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Synthesis, study of biological activity, and hemocompatibility of potential antitumor compounds of thiazolopyrimidinium systems

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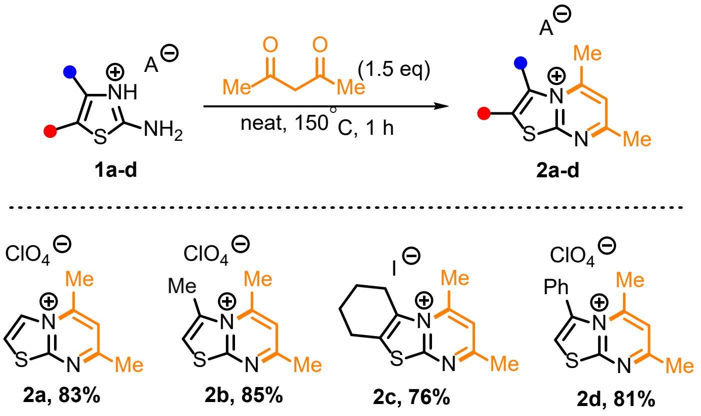
1. Experimental part

^{1}H and ^{13}C NMR spectra of the synthesized compounds were measured on a Agilent 400 MR+ spectrometer (400 MHz for ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra) in DMSO-d_6, internal standard TMS, δ chemical shift scale were used. The mass spectra were obtained on a Finnigan MAT 4615P instrument using a direct inlet system. The temperature of the ionization chamber was 180 °C, the ionization voltage was 70 eV, and the emission current was 100 μA.

1.1 Synthesis of 5,7-dimethylthiazolo[3,2-*a*]pyrimidin-4-ium salts 2a-d

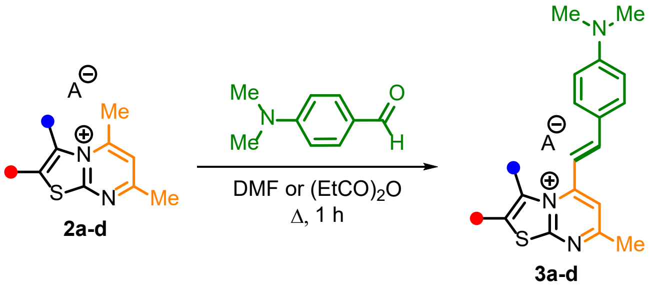


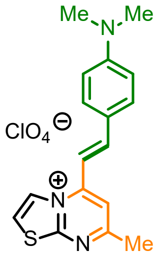
5,7-Dimethylthiazolo[3,2-*a*]pyrimidin-4-ium perchlorate (2a)

A mixture of 2-aminothiazole hydroperchlorate **1a** (300 mg, 1.5 mmol, 1 eq) and acetylacetone (224 mg, 2.24 mmol, 1.5 eq) was heated with stirring for 25-30 min at 150 °C (the reaction mass gradually dissolved, then a white precipitate of 5,7-dimethylthiazolo[3,2-*a*]pyrimidin-4-ium perchlorate **2a** was formed). Isopropyl alcohol (10 ml) was poured, and the mixture was refluxed for a while. White precipitate was filtered under vacuum to give a white powder of **2a**, 328 mg, 83%. The product **2a** can be used for further synthesis without additional purification.

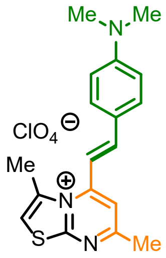
Compounds **2b, 2c, 2d** was synthesized by the same procedure. Yields: **2b** 87%, **2c** 91% and **2d** 72%. Spectral data of the synthesized compounds coincide with those given in reference.[S1]

1.2 Synthesis of (*E*)-5-(4-dimethylaminostyryl)-7-methylthiazolo[3,2-*a*]pyrimidin-4-ium salts 3a-d

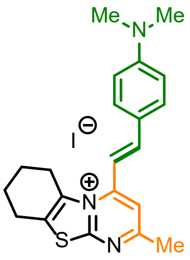




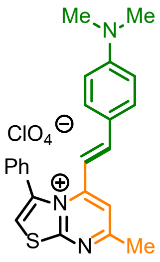
3a, $(EtCO)_2O$, 77%



3b, DMF, 75%



3c, $(EtCO)_2O$, 69%



3d, $(EtCO)_2O$, 72%

1.2.1 (E)-5-(4-dimethylaminostyryl)-7-methylthiazolo[3,2-a]pyrimidin-4-ium perchlorate (3a)

