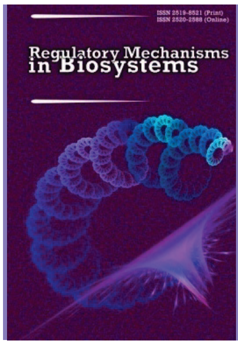

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The effect of low-intensity laser radiation on the sensitivity of *Staphylococcus aureus* to some halogen-containing azaheterocycles

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The rapid rate of acquisition and spread of resistance to antibiotics by microorganisms leads to the search and investigation of new ways of combating infectious agents. Being a modern, minimally invasive method with an almost complete absence of side effects, the use of low-intensity laser radiation can be considered as one of the alternatives to traditional antibiotic therapy. At the same time, the search for new chemical compounds with pronounced antimicrobial activity is also promising. The impact of low-intensity laser of the red spectrum (λ = 660 nm) on the sensitivity of a clinical isolate of *Staphylococcus aureus* to newly synthesized halogen- and chalcogen-halogen-containing derivatives of the quinazoline and benzothiazole series was studied using the serial microdilutions method with the determination of minimal inhibitory and minimal bactericidal concentrations. To evaluate the antimicrobial properties of the investigated substances in dynamic, we investigated their activity two months after the synthesis with subsequent comparison to freshly synthesized compounds. The research results indicate that the most pronounced antimicrobial effect was shown by trichlorotelluromethylthiazoloquinazolinium chloride and propargylthiobenzothiazolium hexachlorotellurate. Evaluating the dynamics of the activity of the studied compounds, it was noted that the absolute majority of substances retained their properties, which indicates their stability. When evaluating the effect of irradiation of microbial inoculum with low-intensity laser radiation on the susceptibility to chemical compounds, an increase in the sensitivity of irradiated microorganisms to some investigated chemicals was noted compared to similar non-irradiated microbial suspensions. In the case of 2 out of 9 studied chemical compounds we noted a 2- to 4-fold decrease in the minimal inhibitory concentration for irradiated microbial suspensions. A decrease in the minimal bactericidal concentration after irradiation was noted for one of the substances. In the cases of butynylthiobenzothiazolium hexabromotellurate, and tribromotelluromethylthiazoloquinazolinium bromide, decrease of both minimal inhibitory and minimal bactericidal concentrations in the irradiated inoculum was observed. The above shows that low-intensity laser radiation under certain parameters increases the susceptibility of microorganisms to antimicrobial agents.

Keywords: opportunistic microorganisms; low-intense laser radiation; thiazoloquinazolin; benzothiazol; antibiotic resistance.

Introduction

The discovery of antibiotics made it easy and effective to treat bacterial infections, and since then antibiotics have had a huge impact on human health and life expectancy (MacLean & San Millan, 2019). But overuse of antibiotics has caused development of multiple mechanisms of resistance for antibacterial drugs used in clinical, agricultural, veterinarian or otherwise practices (Zazharskyi et al., 2019; Berezhna et al., 2021; Vashchenko et al., 2021; Dementieieva et al., 2022). Rapid spread of antibiotic resistance takes place due to the extraordinary genetic capacities of horizontal gene transmission among microbes (Davies & Davies, 2010). This, in turn, leads to the loss of the efficacy of antibiotics, and worsening in treatment results (Rossolini et al., 2014). Besides, expansion of resistant strains has the potential to alter bacterial population genetics at local and global levels (Hernando-Amado et al., 2019).

Staphylococcus aureus is a pathogenic microorganism that causes a variety of diseases ranging from local skin damage to life-threatening toxigenic infections (Otto, 2014). Despite being naturally susceptible to most antibiotics, *S. aureus* has considerable ability to develop resistance to any antibiotic to which it has been exposed (Foster, 2017). The emergence and spread of methicillin-resistant and vancomycin-resistant *S. aureus* has led to the serious review of antimicrobial therapy and development of new agents and approaches to prevent and overcome drug resistance (Rehm & Tice, 2010). Efforts to develop new antimicrobial drugs over the past two decades have been woefully behind the rapid evolution of resistance genes developing among both gram-positive and gram-negative pathogens (Gootz, 2010). To combat antibiotic resistant infections, alternative therapies such as stem cell-AMPs, CRISPR-Cas, probiotics, nanobiotics, antimicrobial photodynamic therapy, etc. (Pantyo et al., 2020, Kumar et al., 2021) should be explored. It is also promising to search for novel compounds which are active against multidrug-resistant pathogens (Xu et al., 2019).

