NEW CATIONIC CHLORIN AS POTENTIAL AGENT FOR ANTIMICROBIAL PHOTODYNAMIC THERAPY

Suvorov N.V.[1], Shchelkova V.V.[2,3], Rysanova E.V.[2], Bagatelia Z.T.[4], Diachenko D.A.[4], Afaniutin A.P.[5], Vasil'ev Yu.L.[1,4], Diachkova E.Yu.[4], Santana Santos I.C.[6], Grin M.A.[1]
[1]Institute of Fine Chemical Technologies, MIREA-Russian Technological University, Moscow, Russia
[2]Moscow Regional Research and Clinical Institute, Moscow, Russia
[3]N. Kosygin Russian State University, Moscow, Russia
[4]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia
[5]AveDent, Dental Clinic, Orel, Russia
[6]São Carlos School of Engineering, University of São Paulo, São Paulo, Brazil

Abstract

Multiple drug resistance is a major global health security risk. Increasing resistance of bacteria to existing drugs puts on the agenda the search for alternative ways to combat antibiotic-resistant pathogens. One of these innovative methods is antimicrobial photodynamic therapy (APDT), which is equally effective against antibiotic-sensitive and antibiotic-resistant pathogens. The most effective photosensitizers (PS) for APDT are molecules containing positively charged groups in their composition. In this work, we have obtained a new cationic derivative of natural chlorin containing a pyridazine group in its composition, the introduction of which occurs using click chemistry approaches. The antimicrobial photoinduced cytotoxicity of the proposed cationic PS, as well as its uncharged precursor, was assessed against a number of gram-positive and gram-negative bacteria: *S. aureus*, *K. pneumoniae*, *E. faecalis*, *P. aeruginosa*. It has been shown that cationic chlorin exhibits an increased bactericidal effect when irradiated with light (λ = 660 nm, P_s = 70.73 mW/cm^2) compared to its base form. When microbial suspensions were incubated with 24 μM cationic PS and subsequently irradiated, a significant bactericidal effect was observed against all of the aforementioned bacteria. As a result of microbiological studies, it was demonstrated that the proposed cationic PS exhibits high photoinduced antimicrobial activity.

Key words: antimicrobial PDT, antibiotic resistance, antimicrobial activity, chlorophyll, chlorin e6.

For citations: Suvorov N.V., Shchelkova V.V., Rysanova E.V., Bagatelia Z.T., Diachenko D.A., Afaniutin A.P., Vasil'ev Yu.L., Diachkova E.Yu., Santana Santos I.C., Grin M.A. New cationic chlorin as potential agent for antimicrobial photodynamic therapy, *Biomedical Photonics*, 2024, vol. 13, no. 3, pp. 14–19. doi: 10.24931/2413–9432–2024–13–3 –14–19

Contacts: Suvorov N.V., e-mail: suvorov.nv@gmail.com

НОВЫЙ КАТИОННЫЙ ХЛОРИН КАК ПОТЕНЦИАЛЬНЫЙ АГЕНТ ДЛЯ АНТИМИКРОБНОЙ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ

Н.В. Суворов[1], В.В. Щелкова[2,3], Е.В. Русанова[2], З.Т. Багателия[4], Д.А. Дьяченко[4], А.П. Афанютин[5], Ю.Л. Васильев[1,4], Е.Ю. Дьячкова[4], И.К. Сантана Сантос[6], Грин М.А.[1]
[1]Институт тонких химических технологий, РТУ МИРЭА, Москва, Россия
[2]Московский областной научно-исследовательский клинический институт им. М.Ф. Владимирского, Москва, Россия
[3]Российский государственный университет им. А.Н. Косыгина, Москва, Россия
[4]Первый МГМУ им. И.М. Сеченова (Сеченовский Университет), Москва, Россия
[5]Стоматологическая клиника АвеДент, Орел, Россия
[6]Инженерная школа Сан-Карлоса, Университет Сан-Паулу, Сан-Паулу, Бразилия

Резюме

Множественная устойчивость микроорганизмов к антибиотикам является одним из основных рисков для безопасности в области глобального здравоохранения. Нарастание резистентности бактерий к уже имеющимся препаратам поставили на повестку дня поиск альтернативных способов борьбы с антибиотикорезистентными возбудителями инфекций. Одним из таких инновационных методов

является антимикробная фотодинамическая терапия (АФДТ) одинаково эффективная против антибиотикочувствительных и антибиотикорезистентных возбудителей. Наиболее эффективными фотосенсибилизаторами (ФС) для АФДТ являются молекулы, содержащие положительно-заряженные группы в своем составе. В настоящей работе нами было получено новое катионное производное природного хлорина, содержащее пиридазиновую группу в своем составе, введение которой происходит с использованием подходов click-химии. Противомикробную фотоиндуцированную цитотоксичность предлагаемого катионного ФС, а также его незаряженного предшественника, оценивали в отношении ряда грамположительных и грамотрицательных бактерий: *S. aureus, K. pneumoniae, E. faecalis, P. aeruginosa*. Показано, что катионный хлорин обладает повышенным бактерицидным действием при облучении светом (λ =660 нм, P_s = 70,73 мВ/см^2) по сравнению со своей основной формой. При инкубировании микробных суспензий с раствором катионного ФС в концентрации 24 мкМ и последующим облучением наблюдался заметный бактерицидный эффект в отношении всех вышеназванных бактерий. В результате проведенных микробиологических исследований показано, что предлагаемый катионный ФС обладает высокой фотоиндуцированной антимикробной активностью.

Ключевые слова: антимикробная ФДТ, антибиотикорезистентность, противомикробная активность, хлорофилл, хлорин е6.

Для цитирования: Суворов Н.В., Щелкова В.В., Русанова Е.В., Багателия З.Т., Дьяченко Д.А., Афанютин А.П., Васильев Ю.Л., Дьячкова Е.Ю., Сантана Сантос И.К., Грин М.А. Новый катионный хлорин как потенциальный агент для антимикробной фотодинамической терапии // Biomedical Photonics. – 2024. – Т. 13, № 3. – С. 14–19. doi: 10.24931/2413–9432–2024–13–3–14–19

Контакты: Суворов Н.В., e-mail: suvorov.nv@gmail.com

Introduction

Multiple antibiotic resistance is a major global health security risk. According to the WHO, already in 2019, 1.27 million deaths worldwide were directly related to drug-resistant infections. If this problem is not addressed, it is predicted that by 2050 the number of such deaths will increase to 10 million annually [1, 2]. According to the Central Research Institute of Epidemiology of Rospotrebnadzor, the number of nosocomial infections in Russia annually affects 2-2.5 million (1.5% of the population). The increasing bacterial resistance to existing drugs, coupled with poor bioavailability and lack of structures that could potentially form the basis of new antibiotics, have put the search for alternative ways to combat antibiotic-resistant pathogens on the agenda.

One such innovative method is antimicrobial photodynamic therapy (APDT) for localized infections [3-6], equally effective against antibiotic-sensitive and antibiotic-resistant pathogens. Antimicrobial photodynamic therapy (APDT) is based on the use of a photosensitizer (PS) drug and harmless visible light. Photosensitizers are capable, when exposed to light of a certain wavelength corresponding to their maximum absorption, to cause the formation of cytotoxic agents, in particular singlet oxygen, stimulating oxidative destruction of the main components (unsaturated lipids, protein channels and enzymes) of cell membranes – the life-supporting structures of microbial cells [7]. Photo-oxidative processes in the lipid bilayer of cell membranes lead to the formation of pores and disruption of cellular permeability barriers. The death of a microbial cell occurs as a result of the leakage of cellular metabolites, primarily potassium ions and protons, and dissipation of the membrane potential.

One of the main advantages of antimicrobial photodynamic therapy is the multiple nature of the oxidative destruction of microbial target cells, which prevents the development of resistance to subsequent cycles of photodynamic effects. In addition, since the bactericidal effect of APDT is local in nature, it does not have a systemic detrimental effect on the saprophytic flora of the body. The use of APDT thus solves two main problems of modern antibiotic therapy: high resistance of pathogenic microorganisms to antibiotics and systemic toxicity [8-10].

To date, many PS have been synthesized for antimicrobial PDT, both based on tetrapyrrole compounds and using other dyes. Unlike synthetic dyes, derivatives of natural chlorins have a number of advantages. The main sources of such compounds are plants and algae containing chlorophyll. Such raw materials are easily accessible and cheap, and methods for extracting pigment from them are not labor-intensive. Thanks to the reduced pyrrole ring, chlorophyll A derivatives exhibit absorption in the near-IR region with high extinction values, which allows PDT of deep-lying zones [5].

The most effective photosensitizers for APDT are molecules containing positively charged groups in their composition. In the 90s, it was shown that there is a difference in susceptibility to APDT between gram-positive and gram-negative bacteria, which is associated with their structure: gram-positive bacteria have a cytoplasmic membrane surrounded by a porous cell wall consisting of peptidoglycan and lipoteichoic acid, which allows the photosensitizer to easily bind with it, and the membrane of gram-negative bacteria consists of outer and inner cytoplasmic membranes separated by a layer of peptidoglycan [11-13]. Neutral, anionic and cationic photosensitizer molecules bind equally well to gram-positive bacteria, while only cationic or neutral photosensitizers can bind to gram-negative bacteria. At the moment, a large number of cationic derivatives of natural chlorins containing both alkylammonium groups

and quaternized heteroaromatic fragments have been synthesized [14-18].

Previously, our research group obtained a derivative of natural chlorin, into which a residue of nicotinic acid was introduced with its subsequent quaternization [19]. This compound had both high photoinduced toxicity towards bacterial cells, as well as biofilms based on them. However, the complexity of the synthesis of this compound complicates its further implementation in practice. In this work, we have proposed an approach that makes it possible to introduce a heterocyclic fragment into the chlorin molecule using one stage inverse electron demand Diels-Alder reaction click-reaction, and also studied the photoinduced toxicity of the resulting compound and its cationic derivative against gram-positive and gram-negative bacteria.

Materials and Methods

Chemistry

Solvents were purified and prepared according to standard procedures. Silica gel 40/60 (Merck, Germany) was used for column chromatography. Absorption spectra were obtained on a Shimadzu spectrophotometer UV 1800 UV/VIS in 10 mm thick quartz cuvettes in chloroform and water. The NMR spectrum was recorded on Bruker DPX 300 spectrometer in $CDCl_3$. The synthesis of photosensitizers **1** and **2** was carried out according to a previously described method [20]. Cationic derivative **3** was obtained by reacting compound **2** (25 mg; 0.033 mmol) with methyl iodide (145.5 mg; 1.025 mmol) in acetonitrile (1 ml) at 60°C for 2 hours. The solvent was evaporated under reduced pressure. The target product was isolated using column chromatography (DCM/MeOH/AcOH, 450/10/1, v/v/v). The yield of compound **3** was 63.3% (19 mg). ^{1}H NMR (300 MHz, $CDCl_3$, δ , ppm): 9.77 (H, s, 10-H) 9.60 (H, d, J = 4.7 Hz, 5′-H), 9.20 (H, s, 5-H), 8.71+8.72 (H, s, 20- H), 8.07 (H, d, J = 4.7 Hz, 6′-H), 7.62 (2H, m, o-Phe), 7.12 (H , m, p-Phe), 7.02 (H, m, o-Phe), 5.42 (H, d, J = 18.8 Hz, 151- CH_2^a) , 4.46 (2H , m, 18-H , 17-H), 4.30 (3H, s 13^2-CH_3), 3.81 (3H, s, 15^3-CH_3), 3.80 (2 H , k, J = 7.7 Hz, 8^1-CH_2), 3.71 (3H, s, 17^4-CH_3), 3.62 (3H, s, 12-CH_3), 3.53 (3H, s, *N*-CH_3) , 3.20 (3H, s, 7-CH_3), 2.95 (3H, s, 2- CH_3), 2.60 (H, m, 17^2-CH_2^a) , 2.28 (2H, m, 17^1-CH_2^a, 17^1-CH_2^b), 1.84 (H, m, 17^1-CH_2^b), 1.75 (3H, d, J = 18.1 Hz, 18-CH_3), 1.74 (3H, t, J = 7.7 Hz, 8^2-CH_3), -1.33 (H, s, I – NH), -1.72 (H, s, III – NH). ESI-MS: m/z calc. for $C_{46}H_{49}N_6O_6$: 781.37, found: $[M+H]^+$, 782.38, $[M+2H]^{2+}$ 391.69. UV/VIS ($CHCl_3$), λ_{max}, nm (ε, M^{-1} cm^{-1}): 395 (180000), 497 (17500), 686 (44000). To prepare a water-soluble form of compounds **2** and **3**, 4% Kolliphor ELP solution in water was used. A weighed portion of the corresponding chlorin (1 mg) was dissolved in dichloromethane (2 ml) and added to 4% Kolliphor solution in water (3 ml). The resulting mixture was stirred in a flow of argon at a temperature of 41°C until the organic phase was completely removed. The resulting emulsion was passed through a syringe filter with a pore diameter of 200 nm.

Microbiology

The source of non-monochromatic LED red radiation was a single-band laser (λ =660 nm, P_s = 70.73 mW/cm^2). Exposure of the PS in the microbial suspension before irradiation is 30 min. The dose of light during irradiation is 20 J/cm^2.

To assess the antibacterial activity of PS, we used suspensions of daily microbial cultures of *S. aureus* ATCC 25923 (n = 15), *K. pneumoniae* ATCC 13883 (n = 15), *E. faecalis* ATCC 29212 (n = 15), *P. aeruginosa* ATCC 27853 (n =15), which are common causative agents of inflammatory processes in the ear and upper respiratory tract. The concentration of the microbial suspension in the experiment was 3 x 10^3 CFU/ml. Control – test strains that were under equal conditions, but were not exposed to PS and irradiation. 200 µl of a suspension of microorganisms was added to the wells of a microplate and 2, 8 and 12 µl of 0.4 mM solution of PS **2** and **3** were injected, respectively. After exposure and irradiation, the microplates were incubated for 24 hours at T = 37^{0}C. To determine the survival rate of microorganisms, the method of surface inoculation using a spatula on solid nutrient media (Koch plate method) was used. After thermostating the control crops, we calculated and expressed the results obtained on solid media.

Formulas for calculating the number of viable microorganisms:

1. If the number of colonies exceeds 10:

$$M = a * 10n/V,$$

where *a* is the average number of colonies grown after sowing from a given dilution; *10* – dilution factor; *n* is the serial number of the dilution from which the sowing was made; *V* – volume of suspension taken for sowing, cm^3.

2. If the number of colonies is less than 10, but more than 4:

$$M = c/V * n,$$

where *c* is the number of colonies counted in the dish, *V* is the volume of the suspension taken for inoculation, cm^3, n is the serial number of the dilution from which the inoculation was made.

3. If there are from 1 to 3 colonies, then we accept as – "microorganisms are present in quantities less than $1 \times V$ per cm^3" (*V* is the dilution factor).

The calculation data is included in Table 1. Statistical analysis of the obtained data was carried out using the SPSS application package Statistics 21.0 and Rstudio 4.2.2. Mean values, standard deviations, and medians were determined. When comparing groups, parametric (ANOVA) and nonparametric methods (Kruskal–Wallis, Mann–Whitney, Wilcoxon) estimates were used depending on the normality of the sample distribution, which was determined using the Shapiro-Wilk test. The results were considered statistically significant at a confidence level of at least 95%.

Results and discussion

Previously, we carried out a one-step functionalization of the chlorin e6 trimethyl ester molecule, which allowed us to introduce a pyridazine group into pyrrole *A* using the inverse electron demand Diels-Alder reaction (Fig. 1) [20]. It was shown that this reaction occurs in high yields without changing the properties of the original PS. In this work, the reaction of chlorin e6 trimethyl ester with 3-phenyl-1,2,4,5-tetrazine was carried out to obtain pyridazine-substituted derivative **2**. The resulting PS was reacted with methyl iodide to obtain quaternized derivative **3**. Thus, we obtained chlorins containing heterocyclic fragments at the periphery of the macrocycle, in neutral and cationic form.

The resulting PS **2** and **3** are insoluble in water, so their water-soluble forms were prepared in the form of an emulsion in 4% Kolliphor ELP, which is a clinically approved solubilizer.

The antimicrobial effect of the obtained PS was assessed *in vitro* against bacteria *S. aureus, K. pneumoniae, E. faecalis* and *P. aeruginosa*. By counting the number of colonies, the probability of the effect of cationic and anionic PS on Gram "-" and "+" microorganisms were determined (Table 1).

Таблица 1
Таблица подсчета колоний
Table 1
Colony count table

Бактерия Bacteria	Концентрация ФС (мкМ) PS concentration (μM)	PS 2		PS 3		Контроль Control
		Среднее количество микроорганизмов Average number of microorganisms M±m	КОЕ/мл CFU/ml	Среднее количество микроорганизмов Average number of microorganisms M±m	КОЕ/мл CFU/ ml	
S. aureus	4	77.5±16.9	$7.6*10^3$	18.3±14.6	$1.8*10^3$	Сливной рост Confluent growth $2*10^6$
	16	16.9±8.6	$1.7*10^3$	3.7±4.4	$4*10^2$	
	24	2.6±2.9	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	0.8±1.4	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	
K. pneumoniae	4	153.1±4.3	$1.5*10^4$	114.4±17.8	$1.1*10^4$	Сливной рост Confluent growth $6*10^6$
	16	20.1±2.5	$2*10^3$	53.6±9.6	$5.4*10^3$	
	24	0.4±1	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	1.8±2.7	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	
E. faecalis	4	149.3±4.2	$1.5*10^4$	43.8±4.3	$4.4*10^3$	Сливной рост Confluent growth $4*10^6$
	16	41.2±3.2	$4.1*10^3$	9.1±1.6	$9*10^2$	
	24	5.5±6.8	$6*10^2$	0.5±1	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	
P. aeruginosa	4	162.1±2.8	$1.6*10^4$	114.4±7.81	$1.4*10^4$	Сливной рост Confluent growth $5*10^6$
	16	17.6±4.8	$1.8*10^3$	13.3±4.9	$1.3*10^3$	
	24	1.8±2.5	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	1.4±2.5	микроорганизмы присутствуют в количестве менее 1 на $см^3$ microorganisms are present in less than 1 per cm^3	

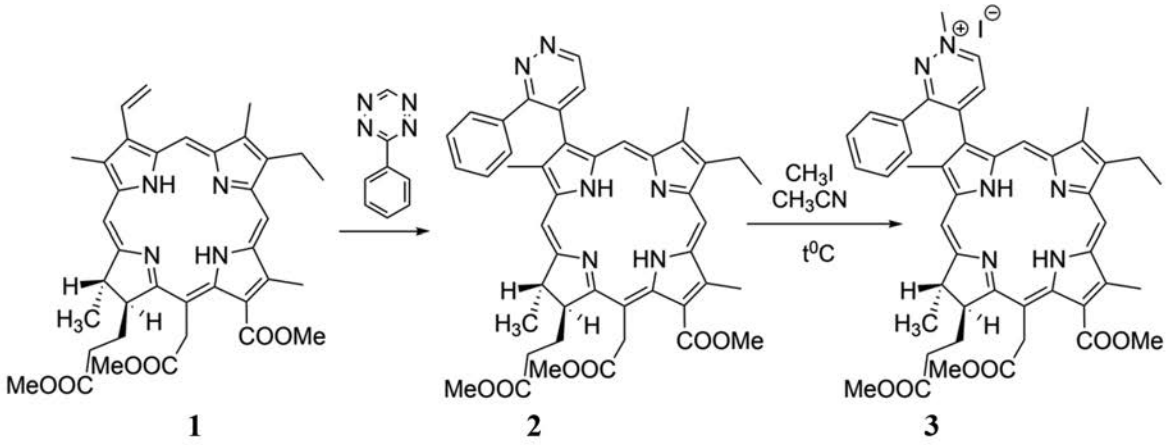

Рис. 1. Синтез пиридазин-замещенных производных хлорина.
Fig. 1. Synthesis of pyridazine-substituted chlorin derivatives.

Analysis of the data obtained showed that all PS have inhibitory properties towards the tested microorganisms. When a microbial suspension was incubated with 4 μM and 16 μM PS **2** and **3** followed by irradiation, a rather bacteriostatic effect was detected or a slight increase in microbial numbers from the initial concentration of microorganisms was observed, while in the control a stable confluent growth was observed. When using 24 μM PS, a noticeable bactericidal effect is observed. Negative dynamics of microorganisms is observed depending on the concentration of PS in the suspension of microorganisms. When comparing the difference between the use of PS **2** and **3**, a significant decrease in the number of colonies can be traced when exposed to cationic PS **3** compared to PS **2** in the case of gram-positive bacteria. When comparing the effectiveness against gram-negative bacteria, the cationic derivative 3 was more effective compared to the pyridazine-substituted derivative **2**, but the same significant difference in biological activity as in the case of gram-positive bacteria was not detected. The results obtained from the study of photoinduced cytotoxicity suggest the high efficiency of PS **3** compared to similar chlorophyll *A* derivatives [21, 22].

Conclusions

Studies have shown that the proposed cationic photosensitizer, obtained using the tetrazine-alkene addition reaction starting from trimethyl ester of chlorin e6, has high photoinduced antimicrobial activity against both gram-positive and gram-negative bacteria. An additional advantage of the proposed PS is the ease of its preparation, and the raw materials for its synthesis are readily available. We are currently planning to conduct a study of the effectiveness of the resulting PS on bacteria in biofilms, as well as to carry out in vivo experiments on models of wound infections.

Acknowledgments

The work was carried out with the support of the Ministry of Science and Higher Education of the Russian Federation (government assignment №075-00701-24-07 dated 04/03/2024; FSFZ-2024-0013).

REFERENCES

1. Macias J., Kahly O., Pattik-Edward R., Khan S., et al. Sepsis: A Systematic Review of Antibiotic Resistance and Antimicrobial Therapies, *Mod Res Inflamm*, 2022, vol. 11, no. 02, pp. 9–23. doi: 10.4236/mri.2022.112002.
2. Murray C. J., Ikuta K. S., Sharara F., Swetschinski L., et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, *Lancet*, 2022, vol. 399, no. 10325, pp. 629–655. doi: 10.1016/S0140-6736(21)02724-0.
3. Hamblin M. R., Hasan T. Photodynamic therapy: A new antimicrobial approach to infectious disease?, *Photochem Photobiol Sci*, 2004, vol. 3, no. 5, pp. 436–450. doi: 10.1039/b311900a.
4. Dai T., Huang Y. Y., Hamblin M. R. Photodynamic therapy for localized infections-State of the art, *Photodiagnosis Photodyn Ther*, 2009, vol. 6, no. 3–4, pp. 170–188. doi: 10.1016/j.pdpdt.2009.10.008.
5. Suvorov N., Pogorilyy V., Diachkova E., Vasil'ev Y., et al. Derivatives of natural chlorophylls as agents for antimicrobial photodynamic

ЛИТЕРАТУРА

1. Macias J., Kahly O., Pattik-Edward R., Khan S., Qureshi A., Shaik A., et al. Sepsis: A Systematic Review of Antibiotic Resistance and Antimicrobial Therapies // Mod. Res. Inflamm. – 2022. – Vol. 11, № 02. – P. 9-23. doi: 10.4236/mri.2022.112002.
2. Murray C.J., Ikuta K.S., Sharara F., Swetschinski L., Robles Aguilar G., et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis // Lancet. – 2022. – Vol. 399, № 10325. – P. 629-655. doi: 10.1016/S0140-6736(21)02724-0.
3. Hamblin M.R., Hasan T. Photodynamic therapy: A new antimicrobial approach to infectious disease? // Photochem. Photobiol. Sci. – 2004. – Vol. 3, № 5. – P. 436–450. doi: 10.1039/b311900a.
4. Dai T., Huang Y.Y., Hamblin M.R. Photodynamic therapy for localized infections-State of the art // Photodiagnosis Photodyn. Ther. – 2009. – Vol. 6, № 3–4. – P. 170-188. doi: 10.1016/j.pdpdt.2009.10.008.
5. Suvorov N., Pogorilyy V., Diachkova E., Vasil'ev Y., Mironov A., et al. Derivatives of natural chlorophylls as agents for antimicrobial pho-

therapy, *Int J Mol Sci*, 2021, vol. 22, no. 12, p. 6392. doi: 10.3390/ijms22126392.
6. de Souza da Fonseca A., de Paoli F., Mencalha A. L. Photodynamic therapy for treatment of infected burns, *Photodiagnosis Photodyn Ther*, 2022, vol. 38, p. 102831. doi: 10.1016/j.pdpdt.2022.102831.
7. Huang L., Xuan Y., Koide Y., Zhiyentayev T., et al. Type i and Type II mechanisms of antimicrobial photodynamic therapy: An in vitro study on gram-negative and gram-positive bacteria, *Lasers Surg Med*, 2012, vol. 44, no. 6, pp. 490–499. doi: 10.1002/lsm.22045.
8. Bustamante V., Palavecino C. E. Effect of photodynamic therapy on multidrug-resistant Acinetobacter baumannii: A scoping review, *Photodiagnosis Photodyn Ther*, 2023, vol. 43, p. 103709. doi: 10.1016/j.pdpdt.2023.103709.
9. Jao Y., Ding S. J., Chen C. C. Antimicrobial photodynamic therapy for the treatment of oral infections: A systematic review, *J Dent Sci*, 2023, vol. 18, no. 4, pp. 1453–1466. doi: 10.1016/j.jds.2023.07.002.
10. Hamblin M. R. Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes, *Curr Opin Microbiol*, 2016, vol. 33, pp. 67–73. doi: 10.1016/j.mib.2016.06.008.
11. Bertolini G., Rossi F., Valduga G., Jori G., et al. Photosensitizing activity of water- and lipid-soluble phthalocyanines on Escherichia coli, *FEMS Microbiol Lett*, 1990, vol. 71, no. 1–2, pp. 149–155. doi: 10.1111/j.1574-6968.1990.tb03814.x.
12. Nitzan Y., Gutterman M., Malik Z., Ehrenberg B. Inactivation of Gram-Negative Bacteria By Photosensitized Porphyrins, *Photochem Photobiol*, 1992, vol. 55, no. 1, pp. 89–96. doi: 10.1111/j.1751-1097.1992.tb04213.x.
13. Merchat M., Bertolini G., Giacomini P., Villanueva A., et al. Meso-substituted cationic porphyrins as efficient photosensitizers of gram-positive and gram-negative bacteria, *J Photochem Photobiol B Biol*, 1996, vol. 32, no. 3, pp. 153–157. doi: 10.1016/1011-1344(95)07147-4.
14. Kustov A. V., Kustova T. V., Belykh D. V., Khudyaeva I. S., et al. Synthesis and investigation of novel chlorin sensitizers containing the myristic acid residue for antimicrobial photodynamic therapy, *Dye Pigment*, 2020, vol. 173, p. 107948. doi: 10.1016/j.dyepig.2019.107948.
15. Ryazanova O., Voloshin I., Dubey I., Dubey L., et al. Fluorescent Studies on Cooperative Binding of Cationic Pheophorbide-a Derivative to Polyphosphate, *Ann NY Acad Sci*, 2008, vol. 1130, no. 1, pp. 293–299. doi: 10.1196/annals.1430.033.
16. Miyatake T., Hasunuma Y., Mukai Y., Oki H., et al. Assemblies of ionic zinc chlorins assisted by water-soluble polypeptides, *Bioorganic Med Chem*, 2016, vol. 24, no. 5, pp. 1155–1161. doi: 10.1016/j.bmc.2016.01.054.
17. Kustov A. V., Belykh D. V., Smirnova N. L., Venediktov E. A., et al. Synthesis and investigation of water-soluble chlorophyll pigments for antimicrobial photodynamic therapy, *Dye Pigment*., 2018, vol. 149, pp. 553–559. doi: 10.1016/j.dyepig.2017.09.073.
18. Morshnev P. K., Kustov A. V., Drondel E. A., Khlydeev I. I., et al. The interaction of chlorin photosensitizers for photodynamic therapy with blood transport proteins, *J Mol Liq*, 2023, vol. 390, p. 123116. doi: 10.1016/j.molliq.2023.123116.
19. Brusov S. S., Koloskova Y. S., Grin M. A., Tiganova I. G., et al. New cationic purpurinimide for photodynamic inactivation of Pseudomonas Aeruginosa biofilms, *Russ Biother J*, 2014, vol. 13, no. 4, pp. 59–63.
20. Suvorov N. V., Cheskov D. A., Mironov A. F., and Grin M. A. Inverse electron demand Diels–Alder reaction as a novel method for functionalization of natural chlorins, *Mendeleev Commun*, 2019, vol. 29, no. 2, pp. 206–208. doi: 10.1016/j.mencom.2019.03.031.
21. Garcia de Carvalho G., Pacheco Mateo R., Costa e Silva R., Maquera Huacho P. M., et al. Chlorin-based photosensitizer under blue or red-light irradiation against multi-species biofilms related to periodontitis, *Photodiagnosis Photodyn Ther*, 2023, vol. 41, p. 103219. doi: 10.1016/j.pdpdt.2022.103219.
22. Yang W., Yoon Y., Lee Y., Oh H., et al. Photosensitizer-peptoid conjugates for photoinactivation of Gram-negative bacteria: structure-activity relationship and mechanistic studies, *Org Biomol Chem*, 2021, vol. 19, no. 29, pp. 6546–6557. doi: 10.1039/d1ob00926e.

todynamic therapy // Int. J. Mol. Sci. – 2021. – Vol. 22, № 12. – P. 6392. doi: 10.3390/ijms22126392.
6. de Souza da Fonseca A., de Paoli F., Mencalha A.L. Photodynamic therapy for treatment of infected burns // Photodiagnosis Photodyn. Ther. – 2022. – Vol. 38. – P. 102831. doi: 10.1016/j.pdpdt.2022.102831.
7. Huang L., Xuan Y., Koide Y., Zhiyentayev T., Tanaka M., et al. Type i and Type II mechanisms of antimicrobial photodynamic therapy: An in vitro study on gram-negative and gram-positive bacteria // Lasers Surg. Med. – 2012. – Vol. 44, № 6. – P. 490-499. doi: 10.1002/lsm.22045.
8. Bustamante V., Palavecino C.E. Effect of photodynamic therapy on multidrug-resistant Acinetobacter baumannii: A scoping review // Photodiagnosis Photodyn. Ther. – 2023. – Vol. 43. – P. 103709. doi: 10.1016/j.pdpdt.2023.103709.
9. Jao Y., Ding S.J., Chen C.C. Antimicrobial photodynamic therapy for the treatment of oral infections: A systematic review // J. Dent. Sci. – 2023. – Vol. 18, № 4. – P. 1453-1466. doi: 10.1016/j.jds.2023.07.002.
10. Hamblin M.R. Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes // Curr. Opin. Microbiol. – 2016. – Vol. 33. – P. 67-73. doi: 10.1016/j.mib.2016.06.008.
11. Bertolini G., Rossi F., Valduga G., Jori G., van Lier J. Photosensitizing activity of water- and lipid-soluble phthalocyanines on Escherichia coli // FEMS Microbiol. Lett. – 1990. – Vol. 71, № 1–2. – P. 149–155. doi: 10.1111/j.1574-6968.1990.tb03814.x.
12. Nitzan Y., Gutterman M., Malik Z., Ehrenberg B. Inactivation of Gram-Negative Bacteria By Photosensitized Porphyrins // Photochem. Photobiol. – 1992. – Vol. 55, № 1. – P. 89-96. doi: 10.1111/j.1751-1097.1992.tb04213.x.
13. Merchat M., Bertolini G., Giacomini P., Villanueva A., Jori G. Meso-substituted cationic porphyrins as efficient photosensitizers of gram-positive and gram-negative bacteria // J. Photochem. Photobiol. B Biol. – 1996. – Vol. 32, № 3. – P. 153-157. doi: 10.1016/1011-1344(95)07147-4.
14. Kustov A. V., Kustova T. V., Belykh D. V., Khudyaeva I.S., Berezin D.B. Synthesis and investigation of novel chlorin sensitizers containing the myristic acid residue for antimicrobial photodynamic therapy // Dye. Pigment. – 2020. – Vol. 173. – P. 107948. doi: 10.1016/j.dyepig.2019.107948.
15. Ryazanova O., Voloshin I., Dubey I., Dubey L., Zozulya V. Fluorescent Studies on Cooperative Binding of Cationic Pheophorbide-a Derivative to Polyphosphate // Ann. N. Y. Acad. Sci. – 2008. – Vol. 1130, № 1. – P. 293-299. doi: 10.1196/annals.1430.033.
16. Miyatake T., Hasunuma Y., Mukai Y., Oki H., Watanabe M., et al. Assemblies of ionic zinc chlorins assisted by water-soluble polypeptides // Bioorganic Med. Chem. – 2016. – Vol. 24, № 5. – P. 1155-1161. doi: 10.1016/j.bmc.2016.01.054.
17. Kustov A. V., Belykh D. V., Smirnova N.L., Venediktov E.A., Kudayarova T. V., et al. Synthesis and investigation of water-soluble chlorophyll pigments for antimicrobial photodynamic therapy // Dye. Pigment. – 2018. – Vol. 149. – P. 553-559. doi: 10.1016/j.dyepig.2017.09.073.
18. Morshnev P.K., Kustov A. V., Drondel E.A., Khlydeev I.I., Abramova O.B., et al. The interaction of chlorin photosensitizers for photodynamic therapy with blood transport proteins // J. Mol. Liq. – 2023. – Vol. 390. – P. 123116. doi: 10.1016/j.molliq.2023.123116.
19. Brusov S.S., Koloskova Y.S., Grin M.A., Tiganova I.G., Pagina O.E., et al. New cationic purpurinimide for photodynamic inactivation of Pseudomonas Aeruginosa biofilms // Russ. Biother. J. – 2014. – Vol. 13, № 4. – P. 59-63.
20. Suvorov N. V., Cheskov D.A., Mironov A.F., Grin M.A. Inverse electron demand Diels–Alder reaction as a novel method for functionalization of natural chlorins // Mendeleev Commun. – 2019. – Vol. 29, № 2. – P. 206-208. doi: 10.1016/j.mencom.2019.03.031.
21. Garcia de Carvalho G., Pacheco Mateo R., Costa e Silva R., Maquera Huacho P.M., de Souza Rastelli A.N., et al. Chlorin-based photosensitizer under blue or red-light irradiation against multi-species biofilms related to periodontitis // Photodiagnosis Photodyn. Ther. – 2023. – Vol. 41. – P. 103219. – doi: 10.1016/j.pdpdt.2022.103219.
22. Yang W., Yoon Y., Lee Y., Oh H., Choi J., et al. Photosensitizer-peptoid conjugates for photoinactivation of Gram-negative bacteria: structure-activity relationship and mechanistic studies // Org. Biomol. Chem. – 2021. – Vol. 19, № 29. – P. 6546-6557. doi: 10.1039/d1ob00926e.