.

Key words: acetals, 1,4-butenediol, cinnamaldehyde, substituted *gem*-dichlorocyclopropanes, cytotoxicity *in vitro*.

Currently, an active search for new biologically active substances among compounds of plant and synthetic origin takes place.[1—4] Compounds that can be prepared from basic compounds such as ethylene glycol and *cis*-but-2-ene-1,4-diol are attracting attention due to their potential for use in medicine.[5,6] These compounds have a fairly wide spectrum of biological activity, their herbicidal, antioxidant, antimicrobial and antiviral properties are known.[7—11]

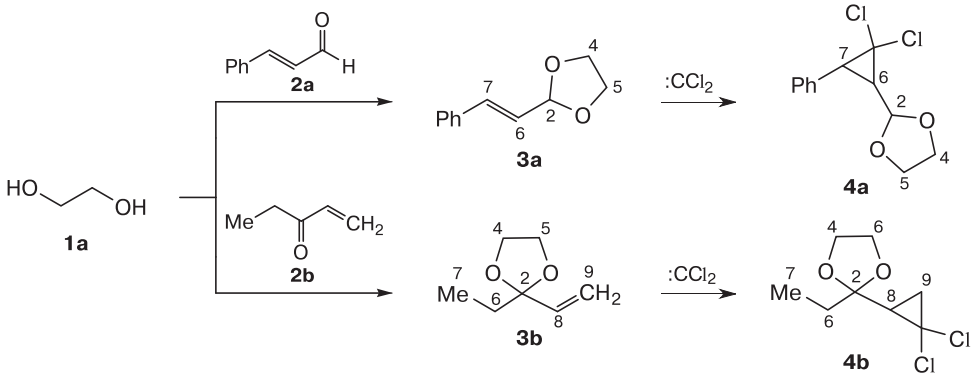
The present study is aimed at the synthesis of cyclic acetals of diols and their dichlorocyclopropyl derivatives and investigation of their cytotoxic characteristics *in vitro*.

Results and Discussion

Condensation of ethylene glycol (**1a**) with cinnamaldehyde (**2a**) and ethyl vinyl ketone (**2b**) in the presence of sulfuric acid in boiling benzene in accordance with a known method[6] produces cyclic unsaturated acetals **3a,b** with quantitative yields. Their subsequent dichlorocarbenation by the Makosza method gave corresponding new cyclic derivatives **4a,b** in yields exceeding 90% (Scheme 1).

Bicyclic compound **4c** was previously prepared (in yield exceeding 90%) by acetalization of isobutanal (**2c**) with *cis*-but-2-en-1,4-diol (**1b**) in the presence of cationite

Scheme 1



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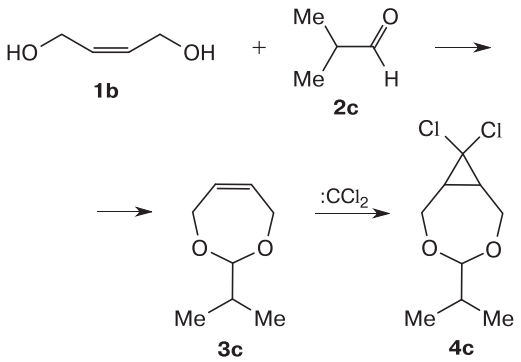
KU-2 (H^+) as a catalyst and subsequent dichlorocarbenation of the obtained 2-isopropyl-4,7-dihydro-1,3-dioxepin (**3c**) under the phase-transfer catalysis conditions using a 50% alkali solution and chloroform[6] (Scheme 2).

Scheme 2



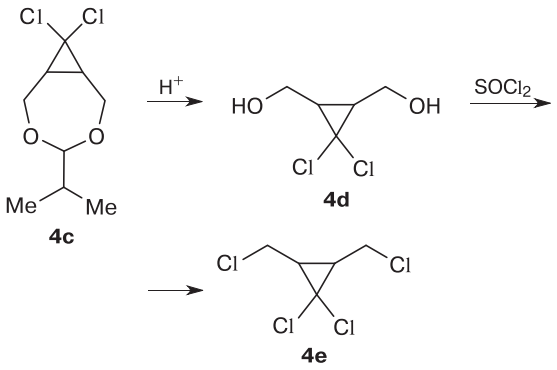
To increase the number of compounds involved in the study of cytotoxic activity, we synthesized[6] compounds **4d** and **4e** from bicyclic derivative **4c** in yields >90% (Scheme 3).