Laser therapy is widely used in the treatment and diagnosis of diseases and various medical fields (Khalkhal et al., 2020), particularly for the treatment of nervous system complications; skin and mucosal disorders, cancers, (Mansouri et al., 2020), inflammation process resolution improvement, pain relief (de Souza da Fonseca et al., 2021), various branches of dentistry (Rajan & Muhammad, 2021). Low-intense laser radiation is also used in the tissue repair processes (Loreti et al., 2015). The mechanism of beneficial effects of low-level laser radiation on biological objects is based on the increase of cellular viability, proliferation rate, as well as DNA integrity, and the repair of damaged DNA (Mussttaf et al., 2019).

A promising direction of using low-intensity laser radiation is to consider it as an alternative to traditional antibiotic therapy (Barbieri et al., 2019). Due to the almost complete absence of side effects on the human body, it is quite noteworthy to study the effect of low-intensity laser radiation on various properties of opportunistic microorganisms and the further possibility of its use in complex therapy of infectious diseases. The purpose of the work was to study the antimicrobial activity of some newly synthesized halogen-containing azaheterocycles and the impact of low-intense laser radiation with red spectrum on the sensitivity of *Staphylococcus aureus* to the specified compounds.

Material and methods

The influence of low-intensity laser radiation (LILR) of the red spectrum on the sensitivity of a clinical isolate of *Staphylococcus aureus* to halogen- and chalcogen-halogen-containing derivatives of the quinazoline and benzothiazole series was studied. The specified microorganism was isolated from a patient with chronic generalized periodontitis. Identification of the isolated strain was carried out according to generally accepted methods using bacterioscopic and bacteriological methods. For the final identification StaphyTest 16 (by Erba Lachema, Czech Republic) test system was used. The investigated substances are halogen- and chalcogen-containing salts of allylthiazoloquinazolinium (1–4), thiazolobenzothiazolinium (9) and alkynylbenzothiazolium (5–8), which contain halogen and chalcogen atoms both covalently bound and ionic (Fig. 1). The synthesis of such salts was carried out in acetic acid from the corresponding alkenyl and alkynyl thioethers of 4-allylquinazoline or benzothiazole by the action of molecular (iodine), hybrid halogens (iodine bromide) or selenium and tellurium dioxides in the corresponding halide acids. The composition and structure of all studied salts were proven by NMR spectroscopy (^{1}H and ^{13}C NMR) and elemental analysis.

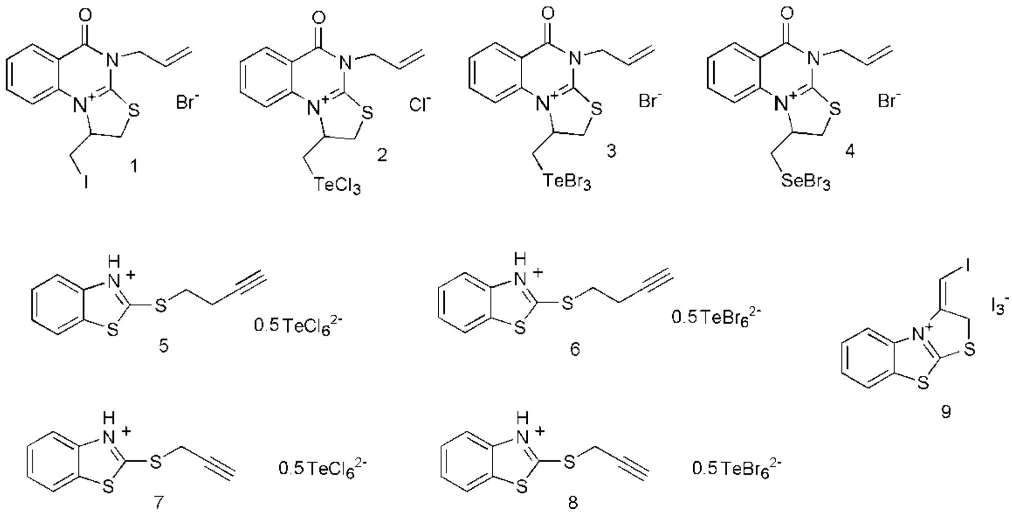


Fig. 1. Structural formulas of the substances

To determine the antimicrobial activity of the investigated substances by the screening method, a standardized suspension of microorganisms was prepared (16–24 hour agar culture brought to a McFarland density standard of 0.5 in Mueller-Hinton broth and diluted 100 fold with sterile broth), which was added into Eppendorf tubes with appropriate substances. To determine the optical density of the microbial inoculum the densitometer (DEN-1, Biosan, Latvia, 2016) was used. The ratio of substances and microbial inoculum volumes was 1:1 (0.1 mL each). The initial dilution of chemicals was 1000 μg/mL, thus after dilution with bacterial suspension it became 500 μg/mL. Subsequently, the test tubes were incubated in a thermostat at 37 degrees for 24 hours. The growth of microorganisms was determined visually (turbidity of the medium) and by inoculating the contents of Eppendorf tubes onto Petri dishes with solid nutrient medium (meat peptone agar). The absence of growth indicated the antimicrobial activity of the studied substances.

For the quantitative study of antimicrobial activity, the method of serial dilutions in a liquid nutrient medium was used. A series of dilutions of chemicals in Mueller-Hinton broth was prepared according to the following scheme. 0.1 mL of sterile broth was added to each Eppendorf tube. Next, 0.1 mL of the examined substance was mixed thoroughly into the first test tube, and 0.1 mL was transferred to the next one. The contents of the second tube were also thoroughly mixed and 0.1 mL was transferred to the next Eppendorf tube. The number of such Eppendorf tubes was 5. In this way, a series of two-fold dilutions of substances from 500 to 31.25 μg/mL was obtained. After that, a standardized inoculum (analogous to the screening method) of the studied strain of *S. aureus* was added into test tubes, and cultivated in the thermostat at 37 degrees for 24 hours. Thus, the final dilutions of substances were 250–15.625 μg/mL. The growth of microorganisms was determined similarly to screening studies. Besides for each investigated chemical, two control test tubes were used – positive control that contained 0.1 mL of sterile broth with 0.1 mL of standardized inoculum and negative control with 0.1 mL of sterile broth and 0.1 mL of appropriate chemical substance. The goal of quantitative studies was to determine the minimum inhibitory concentration (MIC) – the highest dilution of a substance (i.e., the lowest concentration) that inhibits the visible growth of microorganisms and the minimum bactericidal concentration (MBC) – the highest dilution of a substance that completely inhibits the growth of microorganisms. In this way, the classical method of serial microdilutions in a liquid nutrient medium, described in the literature, was used (Syal et al., 2017).

To study the effect of LILR on the sensitivity of the *S. aureus* strain to the investigated chemical compounds, the standardized inoculum was irradiated before it was introduced into the test tubes. The sources of low-intensity laser radiation were the certified devices "LIKA-Therapist" ("Fotonika Plus", Cherkasy, Ukraine, 2010) and "Medic-2K" ("Fotonika Plus", Cherkasy, Ukraine, 2012).

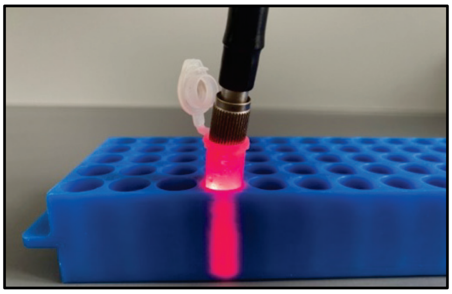
Irradiation of the inoculum by LILR of the "LIKA-therapist" laser was carried out in an Eppendorf tube with the help of a single-fiber quartz-polymer light guide (Fig. 2a). Irradiation by LILR of the "Medic 2K" laser was carried out in sterile Petri dishes with a diameter of 50 mm from a distance of 50 cm, the scanning figure is "a circle that converges to a point" (Fig. 2b). In both cases, continuous radiation of the red spectrum was used at a power density of 50 mW/cm2. The duration of the exposure was 5 minutes; the volume of the irradiated inoculum was 1 mL.

