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ANTIMICROBIAL ACTIVITY STUDY OF NEW QUINAZOLIN-4(3H)-ONES AGAINST *STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS PNEUMONIAE*

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Quinazolin-4(3H)-one derivatives exhibiting a wide spectrum of a pharmacological activity, represent a promising class of substances used to obtain antibacterial agents, which is especially important in the context of the emergence of pathogenic microorganisms' resistance to drugs used in medicine. It has been proved that compounds having a naphthyl radical in the molecule, as well as an amide group bound to the benzene ring as quinazolinone substituents, are characterized by a pronounced antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae.*

The aim of the research is a primary microbiological screening of the *in vitro* antimicrobial activity of new quinazolin-4(3H)-one derivatives against *Staphylococcus aureus* and *Streptococcus pneumoniae*, as well as the assessment of the relationship between the pharmacological effect and the structural transformation of the substance molecule, lipophilicity and the possibility of forming resistance to them.

Materials and methods. The experimental studies have been carried out using well-known nosocomial pathogens of infectious and inflammatory diseases *Staphylococcus aureus* and *Streptococcus pneumoniae* by a serial dilution method.

Results. A compound containing a naphthyl radical in its structure, which contributes to an increase in the hydrophobicity of the substance and its solubility in the membrane of a bacterial cell, has a bacteriostatic effect against both *Staphylococcus aureus* and *Streptococcus pneumoniae.* A similar pharmacological effect is exhibited by a derivative with an amide group as a substituent of the quinazolinone nucleus linked to a phenyl radical, which probably contributes to an increase in the degree of binding to active sites of enzymes involved in the DNA replication, and protein synthesis. Obviously, the increased lipophilicity, which promotes better binding to the efflux protein, cannot serve as objective characteristics of the emergence possibility of the pathogen's resistance to this substance.

Conclusion. Among the synthesized compounds, the leading substances that exhibit an antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pneumonia,* have been identified. The assessment of the chemical structure made it possible to substantiate their pharmacological action and draw conclusions about the possibility of developing resistance to it in microbial cells.

Keywords: quinazolinone derivatives; antimicrobial activity; lead-compound; electron-donating centers; enzyme active site; minimum inhibitory concentration; minimum suppressing concentration; bacteriostatic action; bactericidal activity; resistance; ATP-dependent efflux pump; plasmids; transposones; large mobile element

Abbreviations: PBP – penicillin-binding protein; MRSA – methicillin-resistant *Staphylococcus aureus*; PBP2a – penicillin-binding protein; ATP – adenosine triphosphate, MIC – minimum inhibitory concentration; DMSO – dimethyl sulfoxide; DMF – dimethylformamide; MIB – meat infusion broth; MIA – meat infusion agar; AC – atypical colonies; TC – typical colonies; NMR – nuclear magnetic resonance; TLC – thin layer chromatography; NA – nucleic acid; FnBPs – fibronectin-binding proteins

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ИЗУЧЕНИЕ АНТИМИКРОБНОЙ АКТИВНОСТИ НОВЫХ ХИНАЗОЛИН-4(3*H*)-ОНОВ ПО ОТНОШЕНИЮ К *STAPHYLOCOCCUS AUREUS* И *STREPTOCOCCUS PNEUMONIAE*

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Производные хиназолин-4(3*H*)-она, проявляющие широкий спектр фармакологической активности, представляют перспективный класс веществ, используемых для получения антибактериальных средств, что особенно актуально в условиях возникновения резистентности патогенных микроорганизмов к используемым в медицине лекарственным препаратам. Доказано, что соединения, имеющие в молекуле нафтильный радикал, а также амидную группу, связанную с бензольным кольцом, в качестве заместителей хиназолинона, характеризуются выраженной противомикробной активностью в отношении *Staphylococcus aureus* и *Streptococcus pneumoniae.*

Цель. Первичный микробиологический скрининг антимикробной активности *in vitro* новых производных хиназолин-4(3*H*)-она по отношению к *Staphylococcus aureus* и *Streptococcus pneumoniae*, а также оценка взаимосвязи между проявляемым фармакологическим действием и структурным преобразованием молекулы вещества, липофильностью и возможностью формирования устойчивости к ним.

Материалы и методы. Экспериментальные исследования были выполнены с использованием общеизвестных нозокомиальных возбудителей инфекционно-воспалительных заболеваний *Staphylococcus aureus* и *Streptococcus pneumoniae* методом серийных разведений.

Результаты. Соединение, содержащее в структуре нафтильный радикал, вносящий вклад в увеличение гидрофобности вещества и его растворимости в мембране бактериальной клетки, обладает бактериостатическим действием как в отношении *Staphylococcus aureus*, так и к *Streptococcus pneumoniae*. Сходный фармакологический эффект проявляет производное с амидной группой в качестве заместителя хиназолинонового ядра, связанной с фенильным радикалом, которая, вероятно, способствует увеличению степени связывания с активными сайтами ферментов, принимающих участие в процессах репликации ДНК и синтеза белков. Очевидно, повышенная липофильность, способствующая лучшему связыванию с белком оттока, не может служить объективной характеристикой возможности возникновения резистентности патогенов к данному веществу.

Заключение. Среди синтезированных соединений были выявлены вещества-лидеры, проявляющее антимикробную активность в отношении *Staphylococcus aureus* и *Streptococcus pneumoniae.* Оценка химического строения позволила обосновать их фармакологическое действие и сделать выводы о возможности развития устойчивости к нему у микробных клеток.