A mixture of 5,7-dimethylthiazolo[*3,2-a*]pyrimidin-4-ium perchlorate **2a** (300 mg, 1.13 mmol, 1 eq) and 4-dimethylaminobenzaldehyde (169 mg, 1.13 mmol, 1 eq) was refluxed with stirring for 45-50 min in propionic anhydride (3 ml). The complete conversion of starting material was indicated by TLC analysis (butan-1-ol/acetic acid/water (4:4:1) ternary system was used as the eluent, $R_f = 0.60$, violet spot). A dark-green solid of (*E*)-5-(4-dimethylaminostyryl)-7-methylthiazolo[3,2-*a*]pyrimidin-4-ium perchlorate **3a** was precipitated after the cooling of reaction mixture. Dark-green powder was filtered under vacuum to give 345 mg (77%) of **3a**. The product **3a** can be used without additional purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ_H 2.69 (3H, s, CH_3), 3.07 (6H, s, $N(CH_3)_2$), 6.82 (2H, d, J=9.0 Hz, Ar), 7.55 (1H, d, J=15.5 Hz, styryl), 7.81 (2H, d, J=9.0 Hz, Ar), 8.22 (1H, d, J=15.4 Hz, styryl), 8.30 (1H, s, pyrimidine), 8.37 (1H, d, J=4.8, thiazole), 9.18 (1H, d, J=4.8, thiazole). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 24.7, 40.2, 108.3, 110.6, 112.4, 121.2, 122.5, 125.9, 132.3, 147.5, 152.4, 153.3, 164.2, 166.9. m.p. 300< °C; R_f 0.42 (ethanol/acetic acid/water, 1:1:2). MS (EI, 70 eV) *m/z*: 296 (M^+) (without perchlorate). Found (%): C, 51.56; H, 4.57; N, 10.64. Calc. for $C_{17}H_{18}N_3O_4SCl$. C, 51.58; H, 4.58; N, 10.62.

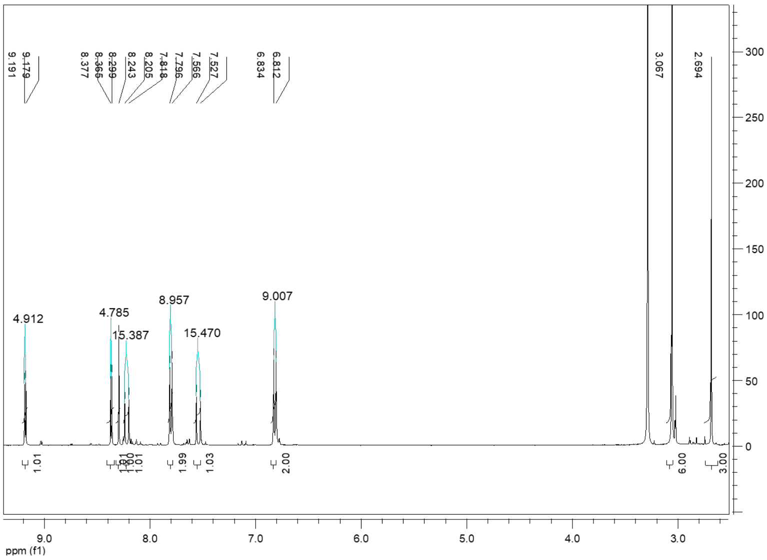


Fig. S1. ^{1}H NMR spectrum of (*E*)-5-(4-dimethylaminostyryl)-7-methylthiazolo-[3,2-*a*]pyrimidin-4-ium perchlorate **3a.**