After irradiation, the microbial inoculum was introduced into test tubes with dilutions of the studied substances. In this way, the antimicrobial activity of the substances was simultaneously investigated in relation to the non-irradiated microbial suspension (control), the inoculum irradiated by the "LIKA-therapist" laser and the inoculum irradiated by the scanning laser radiation of the "Medic 2K" laser devices.

To determine the antimicrobial activity of investigated substances in dynamic, we examined their MIC and MBC 2 months after the synthesis and compared obtained data with freshly synthesized substances.

Results

The data of the screening studies show that all the tested substances at a concentration of 500 μg/mL completely inhibited the growth of the clinical isolate of *S. aureus*. Therefore, quantitative studies were conducted with all investigated halogen- and chalcogen-halogen-containing derivatives of the quinazoline and benzothiazole series. The results of quantitative studies of the antimicrobial activity of substances (freshly synthesized and 2 months after the synthesis), as well as the effect of irradiation of *S. aureus* by LILR on its sensitivity to these substances are presented in Table 1.



a

b

Fig. 2. Irradiation of microbial inoculum in Eppendorf tube by low-intense laser radiation with the fiber-optic tip of "LIKA-therapist" device (*a*) and in 50 mm Petri dish by scanning low-intense laser radiation of "Medic 2K" devise (*b*)

The following substances showed the most pronounced antimicrobial activity: trichlorotelluromethylthiazoloquinazolinium chloride 2, propargylthiobenzothiazolium hexachlorotellurate 7. Their minimal inhibitory concentrations in control series (with non-irradiated inoculum) were 31.25 μg/mL. Intermediate antimicrobial activity showed iodomethylthiazoloquinazolinium bromide 1, tribromoselenomethylthiazoloquinazolinium bromide 4, MIC of which in control was 62.5 μg/mL. The least antimicrobial activity among the studied substances was noticed for butynylthiobenzothiazolium hexachlorotellurate 5, butynylthiobenzothiazolium hexabromotellurate 6, tribromotelluromethylthiazoloquinazolinium bromide 3, iodomethylidenthiazolobenzothiazolium triiodide 9, propargylthiobenzothiazolium hexabromotellurate 8.

Analyzing the dynamics of substance activity, we note that for most of the studied substances it did not change two months after synthesis, that is, it was identical to freshly synthesized compound. Only in cases of propargylthiobenzothiazolium hexachlorotellurate 7, and iodomethylidenthiazolobenzothiazolium triiodide 9 was a two-fold increase in MIC observed. Also for iodomethylthiazoloquinazolinium bromide 1 a two-fold decrease of both MIC and MBC was established compared to the freshly synthesized compound. The latter indicates a partial decrease in activity against the studied strain.

Evaluating the effect of irradiation of the microbial inoculum by LILR on sensitivity to the studied substances, its increase to iodomethylthiazoloquinazolinium bromide 1, trichlorotelluromethylthiazoloquinazolinium chloride 2, tribromotelluromethylthiazoloquinazolinium bromide 3, butynylthiobenzothiazolium hexabromotellurate 6, propargylthiobenzothiazolium hexachlorotellurate 7 was noted.

Thus irradiation of *S. aureus* inoculum by LILR of "LIKA-therapist" devise led to the decrease of both MIC and MBC of butynylthiobenzothiazolium hexabromotellurate 6 from 250 and 500 μg/mL in control to 125 and 250 μg/mL respectively; MBC of trichlorotelluromethylthiazoloquinazolinium chloride 2 decreased from 31.25 μg/mL in control to 15.62 μg/mL; MIC of tribromotelluromethylthiazoloquinazolinium bromide 3 decreased by 4 fold – from 250 μg/mL in control to 62.5 μg/mL in case of irradiated inoculum; MIC of propargylthiobenzothiazolium hexachlorotellurate 7 decreased from 31.25 μg/mL in control to 15.62 μg/mL after irradiation of *S. aureus* inoculum by LILR of "LIKA-therapist" laser.