Ключевые слова: производные хиназолинона**;** антимикробная активность; соединение-лидер; электронодонорные центры; активный сайт фермента; минимальная подавляющая концентрация; минимальная ингибирующая концентрация; бактериостатическое действие; бактерицидная активность; резистентность; АТФ-зависимый эффлюксный насос; плазмиды; транспозоны; большой мобильный элемент

Список сокращений: PBP – пенициллин-связывающий белок; MRSA – метициллин – резистентный *Staphylococcus aureus*; PBP2a – пенициллин-связывающий белок 2а; АТФ – аденозинтрифосфат, МПК – минимальная подавляющая концентрация; ДМСО – диметилсульфоксид; ДМФА – диметилформамид; МПБ – мясопептонный бульон; МПА – мясопептонный агар; АК – атипичные колонии; ТК – типичные колонии; ЯМР – ядерный магнитный резонанс; ТСХ – тонкослойная хроматография; НК – нуклеиновая кислота; FnBPs – фибронектин-связывающие белки

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INTRODUCTION

Currently, multi-resistance of pathogenic bacteria to antimicrobial agents used in medical practice, is a serious public health problem [1-6]. As a rule, the formation of resistance occurs in the course of antibiotic therapy, especially in the departments with more intensive use of this group drugs. Clinical studies have established the dominance of antibiotic-resistant strains in the structure of nosocomial infections. Thus, there is a need to search for new antibacterial substances characterized by high efficacy, low toxicity and insensitive to the suppressing action of pathogens [7–9].

It has been proven that *Staphylococcus aureus* and *Streptococcus pneumonia* are the most common and express various virulence factors. They are pathogens of a wide range of diseases in humans and animals, have the greatest resistance to antibiotics among gram-positive microorganisms [2, 10–14].

The emergence of *Staphylococcus aureus* resistance to β-lactam antibiotics, as well as to other antimicrobial agents, limits its use in medicine due to the following factors: its mutation and selection, the acquisition of new genetic material from other resistant organisms during the processes of transformation, transduction and conjugation, implying a change in the adhesive properties of the cell surface. It is known that functioning of ATP-dependent efflux pumps, which are carrier proteins that push antimicrobial agents out of the cell, contributes to the resistance formation of *Staphylococcus aureus* and *Streptococcus pneumoniae* to fluoroquinolones and the drugs of the tetracycline group [15–18].

Quinazolin-4(3H)-one and its derivatives, which are condensed heterocyclic nitrogen-containing compounds, are known as a promising class of substances exhibiting antibacterial, antifungal, anti-tuberculosis, and antiviral kinds of activity [3]. Its dependence on the nature and number of quinazolinone nucleus substituents has been described. It was found out that the compounds of this group have a pharmacological effect against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* [3, 5, 19].

It has been proven that quinoline derivatives, which are the basis of the quinazolinones structure, inhibit the DNA synthesis, promoting the cleavage of bacterial DNA gyrase and type IV topoisomerase, resulting in the death of a bacterial cell [20–24]. The ability of compounds of the quinazolinone series, similar to β-lactam antibiotics used to prevent pathogenic processes in the body caused by *Staphylococcus aureus* and *Streptococcus pneumoniae,* to participate in the irreversible serine acylation of the active center of transpeptidase – penicillin-binding protein (PBP), catalyzing the formation of peptidaregine, an essential component of the bacterial cell wall, has been described. As a result of the formation of a stable lactam-acyl-enzyme complex, transpeptidase and carboxypeptidase kinds of the enzyme activity are inhibited, leading to the death of the pathogen.

A unique ability of quinazolinones, realized in synergy with piperacillin and tazobactam, to form bonds with the allosteric site of penicillin-binding protein 2a (PBP2a) of methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci, which cannot be inhibited by β-lactams, has been established [15, 25–27]. The possibility of the joint use of quinazoline derivatives with chloramphenicol to increase its intracellular concentration in pathogenic strains applying efflux pumping systems to resist the action of antimicrobial drugs, has been proven [28, 29]. Probably, during their passage, quinazolinone, having a lower polarity, binds to an efflux pump to a greater extent, undergoes an outflow more easily and facilitates the penetration of an antibiotic into a microbial cell with a constant concentration [16–18, 20, 21, 30].

The uniqueness of the structure of quinazolin-4 (3H)-one new derivatives, the possibility of using it together with other antimicrobial agents in order to increase their pharmacological effect and prevent the resistance emergence to them, creates the need for a comprehensive study of their activity.

THE AIM of the research is to study the antimicrobial activity of quinazolin-4 (3H)-one derivatives against *Staphylococcus aureus* and *Streptococcus pneumoniae in vitro* as well as to assess the effect of their structural changes on the biological activity of the analyzed substances, the lipophilicity of their molecules to predict the ability of inducing their resistance by the mechanism of the active outflow.

MATERIALS AND METHODS

Research objects

The objects of the study were new derivatives of quinazolin-4 (3H)-one.

The chemical structure of new quinazolinone compounds can be described by the general formula shown in Fig. 1. The yield and physicochemical properties of the new substances are presented in Table 1.

Synthesis of new derivatives of quinzolin-4(3*H*)-one

The synthesis of new derivatives was carried out according to the classical scheme of the nucleic bases alkylation with alkyl halides in anhydrous dimethylformamide (DMF) in the presence of a potassium carbonate excess. NMR^{1}H spectra were recorded on a BrukerAvance 400 spectrometer (400 MHz) in DSMO-d6, tetramethylsilane as the internal standard. The spectra were interpreted using the ACD/HNMR PredictorPro 3.0 licensed program from Advanced Chemistry Development (Canada). The melting points were measured in glass capillaries on a Mel-Temp 3.0 instrument (Laboratory Devices Inc., USA). The purity and individuality of the compounds were monitored by the TLC method.

N-[4-(Dimethylamino)phenyl]-2-[4-oxo-3(4H)-quinazolinyl] acetamide (Laboratory code: VMA-10-10).

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A mixture of 2.0 g (13.7 mmol) of quinazolin-4(3H)-one, 4.0 g (28.9 mmol) of anhydrous potassium carbonate and 50 ml of DMF is stirred at the temperature of 100-105°C for 30 min., then 3.2 g (15.1 mmol) of 2-Chloro-N-[4-(dimethylamino)phenyl]acetamide is added and stirred at the same temperature for 1 hour. After that, the mixture is cooled down to room temperature and filtered.

The filtrate is kept at the temperature of 0–5°C within 24 hours. The separated precipitate is filtered off, washed with cold DMF, water, and dried in air. It is recrystallized from DMF to get 2.95 g of the VMA-10-10compound, the yield is 67%, the mp. is 261–264°C.

The NMR^{1}H spectrum, δ, ppm, is the following: 2.78 s (6H, CH_3). 4.76 s (2H, CH_2); 6.63 d (8 Hz, 2H, phenyl); 7.34 d (8 Hz, 2H, phenyl); 7.51 t (7 Hz, 1H, H^6); 7.66 d (8 Hz, 1H, H^8); 7.78 t (7 Hz, 1H, H^7); 8.09 d (8 Hz, 1H, H^5); 8.29 s (1H, H^2); 10.08 s (1H, NH).

The rest of the compounds are obtained in the same way.

N-(4-Methoxyphenyl)-2-[4-oxo-3(4H)-quinazolinyl] acetamide (Laboratory code: VMA-10-18). The NMR^{1}H spectrum, δ, ppm is the following: 3.72 s (3H, OCH_3); 4.85 s (2H, CH_2); 7.51 d (8 Hz, 2H, phenyl); 6.90 d (8 Hz, 2H, phenyl); 7.57 t (7 Hz, 1H, H^6); 7.73 d (8 Hz, 1H, H^8); 7.86 t (7 Hz, 1H, H^7); 8.16 d (8 Hz, 1H, H^5); 8.37 s (1H, H^2); 10.31 s (1H, NH).

3-[2-Oxo-2-(4-phenylpiperazin-1-yl)ethyl] quinazolin-4(3H)-one (Laboratory code: VMA-10-21). The NMR^{1}H spectrum, δ, ppm is as follows: 3.14-3.32 m (4H, piperazine); 3.62-3.78 m (4H, piperazine); 5.01 s (2H, CH_2); 6.96-7.01 m (2H, phenyl); 7.23-7.29 m (3H, phenyl); 7.55 t (7.5 Hz, 1H, H^6); 7.71 d (8 Hz, 1H, H^8); 7.86 t (7.5 Hz, 1H, H^7); 8.17 d (8 Hz, 1H, H^5); 8.26 s (1H, H^2).

N-(2-Naphthyl)-2-[4-oxo-3(4H)-quinazolinyl] acetamide (Laboratory code: VMA-13-05). The NMR^{1}H spectrum, δ, ppm is as follows: 5.81 s (2H, CH2); 7.55-8.89 m (11H, H^5, H^6, H^7, H^8, naphthyl); 8.42 s (1H, H^2).

N-Phenyl-2-[4-oxo-3 (4H)-quinazolinyl]acetamide (Laboratory code: VMA-17-01). The NMR^{1}H spectrum, δ, ppm is as follows: 5.67 s (2H, CH_2); 7.54-7.77 m (5H, H^6, H^8, phenyl); 7.87 t (1H, 8 Hz, H^7); 8.07–8.19 m (3H, H^5, phenyl); 8.39 s (1H, H^2).

N-Phenyl-2-[4-oxo-3(4H)-quinazolinyl]propanamide (Laboratory code: VMA-17-04). The NMR^{1}H spectrum, δ, ppm is as follows: 1.53 d (3H, 7 Hz, CH_3) 5.49 q (1H, 7 Hz, CH); 7.56–7.80 m (5H, H^6, H^8, phenyl); 7.85 t (1H, 8 Hz, H^7); 8.06–8.19 m (3H, H^5, phenyl); 8.40 s (1H, H^2).

N- [6-Bromoquinazolin-3 (4H) -yl] acetylguanidine (Laboratory code: VMA-13-17). The NMR^{1}H spectrum, δ, ppm is as follows: 4.37 s (2H, CH2); 7.47 br. s (4H, NH); 7.60 d (1H, 8 Hz, H^8); 7.90 d (1H, 8 Hz, H^7); 8.17 s (1H, H^2); 8.28 s (1H, H^5).

Test cultures

A primary microbiological screening of the antimicrobial activity of the synthesized compounds in order to identify the lead compound, was carried out using cultures of *Staphylococcus aureus* and *Streptococcus pneumoniae* isolated from sick patients provided by the clinical diagnostic laboratory, City Clinical Hospital No. 3 n. a. S.M. Kirov, Astrakhan. The studies were approved by the Ethics Committee of Astrakhan State Medical University of the Ministry of Health of Russia (protocol No. 6 dated November 27, 2018).

Research methods

The analysis of substances with the assigned codes – VMA-10-10, VMA-10-18, VMA-10-21, VMA-13-05, VMA-17-01, VMA-17-04, VMA-13-17 – was carried out *in vitro* by the serial dilutions method in accordance with the requirements of the international standard ISO 20776-1:2006[1] and the National Standard GOST R ISO 20776-1-2010[2], identical to the international one.

The determination of the microorganism's sensitivity to quinazolinone derivatives was carried out by the macro method (test tube) in the medium of meat infusion broth (MIB) prepared in accordance with GOST 20729-75.

Preparation process of working solutions

The working solution was prepared by dissolving a 4 mg sample of the test substance in 0.5 ml of dimethyl sulfoxide (DMSO), followed by adding 4.5 ml of a physiological solution to it. The choice of the solvent was carried out in accordance with the Methodological Recommendations "Sensitivity determination of microorganisms to antibacterial drugs"[3], as well as taking into account the solubility of the compounds under study, with a preliminary assessment of DMSO effect on the strains of the microorganisms used [33]. It was found out that the compounds under study are insoluble in water, slightly soluble in 40 and 90% ethyl alcohol, and freely soluble in DMSO. A series of solutions with an exponentially decreasing concentration was obtained from the resulting initial solution: 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μg/ml. A solution of ceftriaxone (JSC Sintez, Kurgan, P N000750/01) with the concentration equivalent to the process solution was used as a reference drug. Process solutions were introduced into 1 ml test tubes.

[1] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25. Wayne, PA: Clinicaland Laboratory Standards Institute; 2015.

[2] National Standard GOST R ISO 20776-1-2010 Clinical laboratory research and in vitro diagnostic test systems. Investigation of the sensitivity of infectious agents and assessment of the functional characteristics of products for the study of sensitivity to antimicrobial agents. Part 1. Reference method for laboratory study of the activity of antimicrobial agents against fast-growing aerobic bacteria that cause infectious diseases. Russian

[3] Methodical instructions 4.2.1890-04. Determination of the sensitivity of microorganisms to antibacterial drugs: Guidelines. M.: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of Russia, 2004: 91 p. Russian

Inoculum preparation

Inoculum preparation was carried out in accordance with the requirements for the method of direct suspension of morphologically similar colonies collected using a sterile bacteriological loop in a sterile isotonic solution.

Methodology

Suspensions of *Staphylococcus aureus* and *Streptococcus pneumoniae,* diluted in a liquid nutrient medium to 10^6 cfu/ml, were added 1 ml each into the test tubes with the solutions of the studied substances.

The inoculations in the tubes closed with sterile cotton-gauze stoppers, were incubated for 24 hours at the temperature of + 37°C. At the end of the incubation period, they were visually assessed in the transmitted light. In the control tubes, in which a native culture had been grown without adding a reference drug or test compounds, complete turbidity of the culture medium indicating an intensive growth of the culture, was noted.

The determination of the minimum inhibitory concentration (MIC) of the test substance assumed the establishment of the lowest substance concentration at which there had been no bacterial growth. That was evidenced by the absence of turbidity of the solution, which was recorded visually.

Assessment of microorganisms growth

The assessment of the bacteria viability was carried out according to the value of the lowest concentration of the test substance that prevents the visible growth of bacteria, or, in other words, the minimum inhibitory concentration. The inoculation of 0.05 ml of the precipitate obtained by centrifuging the contents of each tube of the series at 1500 rpm for 10 min and separating the supernatant, was carried out on the meat infusion agar (MIA) placed in Petri dishes. The nutrient medium was prepared by dissolving the dry agar followed by autoclaving. After the inoculation, the Petri dishes were placed in a thermostat. The analysis of the characteristic growth was performed 24 hours after the incubation of the inoculation at the temperature of +37°C [33].

The determination of the antimicrobial activity of the test substances hypothesized a sixfold reproduction of the selected analysis method [31-32]. The absence of the culture growth was taken for the bactericidal effect manifested by the substance, while the inhibition of the culture growth, its intermittent growth, and the formation of single colonies indicated a bacteriostatic effect.

Statistical processing of research results

Statistical processing of the research results was carried out using the following software packages: Microsoft Office Excel 2007 (Microsoft, USA), BIOSTAT 2008 Professional 5.1.3.1. ("Analyst-Soft" Inc., USA). When processing the results obtained, a parametric method with the determination of the Student's t-test with the Bonferroni correction was used. The differences in the comparison groups were assessed at the constantly chosen significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The analysis of the antimicrobial activity of the substances with codes VMA-10-10, VMA-10-18, VMA-10-21, VMA-13-05, VMA-17-01, VMA-17-04, VMA-13-17 showed that their manifestation depends on the multiplicity of dilution and a type of a pathogenic microorganism.

The experimental data obtained are summarized in Tables 2–5.

The growth pattern analysis of *Staphylococcus aureus* and *Streptococcus pneumoniae* in the meat infusion broth and on the meat infusion agar with DMSO showed a moderate growth of microorganisms at the concentration of 128 and 64 μg/ml, as well as an intensive growth in the concentration range from 32 to 0.25 μg/ml.

During the visual control of *Staphylococcus aureus* cultures in the meat infusion broth, the signs of growth in the test tubes with ceftriaxone were observed at low drug concentrations – 2–0.5 μg/ml. A moderate growth of the culture was observed in the presence of the VMA-10-10 compound in the concentration range of 128-4 μg/ml and in the case of VMA-17-01 – in the concentration range of 16–8 μg/ml. The intensive development of cells, accompanied by strong turbidity of the nutrient medium, the formation of flakes and abundant sediment, were observed in test tubes at the concentrations of the compound VMA-10-21 in the range of 128–0.25 μg/ml.

A significant culture growth was also recorded in the test tubes with substances VMA-17-04 with a concentration of 4–0.25 μg/ml and VMA-13-17 with its content of 2–0.25 μg in 1 ml of the solution.

Table 3 shows the results of inoculating *Staphylococcus aureus* on a solid nutrient medium – meat infusion agar.

The Table 3 data indicate that in the presence of the control, ceftriaxone at the concentrations of 128–64 μg/ml, the growth of the culture is completely suppressed, while at its content of 32–4 μg in 1 ml of the solution, the growth of single colonies of the pathogen is observed.

An intensive growth is recorded on the *Staphylococcus aureus* meat infusion agar when using VMA-10-10 at the concentrations of 128–0.25 μg/ml and VMA-10-21 at 64–0.25 μg/ml. No growth of colonies was observed in the concentration ranges of 128–16 μg/ml of the substance VMA-17-04, 128–64 μg/ml – VMA 13-05, 128–32 μg/ml – VMA-17-01. The results indicate the ability of these compounds to inhibit the development of *Staphylococcus aureus* and, as a consequence, to exhibit a pronounced antimicrobial activity against the pathogen.

Table 4 shows the results of *Streptococcus pneumo-*

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niae inoculations on a liquid nutrient medium (meat infusion broth).

During the visual control of *Streptococcus pneumonia* inoculations on the meat infusion broth, the signs of growth in the test tubes with ceftriaxone were observed at the concentration of 4–0.25 μg/ml. A moderate growth of the culture was observed in the presence of the VMA-10-21 compound in the concentration range of 64–0.25 μg/ml, and of the VMA-10-18 substance – at its content of 8–0.25 μg in 1 ml. Lower values were set for the VMA-13-17, VMA-13-05 derivatives – 2–0.25 μg/ml and for VMA-10-10, VMA-17-01 derivatives – 1–0.25 μg/ml.

A complete transparency of the medium was observed in the tubes with quinazolinone derivative VMA-13-05 at the concentration of 128–32 μg/ml, of compounds VMA-17-01 and VMA-17-04 – in the content of the active ingredient of 128–64 μg in 1 ml. The results obtained indicate a pronounced antipneumococcal activity of the substances.

Table 5 shows that the culture of *Streptococcus pneumoniae* gives a heavy growth on the MIA in the presence of VMA-10-10, VMA-10-18 compounds at the concentrations of 4–0.25 μg/ml, in the presence of VMA-13-05 substances – at 8–0.25 μg/ml and in the presence of the VMA-17-04 derivative – at the concentrations of 2–0.25 mg/ml. The results obtained indicate the lack of sensitivity of the pathogen to these substances in the given dilution.

When the content of VMA-13-05 is at the concentration of 128–16 μg/ml, the growth of the pathogenic strain colonies is not observed. This is similar to the effects of VMA-17-04 and VMA-17-01 in the concentration range of 128–64 μg/ml. Consequently, in this content in the solution, the substances are characterized by a high antimicrobial activity against *Streptococcus pneumoniae.*

Table 6 shows the average results of assessing the antibacterial action of the most active substances against the strains of *Staphylococcus aureus* and *Streptococcus pneumoniae.*

The analysis of the average results of the antibacterial action of the most active substances against pathogenic microorganisms, makes it possible to conclude the following. The bactericidal activity of the compounds VMA-13-05, VMA-17-01 and VMA-17-04 is comparable to the action of ceftriaxone at the concentrations of 128 and 64 μg/ml; their bactericidal activity against *Staphylococcus aureus* manifests itself at the concentration of 32 μg/ml. When analyzing the antimicrobial action of the most active quinazoline compounds in subsequent concentrations, it was found out that the bactericidal activity of VMA-13-05, VMA-17-01 and VMA-17-04 statistically significantly decreases in proportion to the decrease of the substances concentration in relation to the reference drug – ceftriaxone.

The heterocyclic nature of quinazolinone compounds determines their ability to inhibit a PBP2a activity due to the formation of hydrogen bonds with the amino acids of the allosteric enzyme site: lysine, glutamine and asparagine. As a result of this interaction, an active site, where the carbonyl group and the nitrogen atom of another molecule of the antimicrobial agent are covalently bound to the carboxyl and amino groups of lysine and arginine, is opened. The enzyme is suppressed and, therefore, the biosynthesis of the bacterial cell wall is blocked [37-40]. The analysis of various substituents effect in the molecule of quinazolinone derivatives made it possible to identify the functional groups and structural fragments that take part in the formation of a chemical bond with the amino acid residues of the enzyme, due to which the pharmacological effect of the substances is probably realized. The studies of the relationship between the structure and activity of quinazolinone derivatives have shown that the presence of a substituted aromatic ring at position 3 and a methyl group is essential for the compound to exhibit the antimicrobial activity [34]. In this case, the quinazolinone compounds containing a phenyl radical are characterized by a higher binding affinity than the substances with a methyl group, which can be explained by an increase in the number of hydrophobic bonds with amino acids of the active site [35]. It has been shown that the substituent in the phenyl ring also has a significant effect on the antibacterial activity. Methoxy, methyl, hydroxy groups, as well as bromine and chlorine atoms, increase the antimicrobial effect [24]. It has been proven that the combination of two or more biologically active fragments in one molecule also contributes to an increase in the antibacterial effect due to a change in the degree of polarity of the drug molecule [1].

The mechanism of the substances interaction with DNA gyrase has been described. It also depends on the substituents nature determining the polarity of the molecule, its ability to form various chemical bonds with the enzyme. In this case, the death of a bacterial cell is known to be mediated by a violation of the DNA synthesis during the DNA gyrase inhibition involved in the reduction (negative supercoiling) of a nucleic acid (NA) molecule, with a quinazolinone derivative [37]. It has been established that its effect can be explained by the formation of an intermediate complex “DNA-topoisomerase-quinazolinone” due to the donor-acceptor interaction of the carbonyl group oxygen atom of the antimicrobial agent and the phosphate group of DNA, nitrogen with guanine and NA asparagine, and the substituents of the quinazolinone molecule with its non-polar groups. Binding to the active site of the enzyme occurs due to the hydrogen bonds of the quinazolinone derivative with the amino acid residues of serine and arginine [37].

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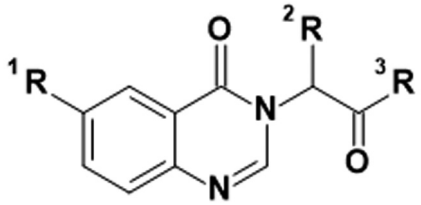

Figure 1 – General formula of quinazolin-4 (3H)-one derivatives

Table 1 – Chemical structure of new quinzolin-4 (3H)-one derivatives

Compound	R^1	R^2	R^3	Yield, %	Mp., °C
VMA-10-10	H	H	4-dimethylaminophenyl	67	261–264
VMA-10-18	H	H	4-methoxyphenyl	61	228–229
VMA-10-21	H	H	4-phenylpiperazin-1-yl	73	222–224
VMA-13-05	H	H	β-naphthyl	56	199–201
VMA-17-01	H	H	phenylamino	83	156–158
VMA-17-04	H	CH_3	phenylamino	72	222–224
VMA-13-17	Br	H	$NHC(NH)NH_2$	89	242–244

Table 2 – Indicators of visual assessment of compounds activity against growth of *Staphylococcus aureus* (MIB medium)

Series (compounds, drugs)	Concentration, μg/ml									
	128	64	32	16	8	4	2	1	0.5	0.25
DMSO	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Ceftriaxone	–	–	–	–	–	+	++	+++	+++	+++
VMA-10-10	++	++	++	++	++	++	+++	+++	+++	+++
VMA-10-18	+	+	+	+	+++	+++	+++	+++	++++	++++
VMA-10-21	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
VMA-13-05	–	–	–	++	++	++	+++	+++	+++	+++
VMA-17-01	–	–	–	++	++	+++	+++	+++	+++	+++
VMA-17-04	–	–	–	–	++	++	++++	++++	++++	++++
VMA-13-17	+	+	++	++	++	++	+++	+++	+++	+++

Note: "–" – full medium transparency; "+ –" – incomplete medium transparency; "+" – weak growth; ++ – moderate growth; +++ – intensive growth

Table 3 – Indicators of visual assessment of compounds activity against growth of *Staphylococcus aureus* (MIA medium)

Series (compounds, drugs)	n	Concentration, μg/ml									
		128	64	32	16	8	4	2	1	0.5	0.25
DMSO	6	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Ceftriaxone	6	–	–	+AC	+AC	+AC	+AC	+++AC	+++AC	+++AC	+++AC
VMA-10-10	6	+++AC	+++AC	+++AC	+++AC	+++AC	++++AC	++++AC	++++AC	++++AC	++++AC
VMA-10-18	6	+ AC	+AC	+ AC	+AC	+++AC	+++TC	+++TC	+++TC	++++TC	++++TC
VMA-10-21	6	++AC	+++TC	+++TC	+++TC	++++TC	++++TC	++++TC	++++TC	++++TC	++++TC
VMA-13-05	6	–	–	+AC	+++AC	++++AC	++++AC	++++AC	++++AC	++++AC	++++AC
VMA-17-01	6	–	–	–	++ AC	++ AC	+++ TC	+++ TC	+++ TC	+++ TC	+++ TC
VMA-17-04	6	–	–	–	–	++ AC	++AC	++++ AC	++++ AC	++++ AC	++++AC
VMA-13-17	6	+ AC	+ AC	++ AC	++ AC	++ AC	++AC	+++ TC	+++ TC	+++ TC	+++TC

Note: "–" – no colonies; "+" – single colonies; "++" – ≤ 50%, "+++" – ≤ 75%; "++++" – ≤ 100% of colonizating the Petri dish area; AC – atypical colonies; TC – typical colonies

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Table 4 – Indicators of visual assessment of compounds activity against growth of *Streptococcus pneumoniae* (MIB medium)

Series (compounds, drugs)	Concentration, μg/ml									
	128	64	32	16	8	4	2	1	0.5	0.25
DMSO	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Ceftriaxone	–	–	–	–	–	+	+	+	+	+
VMA-10-10	+ –	+ –	+ –	+	+	++	++	+++	+++	+++
VMA-10-18	+	+	+	+	+++	+++	+++	+++	++++	
VMA-10-21	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
VMA-13-05	–	–	–	+	+ –	++	+++	+++	+++	+++
VMA-17-01	–	–	+ –	+ –	+	++	++	+++	+++	+++
VMA-17-04	–	–	+	+	+	+	++	++	+++	+++
VMA-13-17	+	+	+	+	+	+	+++	+++	+++	+++

Note: "–" – full medium transparency; "+ –" – incomplete medium transparency; "+" – weak growth; ++ – moderate growth; +++ – intensive growth

Table 5 – Indicators of visual assessment of compounds activity against growth of *Streptococcus pneumoniae* (MIA medium)

Series (compounds, drugs)	n	Concentration, μg/ml									
		128	64	32	16	8	4	2	1	0.5	0.25
DMSO	6	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Ceftriaxone	6	–	–	–	+AC	+AC	+AC	++AC	++AC	++AC	++AC
VMA-10-10	6	++AC	++AC	++AC	++AC	++AC	++++AC	++++AC	++++AC	++++AC	++++AC
VMA-10-18	6	++AC	++AC	++ AC	++ AC	+++AC	+++TC	+++TC	+++TC	++++TC	++++TC
VMA-10-21	6	+AC	+TC	++TC	++TC	+++TC	+++TC	+++TC	+++TC	++++TC	++++TC
VMA-13-05	6	–	–	–	–	++++AC	++++AC	++++AC	++++AC	++++AC	++++AC
VMA-13-17	6	+ AC	+ AC	++ AC	++ AC	++ AC	++ AC	+++ TC	+++ TC	+++ TC	+++ TC
VMA-17-04	6	–	–	+ AC	+AC	++ AC	++ AC	++++ TC	++++ TC	++++ TC	++++ TC
VMA-17-01	6	–	–	+AC	++ AC	++ AC	+++ TC	+++ TC	+++ TC	+++ TC	+++ TC

Note: "–" – no colonies; "+" – single colonies; "++" – ≤ 50%, "+++" – ≤ 75%; "++++" – ≤ 100% of colonizating the Petri dish area; AC – atypical colonies; TC – typical colonies

Table 6 – Average results of antibacterial action of the most active substances against *Staphylococcus aureus* and *Streptococcus pneumoniae* strains

Series (compounds, drugs)	Concentration, μg/ml									
	128	64	32	16	8	4	2	1	0.5	0.25
Ceftriaxone	0	0	0	0	0	18.1±2.3	18.8±2.2	19.3±2.2	22.5±3.6	22.7±3.2
Against *Staphylococcus aureus* strains										
VMA-13-05	0	0	0	29.5±2.4 ***	32.1±3.1 ***	38.4±3.8 **	59.4±4.7 ***	65.3±4.2 ***	65.8±5.6 ***	68.3±5.4 ***
VMA-17-01	0	0	0	28.3±2.1 ***	33.8±3.7 ***	39.9±4.2 **	64.4±4.3 ***	65.7±4.1 ***	65.6±6.6 ***	69.3±6.1 ***
VMA-17-04	0	0	0	0	27.3±3.1 ***	28.1±2.8 *	78.4±5.9 ***	81.3±7.1 ***	83.6±7.3 ***	85.2±6.5 ***
Against *Streptococcus pneumoniae* strains										
VMA-13-05	0	0	0	14.3±1.8 ***	16.4±2.1 ***	26.3±1.8 *	61.3±4.8 ***	63.8±5.6 ***	66.4±5.2 ***	71.6±6.9 ***
VMA-17-01	0	0	12.3±1.8 ***	14.9±2.0 ***	15.7±1.9 ***	25.9±1.8 *	27.3±2.0 *	56.4±4.6 ***	62.3±4.9 ***	68.3±6.0 ***
VMA-17-04	0	0	10.2±1.3 ***	12.7±1.8 ***	12.8±1.4 ***	13.2±1.9	26.2±1.9 *	28.6±2.2 **	53.8±5.2 ***	55.7±5.2 ***

Note: * – p <0.05; ** – p <0.01; *** – p <0.001 – by reference to the indicators of the antibacterial ceftriaxone action

The possibility of the quinazolinone derivatives interaction with peptidoglycan precursors cannot excluded. That leads to the inhibition of its polymerization (transglycosylation) and the subsequent stage of cross-linking (transpeptidation). The bactericidal effect of the drug is realized during the formation of an intermediate complex "quinazolinone – peptidoglycan derivative", as a result of which depolarization of the membrane occurs, its permeability increases, leakage of potassium ions and cytoplasmic ATP occurs resulting in the cell death [41, 42].

The idea of the efflux pumps functioning increases a number of requirements for the investigated antimicrobial substances, in the form of a combination of high efficacy with resistance to outflow. One of the options for achieving it can be the dissipation of the membrane potential [29, 34]. It has been proven that the presence of a keto group, a benzyl radical and nitrogen atom in the quinazolinone structure, contributes to a decrease in lipophilicity; covalently bound bromine in the quinazoline core; methoxyphenyl and methyl substituents, on the contrary, increase hydrophobicity [35, 36]. The saturation of the quinazolinone derivatives molecules by the centers that reduce hydrophobicity, suggests an insignificant degree of binding to efflux proteins and, as a consequence, a low probability of resistance to these substances from the point of view of the efflux theory [5, 7, 28].

The analysis of the results obtained shows that the compound VMA-17-04, and, to a lesser extent, VMA-13-05, are active against *Staphylococcus aureus* and have a bacteriostatic effect. The structure of the substance VMA-13-05 contains a naphthyl substituent, which makes the molecule more lipophilic and, as a result, increases its penetration into the cell membrane of the pathogenic culture. The polarity of VMA-17-04, due to the amide group associated with the quinazolinone moiety and the benzene ring, causes an increase in the interaction degree of the electron donor center in the form of a nitrogen atom with the active sites of enzymes that catalyze the DNA replication and protein synthesis.

The assessment of the test compounds antimicrobial activity against *Streptococcus pneumoniae* shows the manifestation of the bacteriostatic effect of the VMA-13-05 derivatives. The VMA-17-04 and VMA-17-01 compounds are characterized by a weakly expressed antimicrobial effect.

The VMA-10-10 substance has practically no effect on *Staphylococcus aureus* and *Streptococcus pneumoniae.*

Probably, the difference of the membrane components of gram-positive bacteria in the chemical composition can be the reason for the unequal manifestation of the pharmacological activity of the VMA-17-04 and VMA-13-05 substances in relation to the pathogens. The presence of the quinazolinone derivatives in the molecules differing from their substituents in the structure, determines the difference in the mechanism of their binding to the substances of the pathogens cell membrane acting as adhesives, which are one of the virulence factors of these microorganisms. It has been established that the main role in the adhesion process of *Streptococcus pneumonia,* is played by collagen-binding and fibronectin-binding proteins, lipoteichoic acid, as well as surface phosphoryl-choline, which is a part of teichoic acid with choline-binding proteins attached to it. The adhesive activity of Staphylococcus aureus is carried out due to fibrinogen-binding protein, the molecules of which are bound to the peptidoglycan of the cell wall, collagen adhesin, extracellular protein, fibronectin-binding proteins, teichoic acid, as well as staphylococcal haptoglobin receptor residues, consisting of 145 amino acid residues [43].

The nature of the substituents in the molecule determines the varying degrees of lipophilicity of the compounds, which, according to Gibbonson, is an important property of the substance that characterizes its solubility in the bacterial membrane, and the degree of binding to efflux proteins or pump substrates. The hydrophobicity of derivatives serves as a factor that reduces the recognition and transport of antimicrobial agents by a suction pump, which is especially important in the search for the inhibitors of their outflow [29]. Although the lipophilicity of the VMA-13-05 structure suggests better binding to the efflux pump protein, which can lead to the emergence of resistance in *Staphylococcus aureus* and *Streptococcus pneumoniae* due to a decrease in the concentration of the antimicrobial agent, it cannot serve as an objective characteristic of this process without additional data obtained by an alternative methods analysis.

CONCLUSION

Thus, among the synthesized derivatives of quinazolin-4(3H)-one, the substances that exhibit a pronounced antimicrobial activity against *Staphylococcus aureus* (VMA-17-04) and *Streptococcus pneumoniae* (VMA-13-05), have been identified. This is apparently due to the effect of the lipophilic site of their molecules on the manifestation of the antimicrobial action. The results obtained in the course of this study, determine the prospects for further research of the antimicrobial properties of new quinazoline-4(3H)-one compounds in order to increase their pharmacological effect and prevent the development of pathogenic microorganisms' resistance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Marina A. Samotrueva – research concept and design, research planning, critical intellectual content review, final approval of manuscript for publication; Alexander A. Ozerov – scheme development of derivatives synthesis, obtaining data on physicochemical properties and spectral characteristics of substances, manuscript editing, its final approval for publication; Alla A. Starikova – data collection, text writing, chemical substantiation of ongoing processes based on structures of investigated substances, preparation of manuscript draft; Narmina Mutallimagakyzy Gabitova – carrying out microbiological research, assessment, substantiation and statistical processing of data obtained; Daria V. Merezhkina – implementation of quinazoline derivatives synthesis; Alexandra A. Tsibizova – data collection, assessment, substantiation and statistical processing of data obtained; Ivan N. Tyurenkov – research planning, research methodology, manuscript editing, assessment of results obtained by microbiological methods; final approval of manuscript for publication.

REFERENCES

1. Abrusán G, Marsh JA. Ligands and Receptors with Broad Binding Capabilities Have Common Structural Characteristics: An Antibiotic Design Perspective. J Med Chem. 2019 Nov 14;62(21):9357–74. DOI: 10.1021/acs.jmedchem.9b00220.
2. Beyzaei H, Ghasemi B. *In vitro* Antibacterial evaluation of newly synthesized heterocyclic compounds against Streptococcus Pneumoniae. Journal of Sciences, Islamic Republic of Iran. 2018; 29(3):211–20. DOI: 10.22059/JSCIENCES.2018.67436.
3. Tsibizova AA, Yasenyavskaya AL, Ozerov AA, Samotrueva MA, Tyurenkov IN. Acute toxicity assessment a new pyrimidine derivative. Astrakhan medical journal. 2021;16(1):82–87. DOI: 10.17021/2021.16.1.82.87. Russian
4. Jampilek J. Heterocycles in Medicinal Chemistry. Molecules. 2019 Oct 25;24(21):3839. DOI: 10.3390/molecules24213839.
5. Patel PR, Joshi H, Shah U, Bapna M, Patel B. New generation of quinazolinone derivatives as potent antimicrobial agents. Asian Pac J Health Sci. 2021;8(2):61–6. DOI: 10.21276/apjhs.2021.8.2.12.
6. Etebu E, Arikekpar I. Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Int. J. Appl. Microbiol. Biotechnol.Res. 2016;4:90–101.
7. Alanazi AM, Abdel-Aziz AA, Shawer TZ, Ayyad RR, Al-Obaid AM, Al-Agamy MH, Maarouf AR, El-Azab AS. Synthesis, antitumor and antimicrobial activity of some new 6-methyl-3-phenyl-4(3H)-quinazolinone analogues: *in silico* studies. J Enzyme Inhib Med Chem. 2016 Oct;31(5):721–35. DOI: 10.3109/14756366.2015.1060482.
8. El-Sayed NNE, Al-Otaibi TM, Alonazi M, Masand VH, Barakat A, Almarhoon ZM, Ben Bacha A. Synthesis and Characterization of Some New Quinoxalin-2(*1H*)-one and 2-Methyl-3*H*-quinazolin-4-one Derivatives Targeting the Onset and Progression of CRC with SRA, Molecular Docking, and ADMET Analyses. Molecules. 2021 May 23;26(11):3121. DOI: 10.3390/molecules26113121.
9. Hassan KA, Liu Q, Elbourne LDH, Ahmad I, Sharples D, Naidu V, Chan CL, Li L, Harborne SPD, Pokhrel A, Postis VLG, Goldman A, Henderson PJF, Paulsen IT. Pacing across the membrane: the novel PACE family of efflux pumps is widespread in Gram-negative pathogens. Res Microbiol. 2018 Sep–Oct;169(7–8):450–4. DOI: 10.1016/j.resmic.2018.01.001.
10. Vila J, Moreno-Morales J, Ballesté-Delpierre C. Current landscape in the discovery of novel antibacterial agents. Clin Microbiol Infect. 2020 May;26(5):596–603. DOI: 10.1016/j.cmi.2019.09.015.
11. Nagaraja V, Godbole AA, Henderson SR, Maxwell A. DNA topoisomerase I and DNA gyrase as targets for TB therapy. Drug Discovery Today. 2017;22(3):510–8. DOI: 10.1016/j.drudis.2016.11.006.
12. D'Atanasio N, Capezzone de Joannon A, Di Sante L, Mangano G, Ombrato R, Vitiello M, Bartella C, Magarò G, Prati F, Milanese C, Vignaroli C, Di Giorgio FP, Tongiani S. Antibacterial activity of novel dual bacterial DNA type II topoisomerase inhibitors. PLoS One. 2020 Feb 19;15(2):e0228509. DOI: 10.1371/journal.pone.0228509.
13. Karaman R, Jubeh B, Breijyeh Z. Resistance of Gram-Positive Bacteria to Current Antibacterial Agents and Overcoming Approaches. Molecules. 2020 Jun 23;25(12):2888. DOI: 10.3390/molecules25122888.
14. Lepak AJ, Seiler P, Surivet JP, Ritz D, Kohl C, Andes DR. In Vivo Pharmacodynamic Target Investigation of Two Bacterial Topoisomerase Inhibitors, ACT-387042 and ACT-292706, in the Neutropenic Murine Thigh Model against Streptococcus pneumoniae and Staphylococcus aureus. Antimicrob Agents Chemother. 2016 May 23;60(6):3626–32. DOI: 10.1128/AAC.00363-16.
15. Li L, Wang Q, Zhang H, Yang M, Khan MI, Zhou X. Sensor histidine kinase is a β-lactam receptor and induces resistance to β-lactam antibiotics. Proc Natl Acad Sci U S A. 2016 Feb 9;113(6):1648–53. DOI: 10.1073/pnas.1520300113.
16. Qiao Y, Srisuknimit V, Rubino F, Schaefer K, Ruiz N, Walker S, Kahne D. Lipid II overproduction allows direct assay of transpeptidase inhibition by β-lactams. Nat Chem Biol. 2017 Jul;13(7):793–8. DOI: 10.1038/nchembio.2388.
17. Janardhanan J, Bouley R, Martínez-Caballero S, Peng Z, Batuecas-Mordillo M, Meisel JE, Ding D, Schroeder VA, Wolter WR, Mahasenan KV, Hermoso JA, Mobashery S,

Chang M. The Quinazolinone Allosteric Inhibitor of PBP 2a Synergizes with Piperacillin and Tazobactam against Methicillin-Resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2019 Apr 25;63(5):e02637–18. DOI: 10.1128/AAC.02637-18.

18. Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, Shirliff ME. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus. Microb Pathog. 2016 Dec;101:56–67. DOI: 10.1016/j.micpath.2016.10.028.
19. Cai ZQ, Jin ZS, Zheng DQ, Hou L, Huang GW, Tian JQ, Wang GJ. Synthesis of several new quinazolin-4-amines containing p-toluenesulfonate moiety. Journal of chemical research. 2016;40:573–5. DOI: 10.3184/174751916X14725679922221.
20. Khan I, Zaib S, Batool S, Abbas N, Ashraf Z, Iqbal J, Saeed A. Quinazolines and quinazolinones as ubiquitous structural fragments in medicinal chemistry: An update on the development of synthetic methods and pharmacological diversification. Bioorg Med Chem. 2016 Jun 1;24(11):2361–81. DOI: 10.1016/j.bmc.2016.03.031.
21. Badshah SL, Ullah A. New developments in non-quinolone-based antibiotics for the inhibiton of bacterial gyrase and topoisomerase IV. Eur J Med Chem. 2018 May 25;152:393–400. DOI: 10.1016/j.ejmech.2018.04.059.
22. Qian Y, Allegretta G, Janardhanan J, Peng Z, Mahasenan KV, Lastochkin E, Gozun MMN, Tejera S, Schroeder VA, Wolter WR, Feltzer R, Mobashery S, Chang M. Exploration of the Structural Space in 4(3*H*)-Quinazolinone Antibacterials. J Med Chem. 2020 May 28;63(10):5287–5296. DOI: 10.1021/acs.jmedchem.0c00153.
23. Masri A, Anwar A, Khan NA, Shahbaz MS, Khan KM, Shahabuddin S, Siddiqui R. Antibacterial Effects of Quinazolin-4(3*H*)-One Functionalized-Conjugated Silver Nanoparticles. Antibiotics (Basel). 2019 Oct 9;8(4):179. DOI: 10.3390/antibiotics8040179.
24. Bouley R, Ding D, Peng Z, Bastian M, Lastochkin E, Song W, Suckow MA, