https://doi.org/10.18321/ectj929

# Purpurin Esters Containing a Saturated Cyclic Fragment with Antimicrobial Activity

**T.V. Kharlamova*, R.B. Seidakhmetova, K.D. Praliyev**

JSC "Institute of Chemical Sciences named after A.B. Becturov", 106 Ualikhanov str., 050010, Almaty, Kazakhstan

**Article info**

*Received:*
19 March 2019

*Received in revised form:*
15 May 2019

*Accepted:*
26 July 2019

***Keywords:***
Purpurin,
Acid chlorides of saturated cyclic carboxylic acids,
Antimicrobial activity.

**Abstract**

The present study is a continuation of the scientific research works for the synthesis of anthraquinone-containing derivatives with cyclic ring systems by the interaction of 1,2,4-trihydroxyanthraquinone (purpurin) with cyclic carboxylic acid chlorides. Series of purpurin esters containing a saturated cyclic fragment was studied for antibacterial activity about museum strains of microorganisms. The effects of these preparations *in vitro* about S*taphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and fungus *Candida albicans* ATCC 10231 were studied. It has been found that the compounds with the cyclohexane fragment have possess an antimicrobial activity with strains of microorganisms *Staphylococcus aureus* ATCC 6538 for which the minimum inhibitory concentration value was 31.25 μg/ml.

## 1. Introduction

The targeted search and creation of new, highly effective and safe medicines are one of the main and intensively developing areas of synthetic organic chemistry. The number of synthesized organic compounds is steadily growing because of the need to solve both fundamental and applied problems of chemistry. There is a need to expand the arsenal of affordable, reliable and effective drugs for the prevention and treatment of human diseases. Every year there is a need to create new drugs to cope with the new diseases and drug resistance. Every day, a person is faced with a significant number of microorganisms which include bacteria, viruses, fungi, which can cause various infectious diseases. According to the World Health Organization data, every year 17 million people in the world die from infectious diseases. Today infectious diseases occupy 3–4$^{th}$ place in the ranking of causes of death [1]. In modern medicine, antibacterial drugs play one of the leading roles. Since their use, they have revolutionized the treatment of many diseases. However, the formation of resistant strains of pathogens is observed. Nowadays, such factors as the emergence of multi-resistant forms, the emergence of new types of dangerous pathogens are becoming a global health problem [2–4] and determines the relevance of the search and creation of new antimicrobial agents.

Large pharmaceutical companies are increasingly abandoning work in the development of new antibiotics, the reason is the limitation of the circulation of these drugs, the high cost, and the long duration of clinical trials.

Therefore, now the main trend in the search for new biologically active substances with antibacterial properties is the screening of newly synthesized or isolated from natural sources of substances. Screening of newly synthesized compounds is an early approach in the search for new substances with antibacterial activity, which has several undeniable advantages – the ability to study a wide range of compounds, the simplicity of the methods used, and the rapid obtaining of a practical analysis result.

*Corresponding author. E-mail: kharlamovatv@mail.ru

Currently, much attention has been paid to ethnopharmacological studies [5]. The aim of these studies is directed to the search for antimicrobial agents among medicinal plants traditionally used in folk medicine of different countries and the study of the biological activity of natural compounds [6–10] and their chemically modified derivatives. To search for new antimicrobial agents, several secondary metabolites isolated from plants were studied, including alkaloids, terpenoids, phenolic acids, flavonoids, tannins, quinones [11, 12], etc. Moreover, anthraquinone derivatives are of particular interest, due to their diverse biological activity. Derivatives of 9,10-anthraquinone represent a large group of natural and synthetic quinones with great structural diversity and differences in chemical composition [13]. Currently, they are widely used in medicine and industry [14, 15]. These classes of compounds represent promising sources of antimicrobial agents [16].

## 2. Experimental part

### *2.1. Experimental chemical part*

#### *2.2.1. Materials and instrumentation*

The progress of the reaction and the purity of the obtained compounds were monitored by thin-layer chromatography (TLC) on Silufol UV-254 plates in various solvent systems. IR spectrum was recorded on a Nicolet 5700 spectrometer in KBr pellets. 1H NMR spectra were recorded on spectrometer MSL-400 «Bruker» with an operating frequency of 400 MHz for protons at room temperature. The melting point is determined on the device «Boetius». The reaction products were separated on silica gel of the Silica Gel 60 brand (Merck, Germany). Ultrasonic activation was carried out on a Cole-Parmer ultrasonic cleaner (100 W, 42 KHz). The reagents were used as received from commercial suppliers unless otherwise stated (Aldrich).

#### *2.2.2.The method of synthesis derivatives 4, 5*

Purpurin (1.2.4-trihydroxy-9.10-anthraquinone) (1) (0.001 mol) was dissolved in 50 ml of pyridine, 0.001–0.003 mol of the corresponding carboxylic acid chloride 2 or 3 was added. The reaction was carried out with vigorous stirring while heating the reaction mixture (30–65 °C) by monitoring the progress of the reaction using TLC. After the reaction (2–2.5 h), part of the solvent was removed, the resulting concentrated solution was treated with water, acidified with HCl, the precipitate was filtered and dried. The reaction products were isolated by silica gel column chromatography. It was suitable hexane with a gradual transition to a mixture of hexane-ethyl acetate (with a gradient from 100:1 to 1:5, v/v). Recrystallization was carried out from a mixture of hexane-ethyl acetate (1:5). The physical characterization and elemental analysis data are presented in Table 1.

**2-O-cyclopentanecarbonyloxy-1,4-dihydroxy-9,10-anthraquinone 4**.

IR spectrum (KBr, ν, $cm^{-1}$): 1763 (C=Os.), 1668, 1627 ($C=O_{anth}$), 1586 (C=C, Ar).

$^1$H NMR spectrum (DMSO-$d_6$, δ, ppm): 6.42 (s., H-3), 7.70 (m., H-6,7), 7.92 (m., H-5,8), 1,75 (m., 4H), 1,98 (m., 4H), 3.15 (m., 1H) ), 13.26 (s., α-OH), 13.31 (s., α-OH).

**2-O-cyclohexanecarbonyloxy-1,4-dihydroxy-9,10-anthraquinone 5.**

IR spectrum (KBr, ν, $cm^{-1}$): 1764 ($C=O_{s.}$), 1668 and 1626 ($C=O_{anth}$), 1586 (C=C, Ar).

$^1$H NMR spectrum (DMSO-$d_6$, δ, ppm): 6.42 (s., H-3), 7.69 (m., H-6,7), 7.91 (m., H-5,8), 1,47 (m., 2H), 1,59 (m., 6H), 1.88 (m., 2H), 2.66 (m., 1H), 13.26 (s., α-OH), 13.30 (s., α-OH).

### *2.2. Experimental biological part*

The compounds 4, 5 were studied for antibacterial activity with the museum strains of microorganisms, the effects of these preparations *in vitro* about *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and fungus *Candida albicans* ATCC 10231 were studied. The test strains used in the study were obtained from the American Type Culture Collection.

#### *2.2.1. Materials and methods of study*

Nutrient medium and chemicals:

- Nutrient agar M001 (HiMedia), Müller-Hinton broth AM5072;
- Müller-Hinton agar AM5071 (Accumix);
- NaCl (Reaktiv OJSC), ethyl alcohol.

Reference strains of microorganisms:

- *Staphylococcus aureus* ATCC 6538,
- *Bacillus subtilis* ATCC 6633,
- *Escherichia coli* ATCC 25922,
- *Pseudomonas aeruginosa* ATCC 27853,
- *Candida albicans* ATCC 10231.

The methods of study used:

- the Koch's method – determination of the viability of the museum strains, engaged in the experiment;
- an evaluation of physiological and biochemical properties of the museum strains, engaged in the experiment;
- a macro method of two-fold serial dilutions in the nutrient broth – determination of a minimum inhibitory concentration of the studied substance.

### *2.2.2. Justification of the chosen study flow chart*

The study model includes a necessary minimum of tests with different extent of susceptibility. These tests provide reliable and objective information on antimicrobial properties availability of the compounds studied in vitro [17]. All selected tests are adequate from the viewpoint of the results, obtained in the course of their performance. Besides, the study flow chart is carried out following the methodical recommendations and normative documents effective in the territory of Kazakhstan and approved by the State Pharmacological Committees of Kazakhstan [18].

### *2.2.3 Preparation of the museum strains for the study: reactivation, viability test and control of physiological and biochemical properties.*

Before the start of the experiment, the microorganisms have been reactivated (resuscitated), followed by subcultivation. The Koch's method [19] has been used during the experiment to determine the viability of the microorganisms. It has been established that all cultures possess good viability, exceeding $10^{11}$ CFU/ml.

Upon the control of a physiological and biochemical activity [20] it has been proved, that the cultures correspond to the systematic position, they are standard and have not changed their properties while storing.

During the study, 2-day agar cultures of microorganisms were used. They were obtained by serial dilution in Muller-Hinton broth. Also, suspensions of the tested microorganisms with a concentration of $10^8$ CFU/ml were used. Crops were incubated at 28 °C for 24–48 h. The results were taken into account by measuring the diameter (d, mm) of the growth suppression zone around the antibiotic paper disk. By serial dilution of the antibiotic in a nutrient medium, the minimum growth inhibitory concentration (MIC in units/ml or μg/ml) was determined for the test microorganism strain.

### *2.2.4. The study of antimicrobial activity*

The antimicrobial activity of the presented samples was studied in relation to strains of gram-positive bacteria such as *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, gram-negative strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and to *Candida albicans* ATCC 10231 yeast by diffusion into agar (wells). Comparison preparations: benzylpenicillin sodium salt for bacteria and nystatin for *Candida albicans* ATCC 10231 yeast.

The cultures were grown in a liquid medium pH 7.3 ± 0.2 at a temperature of 30 to 35 °C for 18–20 h. The cultures were diluted 1:1000 in a sterile 0.9% isotonic sodium chloride solution. After that, 1 ml was added to the cups with the corresponding elective, nutrient media for the studied test strains and seeded according to the "solid lawn" method. After drying, 6.0 mm wells were formed on the surface of the agar. A solution of the test samples, benzylpenicillin sodium salt of nystatin, was introduced into these wells. Equivolume dimethyl sulfoxide was used in the control. The test samples and the reference drug (benzylpenicillin sodium salt) were tested in quantities of 1 mg. Crops were incubated at 37 °C; counting of growing cultures was carried out after 24 h. The antimicrobial activity of the samples was evaluated by the diameter of the zones of growth inhibition of the test strains (mm).

Antibacterial activity was evaluated by measuring the growth of plants in Petri's cups. If the diameter of the growth inhibition zone was less than 10 mm, it was assessed as the absence of antibacterial activity, 10–15 mm – weak activity, 15–20 mm – moderately pronounced activity, over 20 mm – pronounced. Each sample was tested in three parallel experiments. Statistical processing was performed using parametric statistics methods with calculation of arithmetic mean and standard error.

### *2.2.5. Determination of the minimum inhibitory concentration (MIC)*

Evaluation of the minimum inhibitory concentration (MIC) in relation to the microorganisms, engaged in the experiment, has been carried out following the generally accepted method of two-fold serial dilutions in the Müller-Hinton [19, 21].

Suspensions of test strains at a concentration of $10^6$ CFU/ml were used for the serial dilution method. Primary suspensions of the test strains were

prepared in physiological saline (0.9% NaCl). A suspension of microorganisms test strains was prepared from diurnal cultures grown on agar at a temperature of 37 °C for 24 h. A test culture without a drug were served as a positive control (control 1). A dilutions of a drug without test cultures were used as a negative control (control 2). The antimicrobial activity of the presented compounds was studied at dilutions of samples in the range of 2–500 μg/ml. Freshly prepared suspension of microorganisms at a concentration of $10^6$ CFU/ml. After a series of dilutions, 0.05 ml of the microorganism test strain at a concentration of $10^6$ CFU/ml was added to all tubes. The procedure was repeated for all test cultures. All samples were incubated for 18–24 h at a temperature of 37 °C.

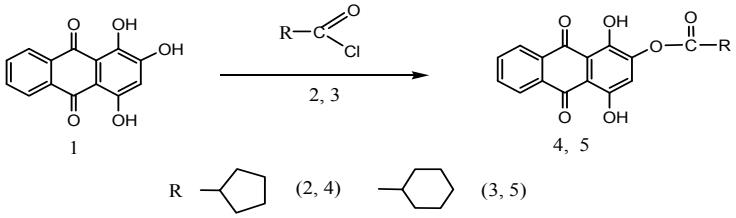
The results were evaluated visually by determining the presence or absence of growth in a medium containing different concentrations of the test compound. The last test tube of a series with growth retardation (clear broth) corresponds to the minimum inhibitory (bacteriostatic concentration) of the drug about this strain. The bactericidal concentration is determined by plating from the last 2 dilutions with no visible signs of growth on a solid nutrient medium – Mueller-Hinton agar to determine viable cells. After seeding, the plates were placed in a thermostat for 18–24 h, cultivation was carried out at a temperature of 37 °C. After the optimal incubation period for each microbial species, the incubation of the crops is noted as the lowest concentration of the substance in the test tube, from which seeding did not give growth. This concentration is taken as the minimum bactericidal concentration. All experiments were performed in three parallels.

## 3. Results and discussion

An interest in the chemistry of 9.10-anthraquinone derivatives has been aroused by the fact, that they have pharmacological activity and they are included in the structure of many natural and synthetic medicinal preparations. Compounds of particular interest were anthraquinone derivatives with different functional groups (carboxy, hydroxy, amino, etc.), whose presence causes different biological effects. Attention is drawn to the interaction of the chemical structure of a substance with its activity, which is an important stage of the directed synthesis of physiologically active preparations.

The present study is a continuation of the scientific research works for synthesis and of derivatives of 9,10-antraquinone derivatives [22–24] and is devoted to the study of the antimicrobial activity of purpurin derivatives containing cyclopentane- and cyclohexane fragments. Recently, there has been an increased interest in compounds containing fragments of saturated cyclic carboxylic acids in the structure due to biological and pharmaceutical applications [25, 26].

Purpurin (1.2.4-trihydroxy-9.10-anthraquinone) (1) is a biologically active anthraquinone derivative witch found in the roots of the madder (Rubia tinctorum) and the Asian madder (Rubia akane) [27]. For the synthesis of derivatives (4, 5) we have used purpurin (1) as the starting material:




Currently, to intensify and increase the efficiency of chemical reactions, various approaches are used, including various factors of physical impact. In recent years, great success has been achieved in the creation of effective ultrasound generators, and therefore there is an increased interest in the use of ultrasound to intensify various chemical presses [28, 29]. The use of ultrasound is one of the promising methods in the process of extraction of various biologically active substances from natural materials [30, 31]. Ultrasound has a significant effect on the speed and direction of reactions and, in some cases, the use of ultrasound improves the selectivity of chemical processes [29, 32, 33]. Synthesis of the compounds 4, 5 has been performed in one stage. To activate the process, ultrasound was used. The reaction mixture was heated to 65 °C. The synthesis with vigorous stirring and heating of the reaction mixture showed that the use of ultrasound reduces the synthesis time. So, if we use the raditional variant of the reaction, the synthesis time varies within 3–3.5 h, whereas during ultrasonic activation the

**Table 1**
Physical characterization of compounds

| Compound code | $R_f$ | Color | Molecular formula | Elemental analysis: Found, %; Calculated, % | |
|---|---|---|---|---|---|
| | | | | C | H |
| 4 | 0.68 (hexane-ethyl acetate, 2:1)<br>0.79 ($CCl_4$ - acetone, 7:3) | Orange | $C_{20}H_{16}O_6$ | 67.90<br>68.21 | 4.27<br>4.54 |
| 5 | 0.71 (hexane-ethyl acetate, 2:1)<br>0.80 ($CCl_4$ - acetone, 7:3) | Orange | $C_{21}H_{18}O_6$ | 68.44<br>68.88 | 4.66<br>4.92 |

process was completed within 2–2.5 h. The yield of products 4 and 5 was 56 and 54% without using ultrasound and 62 and 58% with ultrasonic activation, respectively. The physical and spectral characteristics of the compounds obtained in the traditional synthesis, as well as using ultrasonic treatment, were identical [34]. The physical characteristics and elemental analysis data are presented in Table 1.

The compounds 4, 5 were studied for antibacterial activity in relation to the museum strains of microorganisms. The effects of these preparations in vitro in relation to *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 were studied. The results of the study are presented in Table 2.

According the data from the Table 2 purpurin has a moderately pronounced antimicrobial activity in relation to the test strains of *Staphylococcus aureus* ATCC 6538, *Bacillus subtili*s ATCC 6633 and *Candida albicans* ATCC 10231. It has been found, that the compound (4) possess an antimicrobial activity in relation to museum strain of microorganisms *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, engaged in the experiment. The most active compound in this series of experiments was sample (5), for which a moderately pronounced antimicrobial activity was detected in relation to the test strain *Staphylococcus aureus* ATCC 6538 with a growth inhibition zone of (19 ± 0.1), as well as a moderate activity in relation to the test strain *Escherichia coli* ATCC 25922, which was not demonstrated by other test samples.

Also, samples (4,5) of derivatives of 9,10-anthraquinone were tested in order to determine the minimum inhibitory concentration (MIC). The results of the experiment are presented in Table 3.

**Table 2**
The results of studies of antimicrobial activity compounds

| Bacteria strains | Compound and size of growth retardation in diameter, mm | | | | |
|---|---|---|---|---|---|
| | 1 | 4 | 5 | Benzyl-penicillin sodium salt | Nystatin |
| *Staphylococcus aureus* ATCC 6538 | 17 ± 0.1 | 15 ± 0.1 | 19 ± 0.1 | 16 ± 0.1 | - |
| *Bacillus subtilis* ATCC 6633 | 13 ± 0.1 | 16 ± 0.1 | - | 15 ± 0.1 | - |
| *Escherichia coli* ATCC 25922 | - | - | 14 ± 0.1 | 15 ± 0.1 | - |
| *Pseudomonas aeruginosa* ATCC 27853 | - | - | - | 14 ± 0.1 | - |
| *Candida albicans* ATCC 10231 | 15 ± 0.1 | - | - | - | 21 ± 0.2 |

* «-» No activity

**Table 3**
The results of studies of MIC compounds

| Compound code | MIC, μg/ml | | | | |
|---|---|---|---|---|---|
| | *Staphylococcus aureus* ATCC 6538 | *Bacillus subtilis* ATCC 6633 | *Escherichia coli* ATCC 25922 | *Pseudomonas aeruginosa* ATCC 27853 | *Candida albicans* ATCC 10231 |
| 4 | 125 | 62,5 | - | - | - |
| 5 | 31.25 | - | 250 | - | - |

* «-» No activity

A study of the antimicrobial activity of the presented compounds (4, 5) about the reference strains: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and to the yeast fungus *Candida albicans* ATCC 10231, demonstrated that compound (4) showed a moderately pronounced antibacterial effect for the reference strain of *Bacillus subtilis* ATCC 6633, the MIC for this testing culture was 62.5 μg/ml. The activity of this sample was also observed to the *Staphylococcus aureus* ATCC 6538 strain, and the MIC value was 125 μg /ml.

The results of the study of the antimicrobial activity of the compound (5) showed the high efficiency of this compound with respect to the test strain *Staphylococcus aureu*s ATCC 6538 with MIC = 31.25 μg/ml. A comparative analysis showed a relatively lower antibacterial activity of the sample (5) for the *Escherichia coli* ATCC 25922 intestinal group bacteria. The MIC value for *Escherichia coli* was 250 μg/ml.

## 4. Conclusions

The current research is the first one that studies the antimicrobial activity of purpurin derivatives containing a cyclic carboxylic acid fragment. It was shown that purpurin and its derivatives (4,5) have activity for *Staphylococcus aureus* ATCC 6538. Compound (4), along with purpurin, was also active against *Bacillus subtilis* ATCC strains, and the presence of a cyclohexane fragment leads to activity against *Escherichia coli* ATCC 25922.

Among the presented synthesized compounds, the most active compound is a sample (5), which showed the highest antimicrobial activity against the *Staphylococcus aureus* ATCC 6538 strain, and its MIC value was 31.25 μg/ml. Due to the greatest antimicrobial effect, the compound - (5) may be promising for an additional in-depth study to identify specific antimicrobial activity in a wider range of microorganisms and to further develop a new domestic antimicrobial drug.

## Acknowledgments

The work was carried out under contract No. 83 (Appendix 1.7) dated March 2, 2018, with the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan as part of the project: “Search for new medicinal substances based on available synthetic analogues of natural derivatives of anthraquinone” (IRN AP05131788) and program number BR05234667/PCF.

## References

[1]. Global action plan on antimicrobial resistance (Editors: World Health Organization); 2015. ISBN: 9789241509763

[2]. I.A Rather, B-C. Kim, V.K. Bajpai, Y-H. Park, *Saudi J. Biol. Sci.* 24 (2017) 808–812. DOI: 10.1016/j.sjbs.2017.01.004

[3]. M.S. Morehead, C. Scarbrough, *Primary Care: Clinics in Office Practice* 45 (2018) 467–484. DOI: 10.1016/j.pop.2018.05.006

[4]. Antimicrobial resistance surveillance in Europe. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2012. European Centre for Disease Prevention and Control: Introduced 2013, Stockholm, 208 p. DOI: 10.2900/93403

[5]. D.G. Brown, T. Lister, T.L. May-Dracka, *Bioorg. Med. Chem. Lett.* 24 (2014) 413–418. DOI: 10.1016/j.bmcl.2013.12.059

[6]. S. Mickymaray, M. Saleh Al Aboody, P.K. Rath, P. Annamalai, T. Nooruddin, *Asian Pac. J. Trop. Biomed*. 6 (2016) 185–191. DOI: 10.1016/j.apjtb.2015.12.005

[7]. A. Sharma, R.C. Flores-Vallejo, A. Cardoso-Taketa, M.L. Villarreal, *J. Ethnopharmacol*. 208 (2017) 264–329. DOI: 10.1016/j.jep.2016.04.045

[8]. M. Vambe, A.O. Aremu, J.C. Chukwujekwu, J.F.Finnie, J. Van Staden, *S. Afr. J. Bot.* 114 (2018) 250–259. DOI: 10.1016/j.sajb.2017.11.011

[9]. J.J. Nair, A. Wilhelm, S.L. Bonnet, J. Staden, *Bioorg. Med. Chem. Lett.* 27(2017) 4943–4951. DOI: 10.1016/j.bmcl.2017.09.052

[10]. I. Gutiérrez-del-Río, J. Fernández, F. Lombó, *Int. J. Antimicrob. Ag.* 52(2018) 309–315. DOI: 10.1016/j.ijantimicag.2018.04.024

[11]. S. Gibbons, *Planta Med.* 74 (2008) 594–602. DOI: 10.1055/s-2008-1074518

[12]. R.S. Santhosh, B. Suriyanarayanan, *Planta Med.* 80 (2014) 9–21. DOI: 10.1055/s-0033-1350978

[13]. R.H. Thomson, Naturally Occurring Quinones III. Chapman and Hall, New York, 1987, p. 345–526.

[14]. V.Ya. Fajn 9,10 - Antrahinony i ih primenenie. [9,10 - Anthraquinones and their application.] Centr fotohimii RAN, Moskva, 1999, 92 p. (in Russian).

[15]. Encyclopedia of Medicines 2017. RLS. Vypusk 25. (Ed.: G.L. Vyshkovskogo). Vedanta, Moskva, 2016, 1288 s. (in Russian).

[16]. T.V. Kharlamova, *Chemical Journal of Kazakhstan* [Khimicheskij zhurnal Kazahstana], 4 (2018) 185–215 (in Russian).

[17]. Guidance for experimental (pre-clinical) study of new pharmacological substances, the RF Ministry of Health»], «IIA Remedium», Moscow, 2000, 679 p. (in Russian).
[18]. Pre-clinic tests of medicinal preparations (Methodological recommendations). The Republic of Kazakhstan State Pharm. Committee, Almaty, 1997, 22 p. (in Russian)
[19]. N.S. Yegorov, A guide to practical lessons in microbiology. Guidance for practical training in microbiology. 3rd edition, M.: Moscow State University, 1995, p. 224. (in Russian).
[20]. J. Jolt, N. Crieg, P. Smith, J. Staily, S. Williams. Opredelitel' bakterij Berdzhi [The Berji bacteria determinant] In 2 volumes. v.1: Translated from English. M.: Mir, 1997, p. 432.
[21]. CLSI M07-A9 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard – Ninth Edition. 2012, V. 32 (2) p. 88.
[22]. T.V Kharlamova, *Chem. Nat. Compd.* 43 (2007) 391–394. DOI: 10.1007/s10600-007-0146-6
[23]. T.V. Kharlamova, *Chem. Nat. Compd.* 45 (2009) 629–631. DOI: 10.1007/s10600-009-9443-6
[24]. T.V. Kharlamova, *Chem. Nat. Compd.* 45 (2009) 500–503. DOI: 10.1007/s10600-009-9398-7
[25]. K.A. Kumar, *International Journal of Pharmacy and Pharmaceutical Sciences* 5 (2013) 467-472.
[26]. A. Kleemann, J. Engel Pharmaceutical Substances: Syntheses, Patents, Applications. Thieme, 2001, 2454 p.
[27]. R. Singh, Geetanjali, C.S.M. Chauhan, *Chem. Biodivers.* 1 (2004) 1241–1264. DOI: 10.1002/cbdv.200490088
[28]. E.A. Prutenskaya, E.M. Sul'man, M.G. Sul'man, E.V. Selivanova The use of ultrasound in chemistry and biotechnology. Izd-vo TGTU, Tver', 2011, 92 p. (in Russian).
[29]. Sonam V. Sancheti, Parag R. Gogate, *Ultrason. Sonochem.* 36 (2017) 527–543. DOI: 110.1016/j.ultsonch.2016.08.009
[30]. S.R. Shirsath, S.H. Sonawane, P.R. Gogate, *Chemical Engineering and Processing: Process Intensification* 53 (2012) 10–23. DOI: 10.1016/j.cep.2012.01.003
[31]. C. Wen, J. Zhang, H. Zhang, C. Sedem, D. Manyakara, Z.Y. Duan, H. Ma, X. Luo, *Ultrason. Sonochem.* 48 (2018) 538–549. DOI: 10.1016/j.ultsonch.2018.07.018
[32]. C.M.R. Low, *Ultrason. Sonochem.* 2 (1995) S153-S163. DOI: 10.1016/1350-4177(95)00017-Z
[33]. N.K. Rastogi, *Crit. Rev. Food Sci.* 51 (2011) 705–722. DOI: 10.1080/10408391003770583
[34]. T.V. Kharlamova, R.B. Seidakhmetova, K.D. Praliyev, *Chem. Nat. Compd.* 55 (2019) 622–625. DOI: 10.1007/s10600-019-02763-y