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Erythrocytes Membrane Photodestruction Sensitized by Chlorophyll *a* Derivatives: Some Structure–Activity Regularities

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Dedicated to Academician Oleg Nikolaevich Chupakhin
on the occasion of his 80th birthday

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Photoinduced hemolysis, sensitized by semi-synthetic chlorins, was studied. It was shown that the presence of chlorins without exo-cycle leads to intensive photoinduced hemolysis of erythrocytes which does not depend on the nature of substituents. The presence of exocycle in macrocyclic molecule leads to decrease of hemolysis. The activity of phorbine derivatives becomes comparable with that of compounds without exo-cycle at the presence of substituents in the molecule, promoting hydrogen bonding.

Keywords: Chlorophyll *a* derivatives, methylpheophorbide *a*, chlorin e_6, photosensitizes, erythrocytes membrane, hemolysis.

Фотоповреждение эритроцитарной мембраны, сенсибилизируемое производными хлорофилла *a*: некоторые закономерности «структура–активность»

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В настоящей работе исследован фотогемолиз эритроцитов, сенсибилизируемый полусинтетическими хлоринами на основе фитопорфиринов. Показано, что хлорины, в молекуле которых нет экзоцикла, вне зависимости от природы заместителей вызывают активный фотосенсибилизированный лизис эритроцитов. Наличие в молекуле экзоцикла в большинстве случаев приводит к снижению степени фотогемолиза. Активность форбиновых производных становится сопоставимой с активностью соединений без экзоцикла при наличии в молекуле заместителей, способствующих образованию водородной связи.

Ключевые слова: Производные хлорофилла *a*, метилфеофорбид *a*, хлорин e_6, фотосенсибилизаторы, мембрана эритроцитов, гемолиз.

Introduction

At present several porphyrins are used in clinical practice as diagnostic preparates and photosensitizes (PS) for the photodynamic therapy (PDT) of cancer.[1-9] Some chlorophyll *a* derivatives were found to be highly active PS with low dark toxicity.[1-6,8-29] Thus, new PS searching among chlorophyll *a* derivatives is of a good chance and the investigation of their activity mechanisms and "structure-activity" regularities are of a great interest for new potential PS synthesis planning.[15,21,28-32] Variation of chlorins chemical structure features such as charge, hydrophobicity and steric properties leads to the significant changing of the pigments ability to insert into the cell which can define their photodynamic effectivity.[33,34] The ability of pigments to interact with membrane structures is of a great significance for photosensitizing activity appearance in cell culture and *in vivo*.[35] It is well known that sub-cell localization of PS defines their biological efficiency.[16] It was established that chlorins can insert to lipid membrane and chlorin e_6 derivatives demonstrate high affinity to liposomes.[36] The correlation between membrane bonding and photo hemolytic activity has been shown for chlorin e_6 and its derivatives.[36] From the other hand the increase in hydrophobicity of compounds does not necessarily lead to photodynamic activity increase because of their availability for cell decrease.

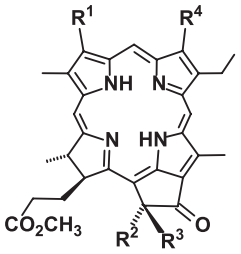
Cell membrane is one of the main target of PS action.[37,38] So, enucleate mammalian erythrocytes can be convenient *in vitro* model for potential PS activity estimation and revealing of the influence of their structure on the biomembrane photodestruction ability.[39-45] According to the literature data the principle factor of membrane photodestruction is an ability of pophyrin to interact with membrane, but spectral properties and the singlet oxygen generation ability are of a much less role in this process.[46] So the erythrocytes photodestruction can be used to investigate features of potential PS to interact with cell membranes. Common application of Photo-RBC test is caused by the correlation of the test results with several PS photodynamic activity *in vivo*.[47] Here we report on the investigation of several chlorophyll *a* derivatives (Scheme 1) with different substituent's ability to induce photohemolysis; also some "structure-activity" regularities were found.

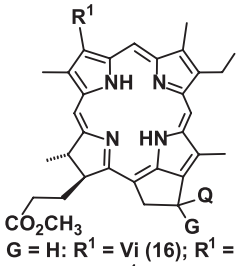
Experimental

^{1}H NMR spectra were recorded in $CDCl_3$ on spectrometer Bruker Avance II (working frequency 300 MHz). IR spectra were recorded on spectrometer Shimadzu IR Prestige 21 in KBr (diffuse reflection). UV-Vis spectra were recorded on spectrometer Shimadzu UV-1700 (PharmaSpec) in $CHCl_3$ in 200-1100 nm range in 10 mm quartz cuvettes, using $CHCl_3$ as comparison sample. Monitoring the reaction proceeding was performed by TLC on Silufol plates, eluent – CCl_4-acetone (4:1 vol). Column chromatography was carried out using silica gel Alfa Aesar 70/230μ.

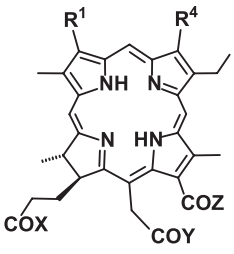
Methylpyropheophorbide *a* (**1**), methylpheophorbide *a* (**2**), methyl-13(2)-hydroxypheophorbide *a* (**5**), chlorin e_6 13(1)-*N*-methylamide 15(2),17(3) dimethyl ester (**19**), chlorin e_6 13(1)-*N,N*-dimethylamide-15(2),17(3)-dimethyl ester (**20**) were obtained according to [48]. Methylpheophorbide *a* 13(2)-*N,N*-dibuthylamide (**3**) was obtained according to [49].

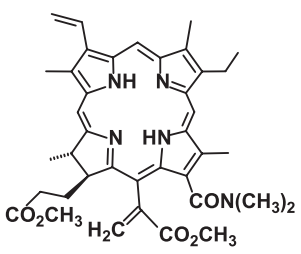
Methyl-13(2)-*N,N*-dimethylaminomethylpheophorbide *a* (**4**), chlorin e_6 13(1)-*N*-(*n*-hexyl)amide-15(2),17(3)-dimethyl ester (**22**), chlorin e_6 13(1)-*N*-(*n*-octyl)amide-15(2),17(3)-dimethyl ester (**23**), chlorin e_6 3(1),3(2)-bis-(*N,N*-dimethylaminomethyl)-13(1)-*N*-methylamide-15(2),17(3)-dimethyl ester (**26**), chlorin e_6 13(1)-*N,N*-dimethylamide-15-(1-methoxycarbomoylvinyl)-17(3)-methyl ester (**35**) were obtained according to [50]. Methyl-mesopheophorbide *a* (**8**),[51] methylpyropheophorbide *b* (**9**),[52] methylpheophorbide *b* (**10**),[52] methylpyropheophorbide *d* (**11**),[53] methylpheophorbide *b* (**12**),[54] methyl-3(1),3(2)-bis-(*N,N*-dimethylaminomethyl)-pyropheophorbide *a* (**15**),[55] and