After irradiation of *S. aureus* inoculum by LILR of "Medic 2K" devise, MIC of iodomethylthiazolo[3,2-a]quinazolinium bromide 1 decreased from 62.5 μg/mL in control to 31.25 μg/mL; both MIC and MBC of butynylthiobenzothiazolium hexabromotellurate 6 decreased from 250 and 500 μg/mL in control to 125 and 250 μg/mL for irradiated *S. aureus* inoculum respectively (similarly to inoculum irradiated by LILR of "LIKA-therapist" devise); MIC and MBC of tribromotelluromethylthiazoloquinazolinium bromide 3 reduced from 250 μg/mL to 62.5 and 125 μg/mL for inoculum irradiated by LILR of "Medic 2K" device; MIC of propargylthiobenzothiazolium hexachlorotellurate 7 decreased from 31.25 μg/mL in control to 15.625 μg/mL.

Table 1
Impact of low-intense laser radiation on the susceptibility of *Staphylococcus aureus* to tested substances

No.	Substance	MIC (control, μg/mL)	MBC (control, μg/mL)	MIC (LT, μg/mL)	MBC (LT, μg/mL)	MIC (LS, μg/mL)	MBC (LS, μg/mL)	MIC-2 (μg/mL)	MBC-2 (μg/mL)
1.	4-allyl-1-iodomethyl-5-oxo-1,2,4,5-tetrahydro[1,3]thiazolo[3,2-a]quinazolinium bromide	62.5	125	62.5	125	31.25	125	125	250
2.	4-allyl-5-oxo-1-[(trichloro-λ^4-tellanyl)methyl]-1,2,4,5-tetrahydro[1,3]thiazolo[3,2-a]quinazolinium chloride	31.25	31.25	31.25	15.62	31.25	31.25	31.25	31.25
3.	4-allyl-5-oxo-1-[(tribromo-λ^4-tellanyl)methyl]-1,2,4,5-tetrahydro[1,3]thiazolo[3,2-a]quinazolinium bromide	250	250	62.5	250	62.5	125	250	250
4.	4-allyl-5-oxo-1-[(tribromo-λ^4-selanyl)methyl]-1,2,4,5-tetrahydro[1,3]thiazolo[3,2-a]quinazolinium bromide	62.5	250	62.5	250	62.5	250	62.5	250
5.	2-(but-3-yn-1-ylthio)-1,3-benzothiazolium hexachlorotellurate	250	250	250	250	250	250	250	250
6.	2-(but-3-yn-1-ylthio)-1,3-benzothiazolium hexabromotellurate	250	500	125	250	125	250	250	500
7.	2-(prop-2-yn-1-ylthio)-1,3-benzothiazolium hexachlorotellurate	31.25	125	15.625	125	15.62	125	62.5	125
8.	2-(prop-2-yn-1-ylthio)-1,3-benzothiazolium hexabromotellurate	250	500	250	500	250	500	250	500
9.	3-(iodomethyliden)-2,3-dihydro[1,3]thiazolo[2,3-*b*][1,3]benzothiazolium triiodide	125	250	125	250	125	250	250	250

Note: numbers of substances correspond to their order in Fig. 1; MIC (control) – minimal inhibitory concentration for control (non-irradiated) *S. aureus* inoculum; MBC (control) – minimal bactericidal concentration for control (non-irradiated) *S. aureus* inoculum; MIC (LT) – minimal inhibitory concentration for *S. aureus* inoculum, irradiated by LILR of "LIKA-therapist" laser device; MBC (LT) – minimal bactericidal concentration for *S. aureus* inoculum, irradiated by LILR of "LIKA-therapist" laser device; MIC (LS) – minimal inhibitory concentration for *S. aureus* inoculum, irradiated by scanning LILR of "Medic 2K" laser device; MBC (LS) – minimal bactericidal concentration for *S. aureus* inoculum, irradiated by scanning LILR of "Medic 2K" laser device; MIC-2 – minimal inhibitory concentration of the substances 2 months after the synthesis; MBC-2 – minimal bactericidal concentration of the substances 2 months after the synthesis.

At the same time irradiation did not lead to any changes in susceptibility to tribromoselenomethylthiazoloquinazolinium bromide 4, butynylthiobenzothiazolium hexachlorotellurate 5, propargylthiobenzothiazolium hexabromotellurate 8, and iodomethylidenthiazolobenzothiazolium triiodide 9.

Discussion

The experimentally found antimicrobial activity of the studied compounds creates prerequisites for determining the "structure-activity" relationship. The most active are chlorotellurium-containing compounds of the quinazoline and benzothiazole series. Thus, trichlorotelluromethylthiazoloquinazolinium chloride 2, which contains a covalently bound trichlorotellurium fragment, proved to be an effective bactericide in cases of both control and series with irradiated inoculum, which was not observed for its bromotellurium-containing analogue. In contrast, propargylthiobenzothiazolium hexachlorotellurate 7 with an anionic chlorotellurate fragment has low MIC values in the control, which decrease upon irradiation. It should be noted that the length of the alkynyl substituent in benzothiazolinium salts significantly reduces antimicrobial activity.

In the literature, 4-methyl(phenyl) substituted thiazolo- and thiazinoquinazolinium salts are known, which have a high bactericidal and fungicidal effect against some gram-positive and gram-negative bacteria and fungi. The authors established the "structure-activity" relationship, showed the influence of the nature of the chalcogen, the type of substituents in the thiazoline and pyrimidine cycle on the biological activity of the studied thiazolo- and thiazinoquinazolines. Halides of 4-methyl-5-oxo-1-((trihalogenthelanyl)methylidene)-8-(trifluoromethyl)thiazolo[3,2-a]quinazolin-10-ium show the highest bactericidal activity against the gram-negative culture of *Escherichia coli* (Kut et al., 2023).

The increase of susceptibility of the examined strain to some chemicals can be explained by the fact that LILR stimulated the growth of microorganisms. Microorganisms are more susceptible to both chemical and physical factors during the period of active growth and division. Methods for determining the sensitivity of bacteria to antibiotics are based on this, in particular (Syal et al., 2017). A certain stimulation of the growth of microorganisms under the influence of short exposures to low-intensity radiation was described in our previous research (Pantyo et al., 2019), as well as in the works of some authors (Roos et al., 2013, de Souza da Fonseca et al., 2021). Exposure of nonphotosynthesizing microorganisms to light could increase cell division in cultures. That phenomenon is known as biostimulation (Roos et al., 2013).

Data about the antimicrobial properties of light (Pantyo et al., 2020, de Souza da Fonseca et al., 2021) in turn indicates its dose-dependent effect. Thus low doses of light stimulate growth of microorganisms while high doses inhibit them. Analyzing data about impact of different types of low-intense radiation such as laser, LED, microwave radiation (Mishra et al., 2013, Mussttaf et al., 2019, Pantyo et al., 2019, Pantyo et al., 2020) etc., can be asserted their athermal effect on different light forms, including microorganisms. Similar patterns of influence of various types of radiation on biological objects in general and microorganisms in particular gives reason to assert that the main factors that affect the properties of biological objects are power density and wavelength. At the same time polarization and coherence play secondary role.

Conclusions

The examined newly synthesized halogen- and chalcogen-halogen-containing derivatives of the quinazoline and benzothiazole series have pronounced antimicrobial activity against clinical isolate *Staphylococcus* aureus. The activity of most substances did not change two months after the synthesis, which indicates their stability and thus their potential for consideration as antimicrobial candidates. Low-intense laser radiation of the red spectrum impacts the biological properties of the investigated strain *S. aureus*. It causes an increase in its susceptibility to 5 out of 9 studied chemical compounds. Taking into account the proven positive role on the human body, as well as the effect on the sensitivity of *S. aureus* to antimicrobial agents, low-intensity laser radiation of the red spectrum can be used in the comprehensive therapy of purulent-inflammatory processes of staphylococcal etiology.

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