The structures of the obtained unsaturated acetals **3a—c**, their dichlorocyclopropyl derivatives **4a—c**, and dichlorocyclopropyl derivatives **4d,e** were proven using ^1H and ^{13}C NMR spectroscopy and gas chromatography—mass spectrometry.

The study of cytotoxicity was carried out *in vitro* using cell lines of normal (HEK293) and tumor (SH-SY5Y, HepG2, MCF-7, A549 and Jurkat) origin. The results are given in Table 1.

Studying the effect of the prepared compounds on the cell viability showed cytotoxic activity of 2-(2,2-dichloro-3-phenylcyclopropyl)-1,3-dioxolane (**4a**) and 8,8-dichloro-4-isopropyl-3,5-dioxabicyclo[5.1.0]octane (**4c**)

Scheme 3



against the HEK293, SH-SY5Y, Jurkat, HepG2, MCF-7, and A549 cell lines. It is important to note that 2-(2,2-dichlorocyclopropyl)-2-ethyl-1,3-dioxolane (**4b**) has a negative effect only on cell lines of tumor origin (SH-SY5Y, HepG2, MCF-7, and A549) and does not affect the cell viability of the conventionally normal HEK293 cell line (IC_{50} 100 μmol L^{-1}). No effect was revealed for other prepared compounds (**3a—c** and **4d,e**) in the concentration range of 1—100 μmol L^{-1}.

The degree of selectivity of the negative effect, the selectivity index (SI), was calculated for compounds exhibiting toxicity (Table 2). This index is important to assess the prospects of studying the antitumor activity of compounds. It should be noted that the selectivity indices of modern antitumor chemotherapeutic drugs are in the range from 4 to 8.[12,13] According to the obtained data, compound **4b** exhibit selective cytotoxicity against the SH-SY5Y (SI = 4.28), Jurkat (SI = 5.14), MCF-7 (SI = 3.19), and A549 (SI = 2.02) tumor cells. Compound **4c** has selective cytotoxicity against the tumor MCF-7 cells (SI = 3.47).

The analysis of the relationship between the chemical structure and cytotoxicity of new compounds, *viz.*, cyclic

Table 1. Effect of compounds on cell viability (48 h, $M\pm m$)

Compound	IC_{50}/μmol L^{-1}					
	HEK293	SH-SY5Y	HepG2	Jurkat	MCF-7	A549
3a	>100	>100	>100	>100	>100	>100
4a	48.08±3.13	58.67±3.67 (p = 0.01)*	59.95±3.68 (p = 0.0006)*	32.65±3.16 (p = 0.00007)*	27.62±0.77 (p = 0.00001)*	33.79±2.80 (p = 0.0001)*
3b	>100	>100	>100	>100	>100	>100
4b	>100	28.19±1.31 (p = 0.00001)*	74.75±4.21 (p = 0.000006)*	23.49±0.88 (p = 0.000005)*	38.29±5.65 (p = 0.000005)*	59.59±0.42 (p = 0.000005)*
3c	>100	>100	>100	>100	>100	>100
4c	73.01±5.71	93.27±9.68 (p = 0.04)*	49.91±7.63 (p = 0.0003)*	37.05±0.88 (p = 0.00001)*	21.06±1.83 (p = 0.000006)*	56.03±4.24 (p = 0.003)*
4d	>100	>100	>100	>100	>100	>100
4e	>100	>100	>100	>100	>100	>100

Note. The results are presented as the arithmetic mean of three independent experiments (M) with indication of the standard error of the mean ($\pm m$). *Statistically significant differences in the IC_{50} values for cells of the tumor origin relative to the IC_{50} values for HEK293 cells of normal origin (ANOVA, Dunnett's test).

Table 2. Selectivity indices for compounds **4a—c**

Compound	SI*				
	SH-SY5Y	HepG2	Jurkat	MCF-7	A549
4a	0.82	0.80	1.47	1.74	1.42
4b	4.28	1.62	5.14	3.19	2.02
4c	1.88	1.48	1.97	3.47	1.30

* Selectivity index (SI) of the test compound is the ratio of IC_{50} obtained using the HEK293 control cells to IC_{50} obtained using tumor cells.

acetals and their dichlorocyclopropyl derivatives, revealed the following. Compounds **3a—c** and **4d,e** containing only 1,3-dioxolane or 1,1-dichlorocyclopropane cycle exhibit no meaningful toxicity, whereas compounds **4a—c** containing both cycles showed strong cytotoxic activity. 2-(2,2-Dichlorocyclopropyl)-2-ethyl-1,3-dioxolane (**4b**) is particularly noteworthy because it has pronounced tumor-specific cytotoxicity combined with low overall toxicity to cells of normal origin.