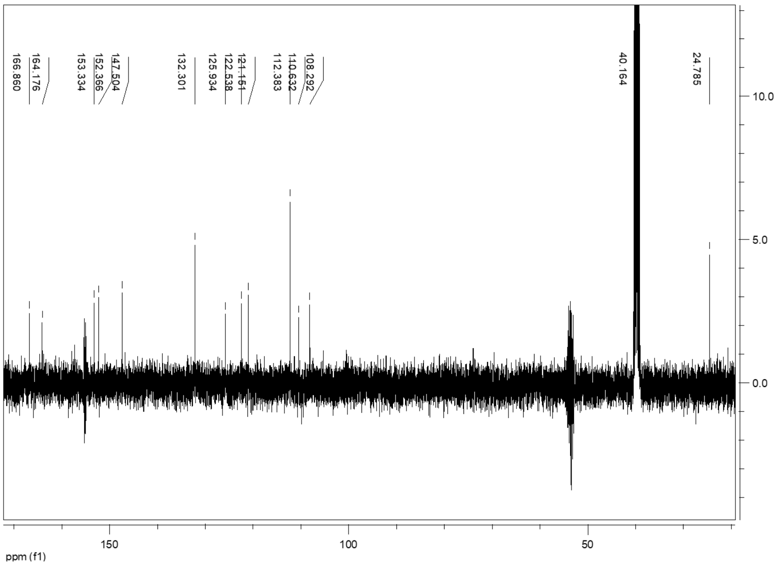


Fig. S2. ^{13}C NMR spectrum of (*E*)-5-(4-(dimethylamino)styryl)-7-methylthiazolo-[*3,2-a*]pyrimidin-4-ium perchlorate **3a.**

Compounds **3c,d** was synthesized analogously to **3a**.

(*E*)-4-(4-Dimethylaminostyryl)-2-methyl-6,7,8,9-tetrahydrobenzo[4,5]thiazolo[3,2-*a*]-pyrimidin-5-ium iodide (3c)

Dark green powder; (421 mg, yield 79 %); (400 MHz, DMSO-*d*$_6$) δ_H 1.84 (4H, s(br), CH$_2$), 2.67 (3H, s, CH$_3$), 2.94 (2H, s(br), CH$_2$), 2.97 (6 H, s, N(CH$_3$)$_2$), 3.03 (2H, s(br), CH$_2$), 6.77 (2H, d, J=8.9 Hz, Ar), 7.48 (1H, d, J=15.7 Hz, styryl), 7.64 (2H, d, J=8.9 Hz, Ar), 8.13 (1H, d, J=15.7 Hz, styryl), 8.11 (1H, s, pyrimidine)., ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ_C 21.4, 22.5, 24.3, 24.9, 28.2, 40.2, 111.8, 112.4, 115.0, 122.7, 130.9, 131.2, 136.1, 144.1, 152.7, 154.3, 163.6, 166.5. m.p. 300< °C; *R$_f$* 0.49 (ethanol/acetic acid/water, 1:1:2). MS (EI, 70 eV) *m/z*: 346 (M$^+$) (without iodide). Found (%): C, 52.86; H, 5.07; N, 8.84. Calc. for $C_{21}H_{24}N_3SI$. C, 52.87; H, 5.03; N, 8.80.

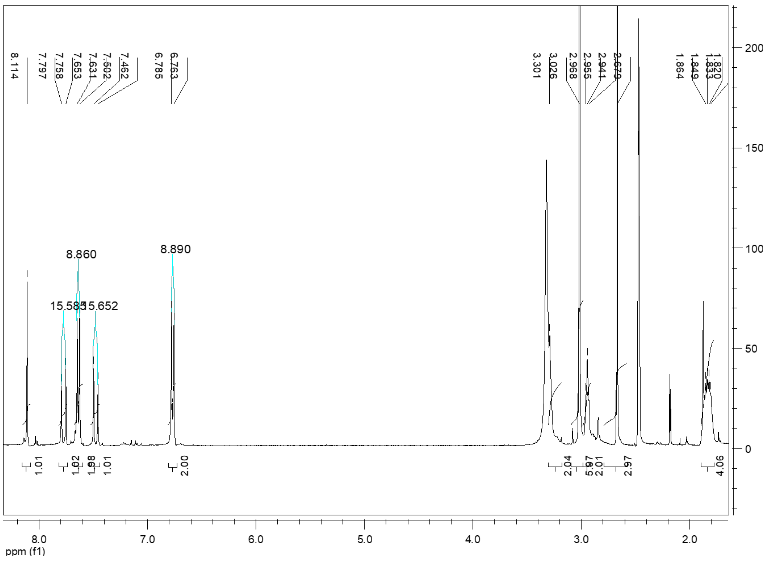


Fig. S3. ^{1}H NMR spectrum of (*E*)-4-(4-dimethylaminostyryl)-2-methyl-6,7,8,9-tetrahydrobenzo[4,5]thiazolo[3,2-*a*]pyrimidin-5-ium iodide **3c.**

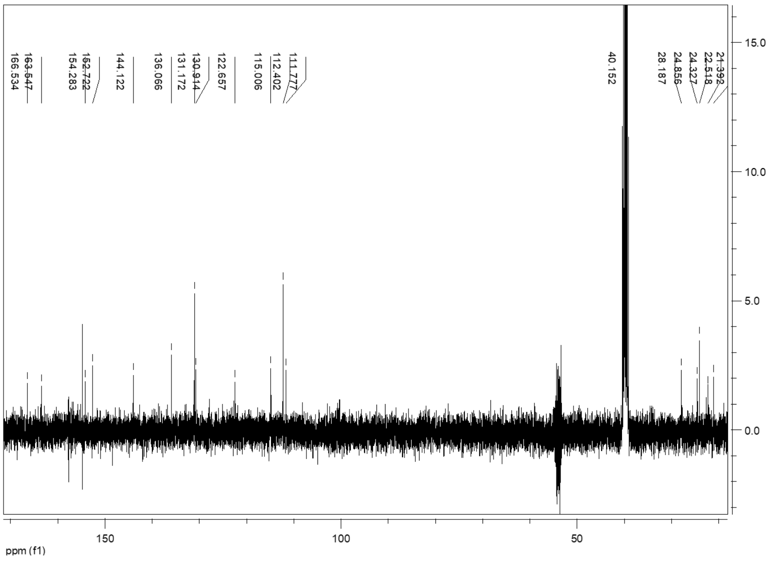


Fig. S4. ^{13}C NMR spectrum of (*E*)-4-(4-dimethylaminostyryl)-2-methyl-6,7,8,9-tetrahydrobenzo[4,5]thiazolo[3,2-*a*]-pyrimidin-5-ium iodide **3c.**

(*E*)-5-(4-Dimethylaminostyryl)-7-methyl-3-phenylthiazolo[3,2-*a*]pyrimidin-4-ium perchlorate (3d)

Dark green powder; (475 mg, yield 82%); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 2.73 (3H, s, CH_3), 2.99 (6H, s, $N(CH_3)_2$), 6.47 (1H, d, J=15.7 Hz, styryl), 6.60 (2H, d, J=8.9 Hz, Ar), 6.88 (2H, d, J=8.9 Hz, Ar), 7.66 (5H, m, Ar), 8.01 (1H, d, J=15.7 Hz, styryl), 8.22 (1H, s, thiazole), 8.23 (1H, s, pyrimidine); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 24.5, 40.1, 111.5, 112.2, 113.6, 120.5, 122.1, 129.4, 130.3, 130.8, 131.0, 131.2, 139.4, 143.0, 152.8, 154.0, 165.1, 166.9. m.p. 300< °C; R_f 0.45. MS (EI, 70 eV) m/z: 372 (M^+) (without perchlorate). Found (%): C, 55.55; H, 4.73; N, 8.87. Calc. for $C_{23}H_{22}N_3O_4SCl$. C, 58.53; H, 4.70; N, 8.90.

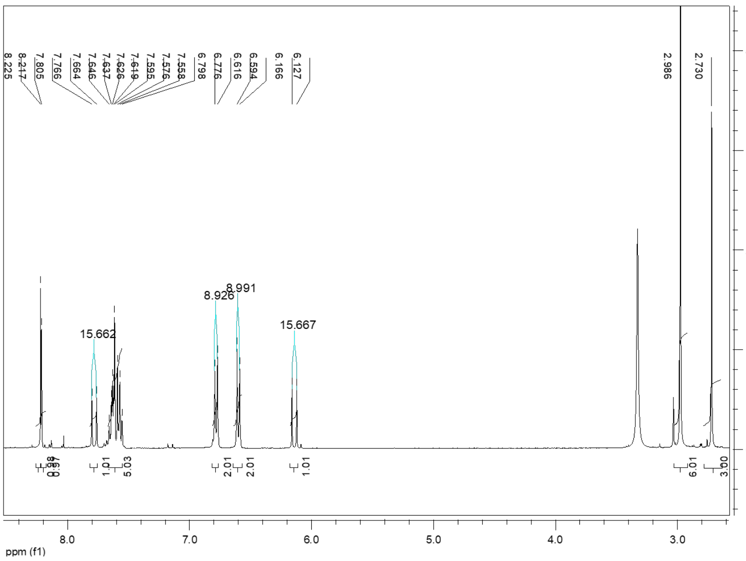


Fig. S5. ^{1}H NMR spectrum of (*E*)-5-(4-dimethylaminostyryl)-7-methyl-3-phenylthiazolo-[*3,2-a*]pyrimidin-4-ium perchlorate **3d.**

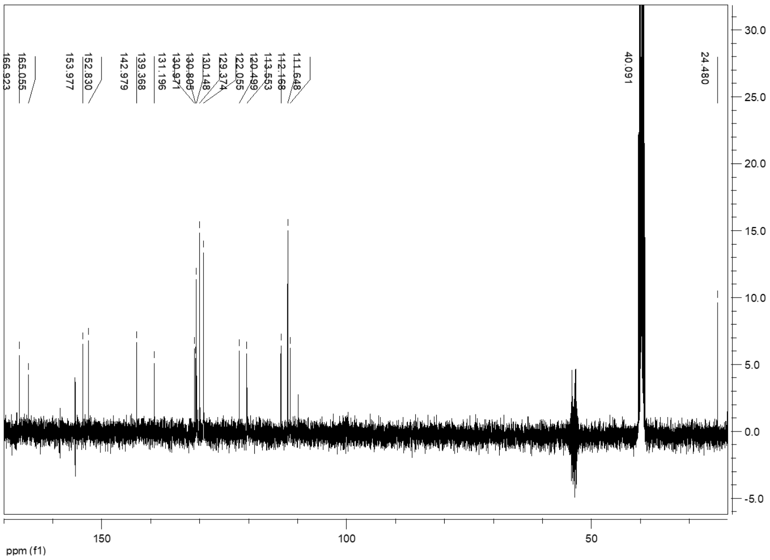


Fig. S6. ^{13}C NMR spectrum of (*E*)-5-(4-dimethylaminostyryl)-7-methyl-3-phenylthiazolo-[3,2-*a*]pyrimidin-4-ium perchlorate **3d.**

1.2.2 (E)-5-(4-Dimethylamino)styryl)-3,7-dimethylthiazolo[3,2-a]pyrimidin-4-ium perchlorate (3b)

A mixture of 3,5,7-trimethylthiazolo[*3,2-a*]pyrimidin-4-ium perchlorate **2b** (300 mg, 1.08 mmol, 1 eq) and 4-dimethylaminobenzaldehyde (161 mg, 1.08 mmol, 1 eq) was refluxed with stirring for 60-80 min in DMF (3 ml). The complete conversion of starting material was indicated by TLC analysis (butan-1-ol/acetic acid/water (4:4:1) ternary system was used as the eluent, $R_f = 0.62$, violet spot). A dark-green solid of (*E*)-5-(4-dimethylaminostyryl)-3,7-dimethylthiazolo[3,2-*a*]pyrimidin-4-ium perchlorate **3b** was precipitated after the cooling of reaction mixture. Dark-green powder was filtered under vacuum to give 331 mg, 71% of **3b**. The product **3b** can be used without additional purification.

Dark green powder; (331 mg, yield 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 2.86 (3H, s, CH_3), 3.03 (9H, s, CH_3, $N(CH_3)_2$), 6.77 (2H, d, J=8.9 Hz, Ar), 7.07 (1H, d, J=15.7 Hz, styryl), 7.64 (2H, d, J=8.9 Hz, Ar), 7.81 (1H, s, pyrimidine), 7.82 (1H, s, thiazole), 8.11 (1H, d, J=15.7 Hz, styryl);

^{13}C NMR (100 MHz, DMSO-d_6) δ_C 18.7, 21.8, 40.1, 112.4, 116.1, 116.8, 117.1, 122.5, 131.7, 138.6, 146.3, 153.0, 154.6, 162.7, 166.0. m.p. 300< °C; R_f 0.39 (ethanol/acetic acid/water, 1:1:2). MS (EI, 70 eV) *m/z*: 310 (M$^+$) (without perchlorate). Found (%): C, 52.77; H, 4.90; N, 10.27. Calc. for $C_{18}H_{20}N_3O_4SCl$. C, 52.75; H, 4.92; N, 10.25.

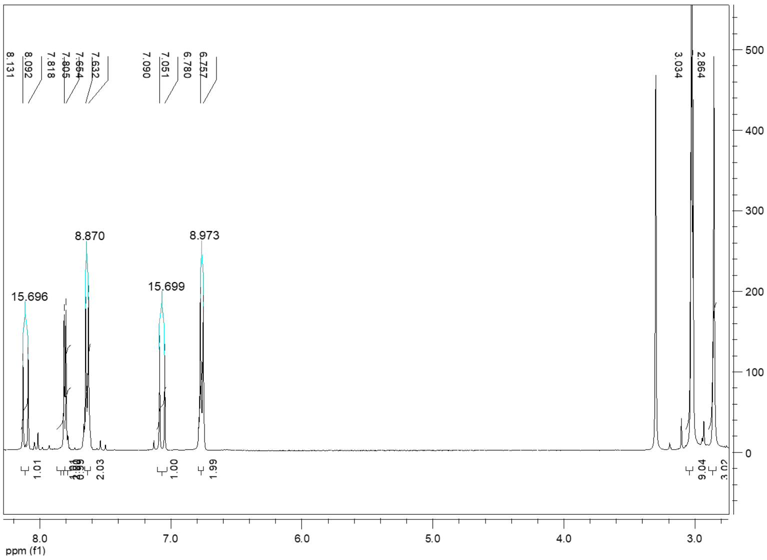


Fig. S7. ^{1}H NMR spectrum of (*E*)-5-(4-dimethylaminostyryl)-3,7-dimethylthiazolo-[3,2-*a*]pyrimidin-4-ium perchlorate **3b.**

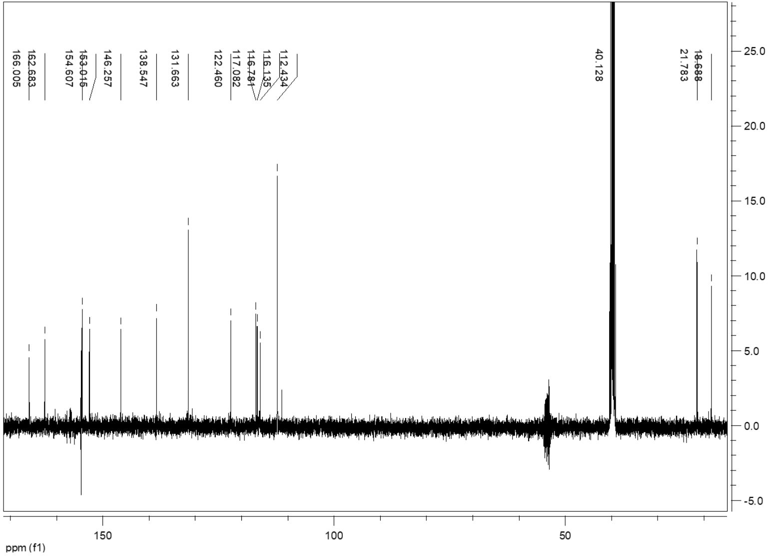


Fig. S8. ^{13}C NMR spectrum of (*E*)-5-(4-dimethylaminostyryl)-3,7-dimethylthiazolo-[3,2-*a*]pyrimidin-4-ium perchlorate **3b.**

1.3 Hemolysis

To assess the biocompatibility of substances **3a-d**, its effect on spontaneous hemolysis was studied. The hemolysis study was performed by measuring the optical density of supernatants at $\lambda = 540$ nm using SF-2000 spectrophotometer (Russia). In the case of substances compatible with blood, the red blood cell membrane remains intact and the cell contents are not released. The effect of the test substance on erythrocyte hemolysis was determined by assessing the released hemoglobin. The degree of hemolysis was calculated using the formula:

$$\%\ \text{hemolysis} = \frac{E_{probe} - E_{control}}{E_{100}} \cdot 100 \qquad (1),$$

where E_{probe} is the optical density of the test sample; $E_{control}$ – optical density of the control sample; E_{100} - optical density of water with a suspension of red blood cells 100% hemolysis. S2–S6 Substances **3a-d** can be considered safe in the concentration range of 0.03-150 μM.

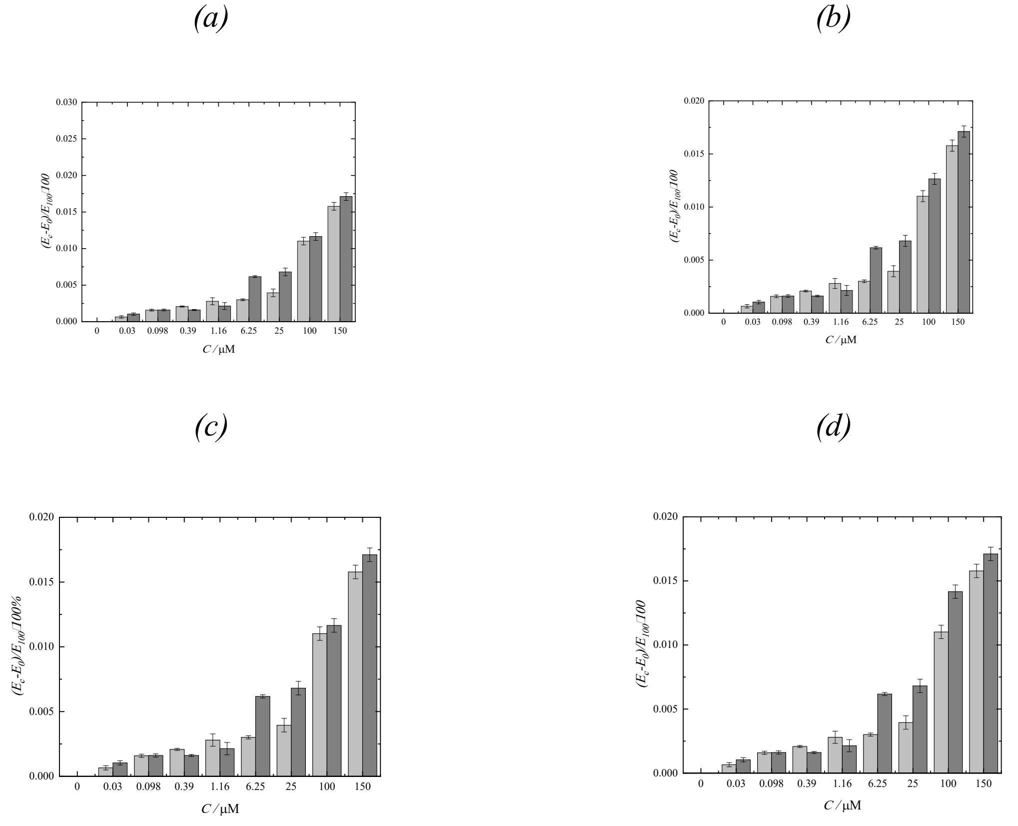


Fig. S9. The effect of substances **3a** (*a*), **3b** (*b*), **3c** (*c*) and **3d** (*d*) on the degree of hemolysis 1 h (light gray) and 3 h (dark gray) after incubation. *C*—concentration, μM.

1.3 Platelet aggregation

To study platelet aggregation, blood was collected in vacuum tubes containing sodium citrate ($C = 0.129$ M) as a stabilizer at a sodium citrate:blood ratio of 1:9 (*v/v*). S4-S7

Table S1. Effect of **3a-d** on ADP-induced platelet aggregation.

		Amplitude / %							
Substance	Control	*C* / μM							
		0.03	0.098	0.390	1.160	6.25	25	100	150
3a	90.4 ± 1.27	92.9 ± 0.2	90.5 ± 0.8	90.7 ± 0.3	84.3 ± 0.4*	89.7 ± 0.2	85.7 ± 0.4*	88.1 ± 0.3	85.8 ± 0.4*
3b	67.3 ± 1.6	64.9 ± 3.6	68.1 ± 0.8	66.0 ± 2.7	85.8 ± 4.3*	77.6 ± 0.8*	64.9 ± 0.7*	59.3 ± 1.2*	51.3 ± 0.3*
3c	78.8±1.6	79.2±0.4	83.7±0.7	84.8±1.6	83.1±0.9*	80.7±0.3	84.0±0.1	62.3±0.5*	53.3±0.4*
3d	81.2±5.6	90.4±4.5	89.9±4.0	84.6±1.	81.9±1.0	83.9±0.8	85.9±0.6	76.4±0.5	77.9±0.1

*$p < 0.05$ compared to control.

1.5 Interactions with DNA

pBR322 plasmid (Thermo Fisher Scientific, USA) was used to analyze the ability of Substances **3a-d** to introduce DNA breaks. The plasmid was propagated using *Escherichia coli* DH5a bacteria strain (Thermo Fisher Scientific, USA), isolated using Plasmid Miniprep kit (Evrogen, Russia) and diluted in water. The concentration of DNA solutions was determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, USA). 5 µl volumes containing 500 ng of plasmid DNA were mixed with 20 µl of aqueous solutions of substances **3a-d**. The final concentration of substances in the experimental samples was 200 µM. Samples were incubated for 24 hours at 37 °C and analyzed by electrophoresis in 1% agarose gel. All experiments were performed in three replicas. [S7]

1.6 MTT analysis

Cells at a concentration of $5 \cdot 10^4$ per well were seeded in a 96-well plate and incubated for 12 h in DMEM-F12 medium supplemented with 10% thermally inactivated fetal bovine serum, 1% L-glutamine, 50 $U \cdot ml^{-1}$ penicillin and 50 $\mu g \cdot ml^{-1}$ streptomycin. After cultivation, fresh DMEM-F12 medium containing various concentrations of Substance **3a-d** was added to the wells. Next, the plate was then incubated at 37°C in a humidified atmosphere CO_2 -incubator in the presence of 20% O_2, 5% CO_2. After 48 h, 0.1 ml of DMEM-F12 and 0.03 ml of the MTT reagent (5 $mg \cdot ml^{-1}$) were added to the wells and the incubation continued for 1 h, after which the supernatant was removed. The formazan crystals formed during MTT reduction by viable cells were dissolved in 0.1 ml of DMSO and the optical density was measured on AMR-100T Microplate Reader (Allsheng, China). [S7]

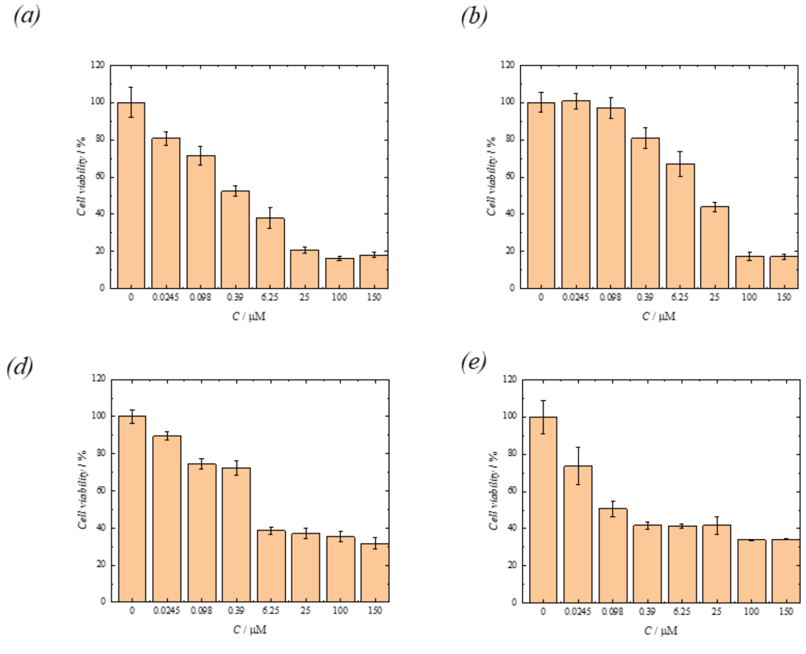


Fig. S10. Effect of substance **3c** on the survival of the HeLa (*a*), PANC-1 (*b*), A549 (*c*), MCF-7 (*d*) cell lines.

References

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