Q = G = H: R^1 = Vi (16); R^1 = Et (17);
Q = OH, G = H, R^1 = Vi (18)





(35)

R^1 = Vi, R^4 = CH_3:
R^2 = R^3 = H (1); R^2 = CO_2CH_3, R^3 = H (2);
R^2 = $CONBu_2$, R^3 = H (3);
R^2 = CO_2CH_3, R^3 = $CH_2N(CH_3)_2$ (4);
R^2 = CO_2CH_3, R^3 = OH (5);
R^2, R^3 = CO_2CH_3, CH_2OH (6);

R^1 = Et, R^4 = CH_3: R^2 = R^3 = H (7); R^2 = CO_2CH_3, R^3 = H (8);
R^1 = Vi, R^4 = CH=O: R^2 = R^3 = H (9); R^2 = CO_2CH_3, R^3 = H (10);
R^1 = CH=O, R^4 = CH_3: R^2 = R^3 = H (11); R^2 = CO_2CH_3, R^3 = H (12);
R^1 = $CH(OH)CH_2OH$, R^4 = CH_3:
R^2 = R^3 = H (13); R^2 = CO_2CH_3, R^3 = H (14);
R^1 = $C(CH_2N(CH_3)_2)$=$CHCH_2N(CH_3)_2$, R^4 = CH_3: R^2 = R^3 = H (15);

R^1 = Vi, R^4 = CH_3, X = Y = OCH_3:
Z = $NHCH_3$ (19); $N(CH_3)_2$ (20);
$NH(CH_2)_nCH_3$ (n = 1 (21), 5 (22), 7 (23)); $NHCH_2CH_2OH$ (24);
N O (25);

R^1 = $C(CH_2N(CH_3)_2)$=$CHCH_2N(CH_3)_2$, R^4 = CH_3, X = Y = OCH_3:
Z = $NHCH_3$ (26); $NHCH_2CH_3$ (27); $NHCH_2CH_2OH$ (28); N O (29);

R^1 = Et, R^4 = CH_3, X = Y = OCH_3:
Z = $NHCH_3$ (30); Z = $N(CH_3)CH_2N(CH_3)_2$ (31)

R^1 = Vi, R^4 = CH_3, X = Z = $NHCH_2CH_2OH$, Y = OCH_3 (32);
R^1 = Vi, R^4 = CH_3, X = Y = Z = $NHCH_2CH_2OH$ (33);
R^1 = Vi, R^4 = CH=O, X = Y = OCH_3, Z = $N(CH_3)_2$ (34)

Scheme 1.

methyl-13(1)-desoxopyropheophorbide *a* (**15**) [55] were obtained according to literature procedures. Methyl-13(1)-hydroxy-13(1)desoxo-pheophorbide *a* (**18**),[57] chlorin e_6 13(1)-*N*-(2-hydroxyethyl)amide-15(3),17(3)-dimethyl ester (**24**),[58] chlorin e_6 13(1)-morpholinylamide-15(3),17(3)-dimethyl ester (**25**),[58] rodin g_7 13(1)-*N,N*-dimethylamide-15(3),17(3)-dimethyl ester (**34**),[59] chlorin e_6 13(1),17(3)-*N,N'*-(2-hydroxyethyl)diamide-15(3),17(3)-dimethyl ester (**32**),[60] and chlorin e_6 13(1),15(2),17(3)-*N,N',N''*-(2-hydroxyethyl)triamide (**33**)[60] were also obtained according to the known procedures.

*Methyl 13(2)-hydroxymethylpheophorbide a (**6**).* To the solution of **2** (100 mg, 0.165 mmol) in THF (5 ml) 110 mg of paraformaldehyde and 1 ml of Na_2CO_3 saturated aqua solution were added and the mixture was stirred for 1 h at room temperature. The reaction was monitored by TLC. The reaction mixture was diluted with 70 ml of chloroform, washed with 10 % aqueous HCl and then with water up to neutral reaction. The chloroform solution was dried by anhydrous Na_2SO_4. The product was isolated by column chromatography (elution by 40:1 (v:v) CCl_4-acetone mixture, TLC monitoring). The yield of compound **6** is 67 mg (70 %). The spectral properties of the compound obtained are the same as described in literature.[61]

*Methyl mesopheophorbide a (**7**)* was obtained as described for **1**. Compound **8** (190.0 mg) was dissolved in 5 ml of pyridine and refluxed for 40 min. The yield of compound **7** is 117 mg (68 %). UV-Vis (CH_2Cl_2) λ nm: 409.0, 469.0, 503.0, 534.0, 600.0, 656.0. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 9.50 s (1H, H^{10}), 9.23 s (1H, H^5), 8.48 s (1H, H^{20}), 5.29 d (1H, $H^{13(2)}_A$, *J* 20.0 Hz), 5.12 d (1H, $H^{13(2)}_B$, *J* 20.0 Hz), 4.49 q (1H, H^{18}, *J* 6.0 Hz), 4.31 br.d (1H, H^{17}, *J* 7.0 Hz), 3.86 q [2H, 8-(CH_2CH_3), *J* 8.0 Hz], 3.77-3.68 m [2H, 3-(CH_2CH_3)], 3.69 s [3H, 17-($CH_2CH_2COOCH_3$)], 3.65 s (3H, 12-CH_3), 3.32 s (3H, 2-CH_3), 3.28 s (3H, 7-CH_3), 2.76-2.52 m [2H, 17-($CH_2CH_2COOCH_3$)], 2.41-2.26 m [2H, 17-($CH_2CH_2COOCH_3$)], 1.84 d (3H, 18-CH_3, *J* 8.0 Hz), 1.81-1.71 m (6H, 3-CH_2CH_3, 8-CH_2CH_3), 0.67 br.s (1H, I-NH), -1.56 br.s (1H, III-NH).

*Methyl-3(1),3(2)-dihydroxyethylpyropheophorbide a (**13**).* 5.6 ml of 3 % aqueous $KMnO_4$ solution was added dropwise to the solution of **1** (312 mg) in acetone (94 ml) during 1 h. The reaction mixture was diluted with 300 ml of chloroform, washed with water. The solution obtained was dried by anhydrous Na_2SO_4, filtrated and evaporated. The product was isolated by column chromatography (elution by 5:1 (v:v) CCl_4-acetone mixture, TLC monitoring). The yield of compound **13** is 171 mg (52 %) (as 3(1)-diastereomer mixture). UV-Vis (CH_2Cl_2) λ nm: 410.1, 472.1, 506.0, 537.0, 607.0, 664.1. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 9.68 s (1H, H^{10}), 9.52 s (1H, H^5), 8.66 s (1H, H^{20}), 6.24-6.10 m [1H, 3-(CH(OH)CH_2OH)], 5.31 d (1H, $H^{13(2)}_A$, *J* 18.0 Hz), 5.15 d (1H, $H^{13(2)}_B$, *J* 18.0 Hz), 4.62-4.05 m [4H, 3-(CH(OH)CH_2OH)], H^{18}, H^{17}), 3.79-3.56 m [2H, 8-(CH_2CH_3)], 3.67/3.66 s [3H, 17-($CH_2CH_2COOCH_3$)], 3.64 s (3H, 12-CH_3), 3.43/3.41 s (3H, 2-CH_3), 3.24 s (3H, 7-CH_3), 2.79-2.54 m [4H, 17-($CH_2CH_2COOCH_3$)], 1.91-1.65 m (6H, 18-CH_3, 8-CH_2CH_3), 0.91 br.s (1H, I-NH), -2.03 br.s (1H, III-NH). MS (EI) *m/z*: (M^+) 582, ($M-H_2O$)$^+$ 564.

*Methyl 3(1),3(2)-dihydroxyethylpheophorbide a (**14**)* was obtained as described for **13**. 51 mg (32 %) of **14** (as 3(1)-diastereomer mixture) was obtained from 152 mg of **2**. UV-Vis (CH_2Cl_2) λ nm: 409.0, 469.0, 504.0, 535.0, 605.5, 662.5. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 9.49 s (1H, H^{10}), 9.36 s (1H, H^5), 8.56/8.52 s (1H, H^{20}), 6.23-6.19 m [1H, 3-(CH(OH)CH_2OH)], 6.22/6.21 s (1H, $H^{13(2)}$), 4.52-4.05 m (4H, 3-(CH(OH)CH_2OH), H^{18}, H^{17}), 3.93/3.92 s (3H, 13(2)-$COOCH_3$), 3.65-3.53 m [2H, 8-(CH_2CH_3)], 3.64/3.63 s [3H, 17-($CH_2CH_2COOCH_3$)], 3.62 s (3H, 12-CH_3), 3.34 s (3H, 2-CH_3), 3.12 s (3H, 7-CH_3), 2.65-2.51 m [2H, 17-($CH_2CH_2COOCH_3$)], 2.34-2.20 m [2H, 17-($CH_2CH_2COOCH_3$)], 1.83 t (3H, 8-CH_2CH_3, *J* 9.0 Hz), 1.69-1.59 m (3H, 18-CH_3), 0.08 br.s (1H, I-NH), -1.98 br.s (1H, III-NH). MS (EI) *m/z*: (M^+) 640 (M^+), 564 ($M-H_2O$)$^+$.

*Methyl-13(1)-desoxomesopheophorbide a (**17**).* To the solution of compound **7** in CH_2Cl_2 TFA (10 ml) was added and the resulted solution was stirred at 0 °C in the ice bath for 10 min. Then 624.8 mg of $NaBH_4$ was added by parts within 30 min. The resulted mixture was stirred at room temperature for 12 h. Then mixture was diluted with 200 ml CH_2Cl_2 and 200 ml of water and stirred for 1 h and washed by water to remove the excess of TFA and $NaBH_4$ reaction products. The resulted solution was dried with anhydrous Na_2SO_4, filtrated and evaporated. The product was isolated by column chromatography (elution by 20:1 (v:v) CCl_4-acetone mixture, TLC monitoring). The yield of compound **17** is 58.9 mg (52 %). UV-Vis (CH_2Cl_2) λ nm: 394.5, 498.0, 526.5, 584.5, 638.5. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 9.85 s (1H, H^{10}), 9.67 s (1H, H^5), 8.95 s (1H, H^{20}), 5.04-4.81 m (2H, $H^{13(2)}_A$, $H^{13(2)}_B$), 4.71 br.q [(1H, H^{18}), 6.3 Hz], 4.53 br.d [(1H, H^{17}), 8.7 Hz], 4.17-4.07 m [4H, $H^{13(1)}_A$, $H^{13(1)}_B$, 3-(CH_2CH_3)], 3.93 q [2H, 8-(CH_2CH_3), 7.8 Hz], 3.62 s [3H, 17-($CH_2CH_2COOCH_3$)], 3.57 s (3H, 12-CH_3), 3.52 s (3H, 2-CH_3), 3.50 s (3H, 7-CH_3), 2.90-2.19 m [4H, 17-($CH_2CH_2COOCH_3$)], 1.90 d (3H, 18-CH_3, *J* 7.9 Hz), 1.87-1.79 m (6H, 3-CH_2CH_3, 8-CH_2CH_3), -1.62 br.s (1H, I-NH), -3.47 br.s (1H, III-NH).

*Chlorin e_6 3(1),3(2)-bis-(N,N-dimethylaminomethyl)-13(1)-N-methylamide 15(2),17(3) dimethyl ester (**27**), chlorin e_6 3(1),3(2)-bis-(N,N-dimethylaminomethyl)-13(1)-N-(2-hydroxyethyl)amide 15(2),17(3) dimethyl ester (**28**), chlorin e_6 3(1),3(2)-bis-(N,N-dimethylaminomethyl)-13(1)-morpholinylamide 15(2),17(3) dimethyl ester (**29**)* were obtained as described for **26**. 50 mg (54 %) of compound **27** was obtained from 79 mg of compound **21**. UV-Vis (CH_2Cl_2) λ nm: 398.0, 498.0, 554.0, 604.0, 659.0. ^{1}H NMR ($CDCl_3$, Me_4Si, *the signals of *cis*- and *trans*-isomers the chemical shifts of which are different, 300 MHz) δ ppm: 9.86 s (1H, H^{10}), 9.74 s (1H, H^5), 8.82/8.83* s (1H, H^{20}), 7.38-7.30 m [1H, 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$)], 6.42 br.t (1H, 13^1-CON$H$$C_2H_5$), 5.58 d (1H, $H^{15(1)}_A$, *J* 19.2 Hz), 5.29 d (1H, $H^{15(1)}_B$, *J* 19.2 Hz), 4.49 q (1H, H^{18}, *J* 6.9 Hz), 4.39 br.d (1H, H^{17}, *J* 9.6 Hz), 3.96-3.85 m (2H, 13^1-CONHCH_2CH_3), 3.85-3.75 m [2H, 8-(CH_2CH_3)], 3.80-3.50 m [4H, 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$)], 3.83 s (3H, 15^2-$COOCH_3$), 3.63/3.62* s (3H, 2-CH_3), 3.60 s [3H, 17-($CH_2CH_2COOCH_3$)], 3.55 s (3H, 12-CH_3), 3.37 s (3H, 7-CH_3), 2.98-2.70 m [2H, 17-($CH_2CH_2COOCH_3$)], 2.60-2.00 m [2H, 17-($CH_2CH_2COOCH_3$)], 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$): 2.30 s (6H), 2.26 s (3H), 2.23 s (3H); 1.78-1.69 m (6H, 18-CH_3, 8-CH_2CH_3), 1.48 t (3H, 13^1-CONHCH$_2$CH_3, *J* 7.5 Hz), -1.91 br.s (1H, I-NH), -1.70 br.s (1H, III-NH). 33 mg (37 %) of compound **28** was obtained from 77 mg of compound **24**. UV-Vis (CH_2Cl_2) λ nm: 399.0, 499.0, 604.0, 660.0. ^{1}H NMR ($CDCl_3$, Me_4Si, *the signals of *cis*- and *trans*-isomers the chemical shifts of which are different, 300 MHz) δ ppm: 9.84 s (1H, H^{10}), 9.73 s (1H, H^5), 8.84 s (1H, H^{20}), 7.36-7.30 m [1H, 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$)], 6.92 t (1H, 13-CON$H$$CH_2CH_2OH$, *J* 4.2 Hz), 5.62 d (1H, $H^{15(1)}_A$, *J* 18.3 Hz), 5.34 d (1H, $H^{15(1)}_B$, *J* 18.3 Hz), 4.47 q (1H, H^{18}, *J* 7.2 Hz), 4.43 br.d (1H, H^{17}, *J* 9.6 Hz), 4.07 t (2H, 13^1-CONHCH$_2$CH_2OH), 4.00-3.92 m (2H, 13^1-CONHCH_2CH$_2$OH), 3.85-3.79 m [2H, 8-(CH_2CH_3)], 3.80-3.40 m [4H, 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$)], 3.77 s (3H, 15^2-$COOCH_3$), 3.62 s (3H, 2-CH_3), 3.61 s [3H, 17-($CH_2CH_2COOCH_3$)], 3.56 s (3H, 12-CH_3), 3.37 s (3H, 7-CH_3), 2.84-2.50 m [4H, 17-($CH_2CH_2COOCH_3$)], 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$): 2.33 s (6H), 2.26/2.23* s (3H), 2.20 s (3H); 1.78-1.68 m (6H, 18-CH_3, 8-CH_2CH_3), -1.88 br.s (1H, I-NH), -1.66 br.s (1H, III-NH). 30 mg (42 %) of compound **29** was obtained from 62 mg of compound **25**. UV-Vis (CH_2Cl_2) λ nm: 398.0, 498.0, 552.0, 605.0, 659.0. ^{1}H NMR ($CDCl_3$, Me_4Si, *the signals of *cis*- and *trans*-isomers the chemical shifts of which are different, 300 MHz) δ ppm, prevalent isomer: 9.89 s (1H, H^{10}), 9.75 s (1H, H^5), 8.87/8.86* s (1H, H^{20}), 7.40-7.29 m [1H, 3-C($CH_2N(CH_3)_2$)=CH($CH_2N(CH_3)_2$)], 5.83 d (1H, $H^{15(1)}_A$, *J* 18.0 Hz), 5.09 d (1H, $H^{15(1)}_B$, *J* 18.0 Hz), 4.54-4.45 m (1H, H^{18}), 4.37 br.d (1H, H^{17}, *J* 9.0 Hz), 13^1-CONC$_4H_8$O: 4.65-4.54 m (1H), 4.17-3.80 m (7H); 3.92-3.78 m [2H, 8-(CH_2CH_3)], 3.89 s (3H, 15^2-$COOCH_3$), 3.68/3.67* s [3H, 17-($CH_2CH_2COOCH_3$)], 3.59 s (3H, 12-CH_3), 3.56 s (3H, 7-CH_3), 3.38/3.37* s (3H, 2-CH_3), 3.18-2.67 m [4H, 3-C($CH_2N(CH_3)_2$)

=CH(CH_2N(CH$_3$)$_2$)], 2.65-2.35 m [4H, 17-(CH_2CH_2COOCH$_3$)], 3-C(CH$_2$N(CH_3)$_2$)=CH(CH$_2$N(CH_3)$_2$): 2.29 s (7H), 2.27/2.26* s (2H), 2.24 s (2H), 2.17 s (1H); 1.80-1.65 m (6H, 18-CH$_3$, 8-CH$_2$CH_3), -1.76 br.s (1H, I-NH), -1.91 br.s (1H, III-NH). Minor isomer: 9.86 s (1H, H^{10}), 9.72 s (1H, H^5), 8.81 s (1H, H^{20}), 7.40-7.29 m [1H, 3-C(CH$_2$N(CH$_3$)$_2$)=C*H*(CH$_2$N(CH$_3$)$_2$)], 5.53/5.52* d (1H, H$^{15(1)}_A$, *J* 18.0 Hz), 5.23 d (1H, H$^{15(1)}_B$, *J* 18.0 Hz), 4.54-4.45 m (1H, H^{18}), 4.37 br.d (1H, H^{17}, *J* 9.0 Hz), 13^1-CONC$_4H_8$O: 4.65-4.54 m (1 H), 4.17-3.80 m (7H); 3.92-3.78 м [2H, 8-(CH_2CH$_3$)], 3.84 c (3H, 15^2-COOCH_3), 3.64/3.63* c [3H, 17-(CH$_2$CH$_2$COOCH_3)], 3.57 s (3H, 12-CH$_3$), 3.56 s (3H, 7-CH$_3$), 3.38/3.37* s (3H, 2-CH$_3$), 3.18-2.67 m [4H, 3-C(CH_2N(CH$_3$)$_2$)=CH(CH_2N(CH$_3$)$_2$)], 2.65-2.35 m [4H, 17-(CH_2CH_2COOCH$_3$)], 3-C(CH$_2$N(CH_3)$_2$)=CH(CH$_2$N(CH_3)$_2$): 2.29 s (7H), 2.27/2.26* s (2H), 2.24 s (2H), 2.17 s (1H); 1.80-1.65 m (6H, 18-CH$_3$, 8-CH$_2$CH_3), -1.76 br.s (1H, I-NH), -1.91 br.s (1H, III-NH).

Chlorin e$_6$ 13(1)-N-ethylamide-15(2),17(3)-dimethyl ester (21) was obtained as described for **19**. 66 mg (61 %) of compound **21** was obtained from 100 mg of compound **2** and 2 ml 70 % aqueous EtNH$_2$. UV-Vis (CH$_2$Cl$_2$) λ nm: 401.5, 500.0, 528.0, 556.0, 607.5, 662.5. ^{1}H NMR (CDCl$_3$, 300 MHz) δ ppm: 9.73 s (1H, H^{10}), 9.67 s (1H, H^5), 8.83 s (1H, H^{20}), 8.12 dd [1H, 3-(C*H*=CH$_2$), *J* 18.0 and 12.0 Hz], 6.40 dd [1H, 3-(CH=CHH_{trans}), *J* 18.0 and 0.9 Hz], 6.17 dd [1H, 3-(CH=CHH_{cis}), *J* 12.0 and 0.9 Hz], 6.40 br.t (1H, 13-CON*H*CH$_2$CH$_3$, *J* 6.0 Hz), 5.58 d (1H, H$^{15(1)}_A$, *J* 18.0 Hz), 5.29 d (1H, H$^{15(1)}_B$, *J* 18.0 Hz), 4.49 q (1H, H^{18}, *J* 9.0 Hz), 4.40 d (1H, H^{17}, *J* 9.0 Hz), 3.92-3.85 m (2H, 13^1-CONHCH_2CH$_3$), 1.06 t (3H, 13^1-CONHCH$_2$CH_3 *J* 7.6 Hz), 3.83 s (3H, 15^2-COOCH_3), 3.63 s [3H, 17-(CH$_2$CH$_2$COOCH_3)], 3.59 s (3H, 12-CH$_3$), 3.52 s (3H, 2-CH$_3$), 3.35 s (3H, 7-CH$_3$), 3.85-3.70 m [2H, 8-(CH_2CH$_3$)], 2.61-2.10 m [4H, 17-(CH_2CH_2COOCH$_3$)], 1.73 m (6H, 18-CH$_3$, 8-CH$_2$CH_3), -1.57 br.s (1H, I-NH), -1.79 br.s (1H, III-NH).

Mesochlorin e$_6$ 13(1)-N-methylamide-15(2),17(3)-dimethyl ester (30) was obtained as described for **19**. 29 mg (87 %) of compound **30** was obtained from 32 mg of compound **8** and 1 ml 33 % aqueous MeNH$_2$. UV-Vis (CH$_2$Cl$_2$) λ nm: 396.5, 496.5, 547.0, 595.0, 650.0. ^{1}H NMR (CDCl$_3$, 300 MHz) δ ppm: 9.72 s (1H, H^{10}), 9.49 s (1H, H^5), 8.76 s (1H, H^{20}), 6.40 m (1H, 13-CON*H*CH$_3$), 5.55 d (1H, H$^{15(1)}_A$, *J* 20.0 Hz), 5.28 d (1H, H$^{15(1)}_B$, *J* 20.0 Hz), 4.48 q (1H, H^{18}, *J* 7.0 Hz), 4.37 br.d (1H, H^{17}, *J* 9.0 Hz), 3.98-3.79 m [4H, 8-(CH_2CH$_3$), 3-(CH_2CH$_3$)], 3.85 s (3H, 15^2-COOCH_3), 3.65 s [3H, 17-(CH$_2$CH$_2$COOCH_3)], 3.58 s (3H, 12-CH$_3$), 3.41 c (3H, 2-CH$_3$), 3.37 s (3H, 7-CH$_3$), 3.28 d (3H, 13-CONHCH_3, *J* 4.0 Hz), 2.69-2.49 m [2H, 17-(CH_2CH$_2$COOCH$_3$)], 2.27-2.10 m [2H, 17-(CH$_2$CH_2COOCH$_3$)], 1.89-1.70 m (9H, 18-CH$_3$, 3-CH$_2$CH_3, 8-CH$_2$CH_3), -1.60 br.s (1H, I-NH), -1.70 br.s (1H, III-NH).

Mesochlorin e$_6$ 13(1)-dimethylaminomethyl-13(1)-N-methyl-amide-15(2),17(3)-dimethyl ester (31) was obtained as described for **19**. To the solution of compound **30** (132.4 mg) in the mixture of THF (7 ml) and AcOH (7 ml) *bis*(*N,N*-dimethylamino)methane (1.5 ml) was added. The mixture was srirred for 24 h at room temperature. Then reaction mixture was diluted with CH$_2$Cl$_2$ (50 ml) and washed by water. The resulted solution was dried with anhydrous Na$_2$SO$_4$, filtrated and evaporated. The product was isolated by column chromatography (elution by 30:1 (v:v) CCl$_4$-acetone mixture, TLC monitoring). The yield of compound **31** is 33.1 mg (30 %). UV-Vis (CH$_2$Cl$_2$) λ nm: 396.5, 496.5, 547.0, 596.0, 650.5. ^{1}H NMR (CDCl$_3$, 4 atropoisomers, 300 MHz) δ ppm: 9.75/9.74/9.72 s (1H, H^{10}), 9.52/9.49 s (1H, H^5), 8.79/8.78/8.74 s (1H, H^{20}), 5.82 d (1H, H$^{15(1)}_A$, *J* 21.0 Hz), 5.30-4.99 m (2H, H$^{15(1)}_B$, 13(2)-CH_2N(CH$_3$)$_2$), 4.53-4.30 m (3H, 13(2)-CH_2N(CH$_3$)$_2$,H^{18}, H^{17}), 3.99-3.81 m [4H, 8-(CH_2CH$_3$), 3-(CH_2CH$_3$)], 3.91/3.87/3.84 s (3H, 15^2-COOCH_3), 3.79/3.76 s (3H, 2-CH$_3$), 3.67/3.65/3.64 s [3H, 17-(CH$_2$CH$_2$COOCH_3)], 3.58/3.57/3.53 s (3H, 12-CH$_3$), 3.44/3.42/3.41 s (3H, 7-CH$_3$), 3.38 s (3H, 13^1-CONHCH_3), 2.84-2.64 m [4H, 17-(CH_2CH_2COOCH$_3$)], 1.91-1.67 m (15H, 18-CH$_3$, 8-CH$_2$CH_3, 3-CH$_2$CH_3, 13(2)-CH$_2$N(CH_3)$_2$), -1.63 br.s (1H, I-NH), -1.78 br.s (1H, III-NH).

The cytotoxic properties and photodynamic activity of compounds were estimated using their ability to induce hemolysis in the dark and in lighting conditions. Laboratory mice erythrocyte suspension (1 %) in PBS (p*H* 7.4) was used in the experiments. Investigated compounds were introduced in erythrocyte suspension as a solution in 96 % ethanol. The obtained suspension was incubated in a thermostatic shaker Biosan ES-20 (Latvia) at 37 °C and gently shaking. The control samples contained 0.1 % ethanol. The filament lamp (60 W) was used for illumination taking into account that photodynamic activity of porphyrins depends on irradiation dose but not on source. Luxmeter Velleman DVM 1300 (USA) was used for illumination estimation. The degree of hemolysis after 1.5 and 3 h of incubation with investigated compounds was determined spectrophotometrically as the hemoglobin transfer in the incubation medium (Genesys 20 ThemoSpectromic, USA). The hemolysis percent was calculated against the total hemolysis of the sample at λ = 524 nm.[62]

Results and Discussion

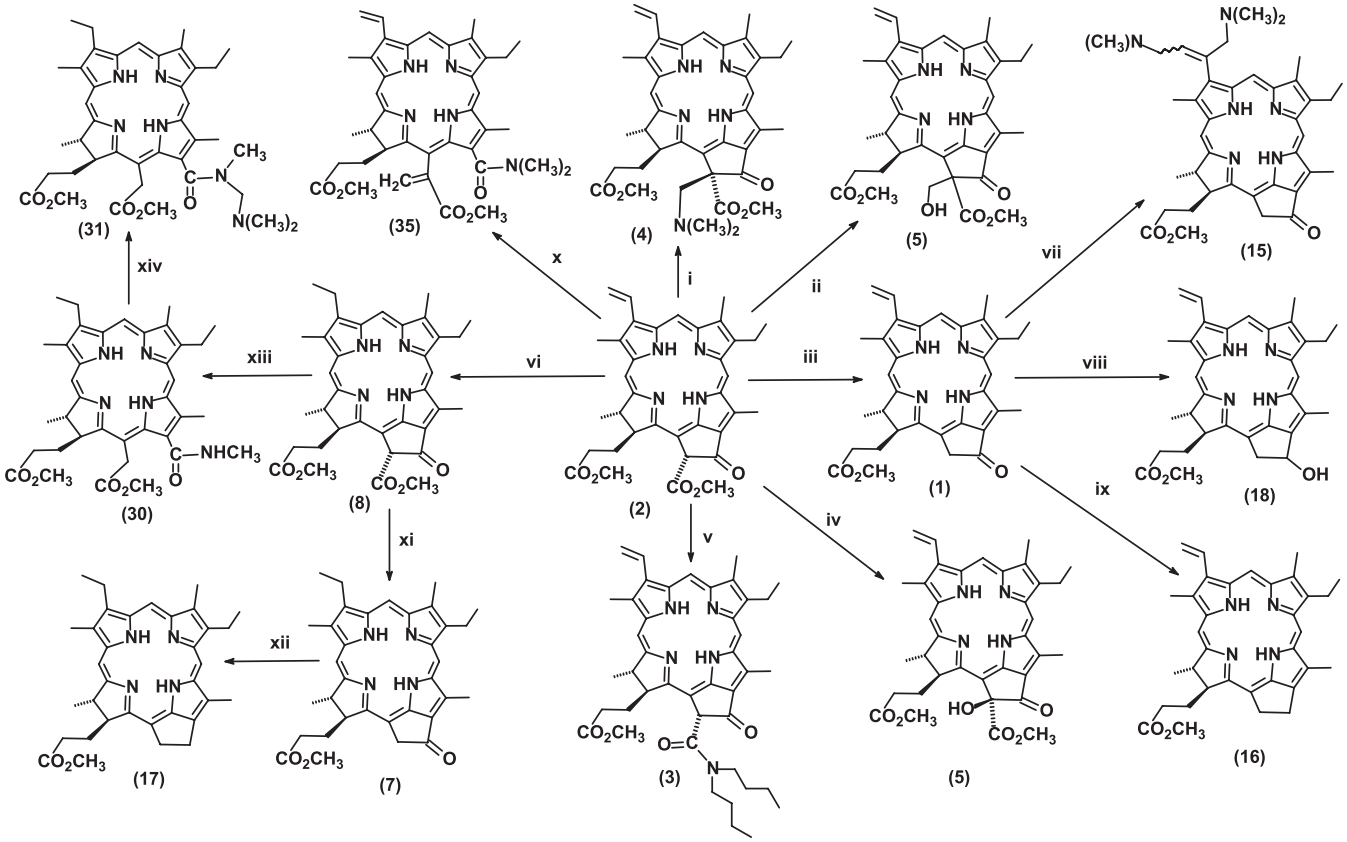
Here the methylpheophorbide *a* (**2**) and its analogs (**8**, **12**) were used us a starting material for the chlorins synthesis. The main reaction centers of these phorbine derivatives (exo-cycle vinyl group, keto group and ester group) were used for peripheral substituent's modification. Phorbine and chlorin derivatives of *a*-series were obtained from methylpheophorbide *a* (**2**). Exo-cycle aminomethylation and hydroxymethylation (compounds **4** and **5**), 13(2)-ester group amidation (compound **3**), exo-cycle decarboxylation lead to compound **1** followed by 13(1)-keto group reduction (compounds **4** and **5**), or complex (**Zn-1**) aminomethylation (compound **15**) was used for the phorbine derivatives synthesis (Scheme 2). Pd/C catalyzed methyl pheophorbide *a* (**2**) hydrogenation leads to corresponding methyl mesopheophorbide *a* (**8**), which was used as a start material for synthesis of *meso*-derivatives (**7**, **17**) (Scheme 2).

Vinyl group oxidation was used for 3-formyl and 3-(1,2-dihydroxyethyl) derivatives synthesis (Scheme 3).

Synthesis of chlorin e_6 13-amide derivatives (**19-25**) was carried out by the reaction of methyl pheophorbide *a* and corresponding amine (Scheme 4).[48,49,58-60] 13-Amide derivatives **30** (Scheme 2) and **34** (Scheme 6) were obtained by the same way.[59] The insertion of additional ethanolamine fragments (compounds **32**, **33**) was carried out using corresponding ester groups amidation of 13-amide (**24**) (Scheme 5).[60] Aminomethylation of chlorin e_6 13-amide derivatives (**19**, **21**, **24**, **25**) with *bis*(*N,N*-dimethylamino) methane (refluxing in AcOH and THF mixture) leads to twice aminomethylated derivatives **26-29** (Scheme 4).[50]

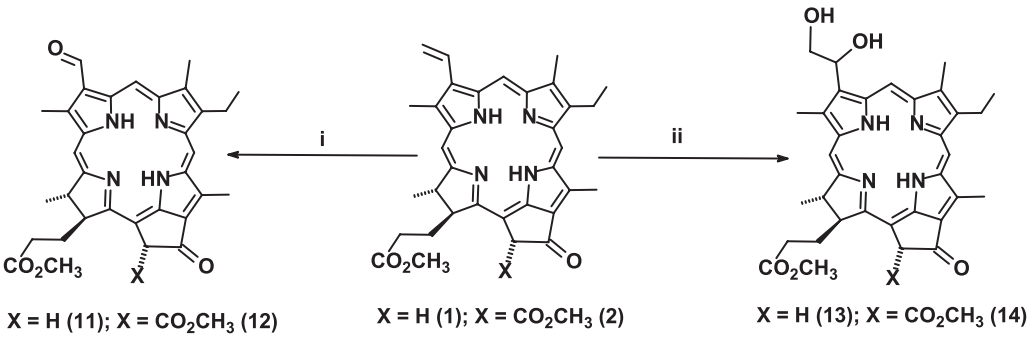
The reaction of *bis*(*N,N*-dimethylamino)methane with mesochlorin e_6 derivative **30** at room temperature leads to aminomethylation of 13-amide group with formation of compound **31** (Scheme 2). Methylpheophorbide *b* (**10**) was used for synthesis of derivatives *b*-series (**9**, **34**) (Scheme 6).

As can be seen from the data presented in Figure 1, in the case of erythrocytes incubation in darkening conditions the chlorophyll *a* derivatives investigated at concentration of 10 mM did not cause hemolysis or it was insignificant. So it indicates their potential low cytotoxicity. The estimation of the photodynamic activity shows (Figure 1) that compounds without exo-cycle (**20-35**) cause active photosensitized lysis



i: $(CH_3)_2NCH_2N(CH_3)_2$, THF/AcOH, 10-12 ºC, 24 h;[50] ii: paraformaldehyde, Na_2CO_3, H_2O-THF, 20 ºC, 1-3 h;[61] iii: pyridine, reflux, 5 h;[48] iv: O_2(air), compound **5** was isolated from polar fractions while obtaining of **2**; v: Bu_2NH, $PhCH_3$, reflux, 1.5 h;[49] vi: H_2, Pd/C;[51] vii: (a) $Zn(OAc)_2$, $CHCl_3/CH_3OH$, 20 ºC, (b) $(CH_3)_2NCH_2N(CH_3)_2$, THF-AcOH, 20 ºC, (c) HCl (30 % aq.)/$CHCl_3$;[55] viii: $NaBH_4$, CH_2Cl_2/CH_3OH; [55] ix: $NaBH_4$, TFA/CH_2Cl_2 0 ºC; x: $(CH_3)_2NCH_2N(CH_3)_2$, THF, reflux, 15 h;[50] xi: pyridine, reflux, 5 h; xii: $NaBH_4$, TFA/CH_2Cl_2 0 ºC; xiii: CH_3NH_2 (33 % aq.)-THF; xiv: $(CH_3)_2NCH_2N(CH_3)_2$, THF/AcOH, 20-22 ºC, 24 h.

Scheme 2.

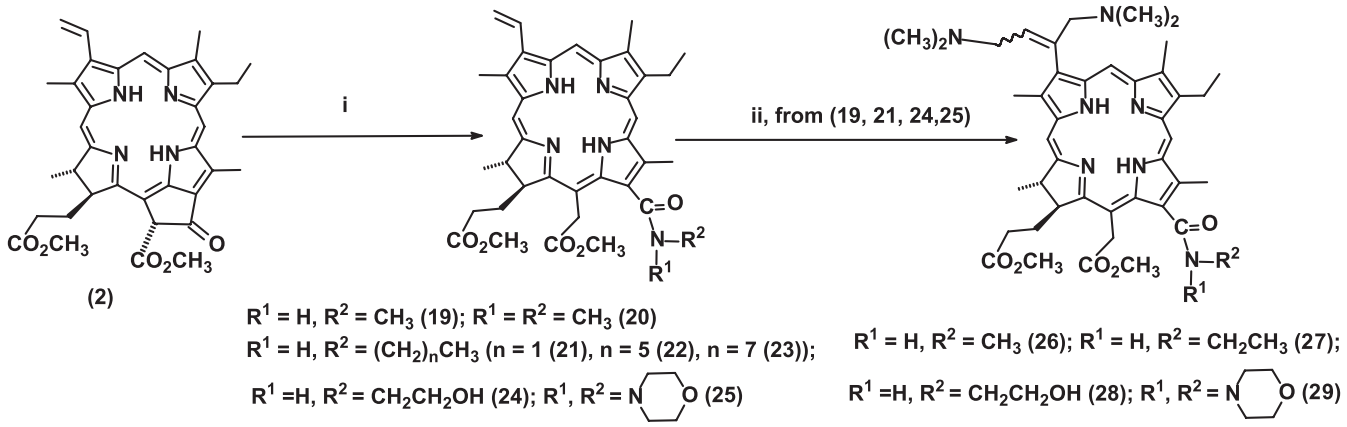


i: $NaIO_4$-OsO_4 (H_2O-AcOH-THF);[53,54] ii: $KMnO_4$, H_2O-acetone.

Scheme 3.

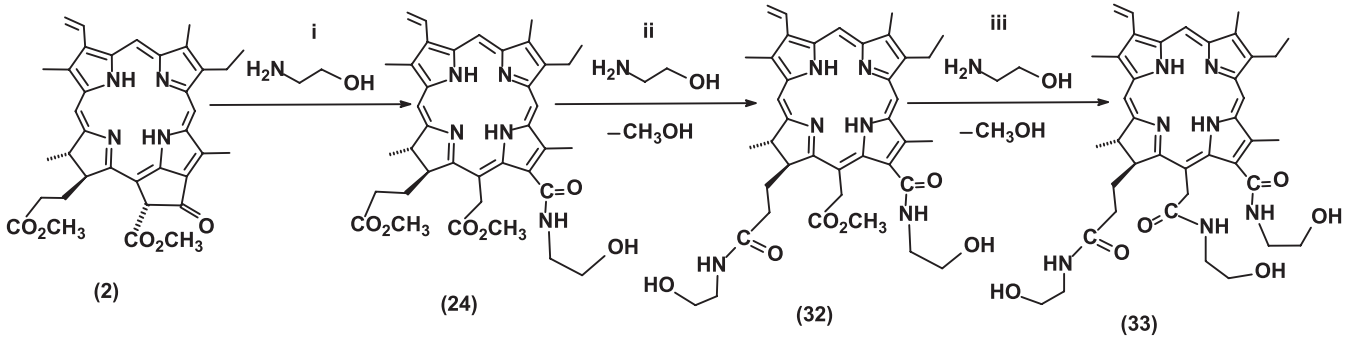
of erythrocytes regardless of the substituent's nature. In all cases a part of dead cells is greater than 80 % after 1.5 hours of incubation. The presence of exo-cycle (compounds **1-19**) in most cases leads to a decrease in photohemolysis power and an increase in its induction period (Figure 1). The phorbine compounds activity becomes comparable with that of compounds without exo-cycle at presence of groups promoting hydrogen bonding in the phorbine derivatives molecules(compounds **4**, **6**, **13-15**). As it was noted above the photo effect is determined mainly by the interaction with the membrane and much less depends on other factors action. Furthermore, the setup of present experiment allows to level factors other than interaction with the membrane.

Therefore, the differences found in photosensitizing effect of peripheral substituents of phorbine derivatives (**1-19**) and chlorins without exo-cycle (**20-35**) suggest the



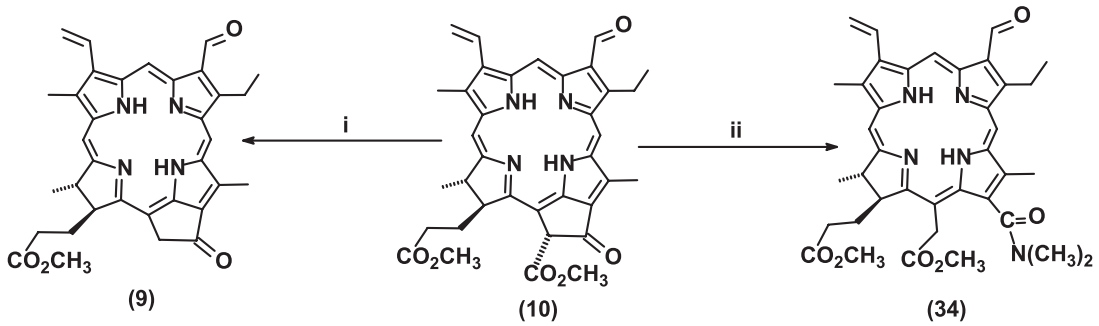
i: amine or its aqueous solution, THF or $CHCl_3$;[48,49,58-60] ii: $(CH_3)_2NCH_2N(CH_3)_2$, THF/AcOH, reflux 20 min.[50]

Scheme 4.



i: ethanolamine, $CHCl_3$, 20 ºC, 3 h;[60] ii: ethanolamine, 20º C , 6-8 h;[60] iii: ethanolamine, 20 ºC, 60 h.[60]

Scheme 5.



i: pyridine, reflux 5 h;[52] ii: CH_3NH_2(33 % aq)-THF.[59]

Scheme 6.

nature of interaction with the erythrocyte membrane to be different for these groups of compounds and the difference is caused by the macrocyclic structure. The phorbine derivatives **1**-**19** seem to interact with membrane by the formation of hydrogen bonds with phospholipids, glycolipids, membrane proteins polar groups, but chlorin derivatives **20**-**35** are known to interact with membrane in some less specific manner. We suggest that the exo-cycle absence in the PS molecule promotes its deeper infiltration into the lipid bilayer. It causes the macrocyclic molecule photoactive region to contact directly with the most sensitive to the oxidation sites of membrane-phospholipid polyunsaturated fatty acids. This may be the reason of independence on the nature of the substituents for chlorin derivatives **20**-**35** to sensitize erythrocytes photohemolysis. Stronger photodynamic effect of the compounds **20**-**35** seems to be caused by the same reason.

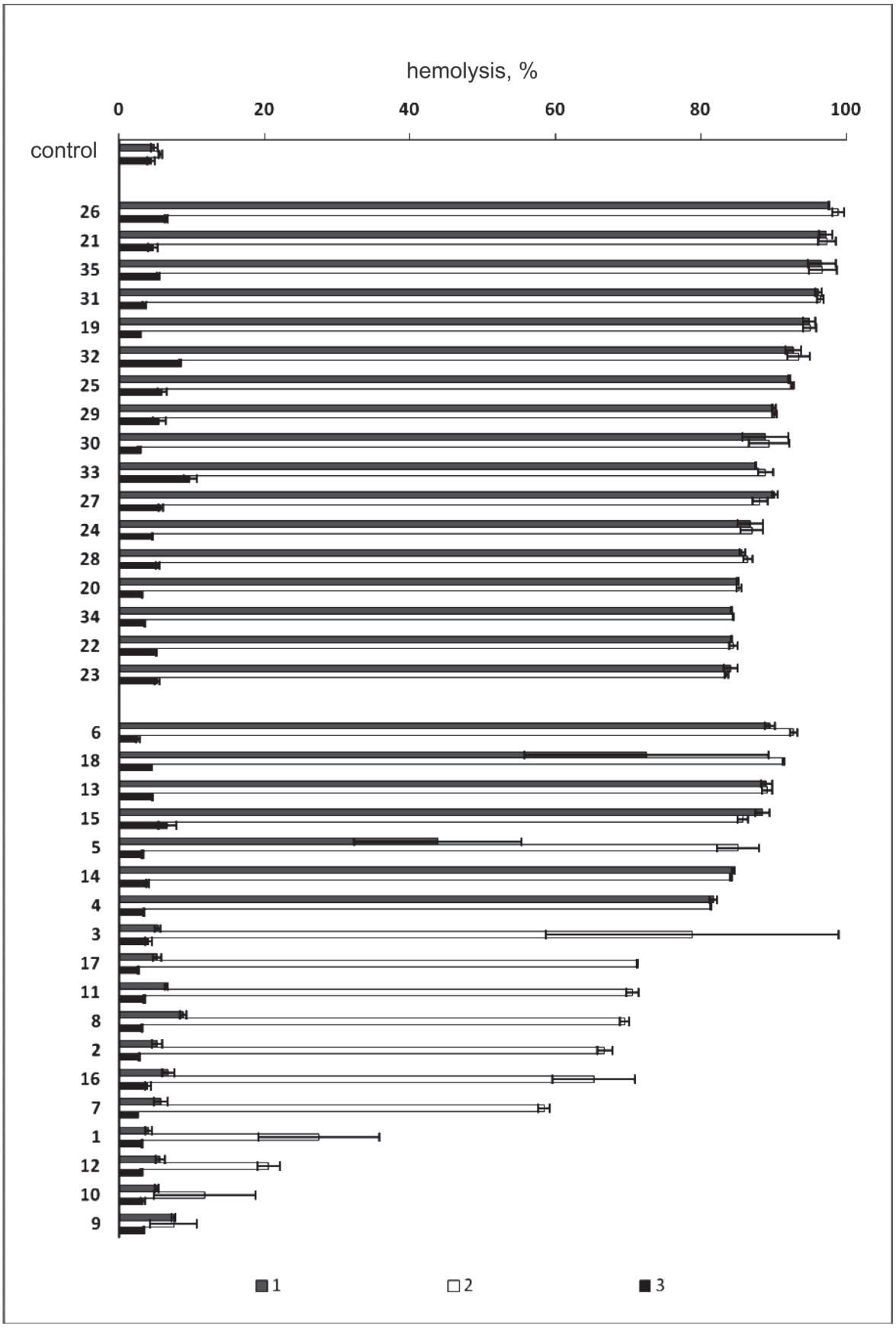


Figure 1. The degree of hemolysis of erythrocytes (%) after incubation with the test compounds at concentration of 10 μM. 1-illuminance 1.7 klux, 1.5 h incubation; 2 - 1.7 klux illumination, 3 h incubation; 3 - incubation conditions dimmer, 3 h.

Conclusions

Thus, the erythrocytes photohemolysis sensitized by semisynthetic chlorins based on phytoporphyrins was studied here. It was shown that chlorins without exo-cycle (**20-35**) cause an active photosensitized lysis of erythrocytes regardless of the substituent's nature. The presence of exo-cycle (compounds **1-19**) in the most cases leads to decrease in photohemolysis power and increase in its induction period. The phorbine compounds activity becomes comparable with activity of that without exo-cycle, but with groups promoting hydrogen bonding (compounds **4**, **6**, **13-15**) in the phorbine derivative molecules.

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