In conclusion, we synthesized cyclic acetals of diols and their dichlorocyclopropyl derivatives. It is found that the simultaneous presence of dioxacycloalkane and dichlorocyclopropane moieties in the structure of the prepared compounds is required for the compound to exhibit cytotoxic activity. Among the studied compounds, those having selective cytotoxic activity against cells of the tumor origin were found. According to the data obtained, compound **4b** is a potential antineoplastic agent. The synthesis of new compounds on its basis in order to improve its characteristics, as well as further study of the biological activity of the series of compounds based on it are very promising tasks.

Experimental

Reaction products were analyzed using a HRGC 5300 Mega Series (Carlo Erba) gas chromatograph equipped with a flame ionization detector and a column of 25 m in length; the temperature was varied within 50—280 °C by heating the column with a rate of 8 deg min^{-1}; helium was used as a carrier gas with a flow rate of 30 mL min^{-1}. Mass spectra were obtained using a Crystal-5000 M mass spectrometer under the following conditions: a 30 m capillary column, temperature increase from 80 to 280 °C with a rate of 20 deg min^{-1}, helium as a carrier gas, the transition line and ion source temperatures were 300 °C. ^1H and ^{13}C NMR spectra were recorded using a Bruker Avance-500 NMR spectrometer (500 MHz, ^1H; 125 MHz, ^{13}C) in $CDCl_3$.

Synthesis of acetals 3a—c (general procedure). Acetals **3a—c** were prepared according to a published method.[6] A mixture containing one of alcohols **1a,b** (0.1 mol), the corresponding carbonyl compound of **2a—c** (0.3 mol), benzene (80 mL) and KU-2 (H^+) (3 g) were stirred at 80 °C until the calculated amount of water (1.8 mL) was collected in the Dean—Stark head. At the end of the reaction, the mixture was washed with water until neutral, dried with calcium chloride; the salt was filtered out; solvent was removed using a rotary evaporator. The products were distilled under reduced pressure.

2-(2-Phenylvinyl)-1,3-dioxolane (3a). Yield 90%. B.p. 134 °C (8 Torr). A colorless liquid. ^1H NMR ($CDCl_3$), δ: 3.45 (d, 2 H, 2 C(4,5)H_a, J = 7.0 Hz); 3.54 (t, 2 H, 2 C(4,5)H_b, J = 7.2 Hz); 5.19 (t, 1 H, C(6)H, J = 6.0 Hz); 5.58 (d, 1 H, C(2)H, J = 6.9 Hz); 6.55 (d, 1 H, C(7)H, J = 6.5 Hz); 7.20—7.30 (m, 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 66.41 (C(4,5)H_2); 101.05 (C(2)H); 121.70 (C(6)H); 126.54 (C(7)H); 127.82 (C(8)); 129.04 (C(9,13)); 131.55 (C(10,12)); 139.04 (C(11)). MS (EI), m/z (I_{rel} (%)): 176 $[M]^+$ (60), 131 $[M - C_3H_5O]^+$ (30), 115 $[M - 2\,CH_2O]^+$ (40), 104 $[C_8H_8]^+$ (100), 77 $[C_6H_5]^+$ (25).

2-Ethyl-2-vinyl-1,3-dioxolane (3b). Yield 92%. B.p. 40 °C (20 Torr). A colorless liquid. ^1H NMR ($CDCl_3$), δ: 0.91 (t, 3 H, C(7)H_3, J = 8.0 Hz); 1.63—1.75 (m, 2 H, C(6)H_2); 3.97 (d, 2 H, 2 C(4,5)H_a, J = 7 Hz); 4.02 (t, 2 H, 2 C(4,5)H_b, J = 6.4 Hz); 5.31 (dd, 1 H, C(9)H, J = 7 Hz, J = 11 Hz); 5.38 (dd, 1 H, C(8)H, J = 6.9 Hz, J = 10.8 Hz); 5.56—5.63 (m, 1 H, CH). ^{13}C NMR ($CDCl_3$), δ: 9.01 (C(7)H_3); 31.03 (C(6)H_2); 65.39 (C(4,5)H_2); 109.60 (C(2)); 115.98 (C(9)H_2); 141.04 (C(8)H). MS (EI), m/z (I_{rel} (%)): 128 $[M]^+$ (10), 99 $[M - C_2H_5]^+$ (100), 67 $[M - 2\,CH_2O - H]^+$ (15), 55 $[C_2H_3CO]^+$ (80).

2-Isopropyl-4,7-dihydro-1,3-dioxepin (3c). Yield 90%. B.p. 62 °C (10 Torr). A colorless liquid. The spectral data corresponds to reported ones.[6]

Synthesis of acetals 4a—c (general procedure). Compounds **4a—c** were synthesized according to a published procedure.[6] A mixture containing one of compounds **3a—c** (0.05 mol), chloroform (150 mL), and a 50% solution of NaOH (160 g) was stirred at 5—7 °C until complete conversion of the starting compound. The mixture was heated to ~20 °C, washed with water until neutral, dried with calcium chloride; the salt was filtered out; solvent was removed using a rotary evaporator. The products were distilled under reduced pressure.

2-(2,2-Dichloro-3-phenylcyclopropyl)-1,3-dioxolane (4a). Yield 90%. B.p. 40 °C (20 Torr). A colorless liquid. ^1H NMR ($CDCl_3$), δ: 2.30 (t, 1 H, C(6)H, J = 7.0 Hz); 2.70 (d, 1 H, C(7)H, J = 7.9 Hz); 3.84 (d, 2 H, 2 C(4,5)H_a, J = 6.7 Hz); 4.02 (t, 2 H, 2 C(4,5)H_b, J = 6.4 Hz); 5.28 (d, 1 H, C(2)H, J = 8.5 Hz); 7.03—7.13 (m , 5 H, Ph). ^{13}C NMR ($CDCl_3$), δ: 39.01 (C(7)H); 44.38 (C(6)H); 66.59 (C(8)); 68.34 (2 C(4,5)H_2); 101.06 (C(2)H); 126.01 (C(8)); 129.77 (C(9,13)); 131.39 (C(10,12)); 133.61 (C(11)). MS (EI), m/z ($I_{отн}$ (%)): 259 (10), 261 (7), 263 (4) $[M]^+$; 135 (50), 137 (30), 139 (10) $[M - H_2O - CH_2O - C_6H_5]^+$; 109 (65), 111 (40), 113 (19) $[C_3H_4Cl_2]^+$; 77 $[C_6H_5]^+$ (55).

2-(2,2-Dichlorocyclopropyl)-2-ethyl-1,3-dioxolane (4b). Yield 9%. B.p. 40 °C (20 Torr). A colorless liquid. ^1H NMR ($CDCl_3$), δ: 0.87 (t, 3 H, C(3)H_3, J = 7.0 Hz); 1.37 (d, 2 H, 2 C(9)H_a, J = 8.0 Hz); 1.45 (t, 2 H, 2 C(9)H_b, J = 7.0 Hz); 1.60—1.66 (m, 2 H, C(6)H_2); 1.72 (dd, 1 H, C(8)H, J = 6.0 Hz, J = 7.0 Hz); 3.95 (d, 2 H, 2 C(4,5)H_a, J = 7 Hz); 4.04 (t, 2 H, C(4,5)H_b, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.45 (C(3)H_3); 27.49 (C(6)H_2); 30.11 (C(9)H_2); 39.56 (C(8)H); 66.49 (C(10)); 69.29 (C(4,5)H_2); 109.00 (C(11)). MS (EI), m/z ($I_{отн}$ (%)): 211 (5), 213 (3), 215 (2) $[M]^+$; 109 (15), 111 (7), 113 (5) $[C_3H_4Cl_2]^+$; 123 (30), 125 (18), 127 (7) $[M - C_2H_5 - 2\,CH_2O]^+$; 73 $[C_3H_5O_2]^+$ (100).

8,8-Dichloro-4-isopropyl-3,5-dioxabicyclo[5.1.0]octane (4c). Yield 98%. B.p. 113 °C (2 Torr). A colorless liquid. The spectral data corresponds to reported ones.[6]

1,1-Dichloro-2,3-bis(hydroxymethyl)cyclopropane (4d). B.p. 144 °C (3 Torr). A colorless liquid gradually solidifying at room

temperature. Compound **4d** was prepared according to a published method.[5] Compound **4c** (0.05 mol) was stirred for 6 h in a 2.0 *M* solution of HCl (100 mL). The reaction was carried out until complete conversion of the starting compound. The solvent was evaporated in *vacuo*, the residue was dissolved in ethyl acetate and filtered to remove insoluble components. The solvent was evaporated. Crystallization of the oily residue from a $CHCl_3$—petroleum ether mixture gave the product. Physicochemical constants of thus obtained compound **4d** correspond to the published data.[6]

1,1-Dichloro-2,3-bis(chloromethyl)cyclopropane (4e). Yield 83%. B.p. 112 °C (5 Torr). A colorless liquid. Compound **4e** was prepared according to a known method.[14] Thionyl chloride (0.012 mol) was added to a mixture of compound **4d** (0.008 mol) and pyridine (0.008 mol) at 0 °C. The reaction mixture was stirred at 10—12 °C until the sulfur dioxide release was finished (5 h). Then chloroform (30 mL) was added, and the mixture was washed with water with caution, avoiding effervescence due to hydrolysis of thionyl chloride. The residue was distilled. Physicochemical constants of thus obtained compound **4e** correspond to the published data.[6]

Assessment of cytotoxic characteristics of the obtained compounds *in vitro*. In the study of the cytotoxicity of the compounds, the following tumor cell lines were used: HepG2 (human hepatocellular carcinoma), SH-SY5Y (human neuroblastoma), A549 (human lung adenocarcinoma), MCF-7 (adenocarcinoma of the human breast duct), and Jurkat (T-lymphoblastic leukemia), as well as the HEK293 cell culture of normal origin (immortalized human embryonic kidney cells). All cell lines were obtained from Russian cell culture collection (Institute of Cytology of the Russian Academy of sciences, Saint Petersburg). Cells of lines HEK293 ($25 \cdot 10^3$ cells per well), HepG2 ($15 \cdot 10^3$ cells per well), SH-SY5Y ($50 \cdot 10^3$ cells per well), MCF-7 ($12 \cdot 10^3$ cells per well), A549 ($10 \cdot 10^3$ cells per well) were seeded in 96 well plates filled with the DMEM cell culture medium (100 μL in a well) containing a 10% fetal bovine serum solution (FBS, Gibco, USA), L-glutamine (2 m*M* PanEco, RF), and gentamicin (50 μg mL^{-1}, Biolot, RF). The Jurkat cells were seeded by $100 \cdot 10^3$ cells per well in 96 well plates filled with the RPMI cell culture medium (100 μL in a well) containing 10% FBS (Gibco, USA), L-glutamine (2 m*M* PanEco, RF), and gentamicin (50 μg mL^{-1}, Biolot, RF). The compounds under study were added to the wells 24 h after seed of the cells in concentrations of 1, 10, 100 μmol L^{-1} in 0.1% DMSO solutions. Then the cell cultures were incubated for 48 h at 37 °C under the 5% CO_2 atmosphere. Cytotoxic characteristics were assessed using PrestoBlue® cell viability reagent according to manufacturer's protocol (Invitrogen, USA). Fluorescence was detected with the use of a 2300 EnSpire® Multimode Plate Reader (Perkin—Elmer, USA). The IC_{50} values (the compound concentration at that the 50% inhibition of cell viability is observed) were calculated using the GraphPad Prizm 4.0 program (GraphPad Software Inc., USA).

In order to identify the possible selectivity of the cytotoxic effect of the test compounds on tumor cells (*i.e.*, potential antitumor effect), the selectivity index (SI) was determined. The HEK293 cell line was used as a control cell line of normal origin. The SI values were calculated as ratios of IC_{50} values obtained for HEK293 cells to the IC_{50} values for tumor cells.

Statistical analysis of the data obtained in the course of the study of the cytotoxic properties of compounds was carried out using the Statistica 6.1 standard software package (StatSoft Inc., USA). The results are presented as the arithmetic mean of three independent experiments (*M*) together with the standard error of the mean (±*m*). Analysis of variance (ANOVA) using Dunnett´s test was used to determine the significance of differences in the IC_{50} values obtained for cells of normal and tumor origin using the test compounds.

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This paper does not contain descriptions of studies on animals or humans.

The authors declare no competing interests.

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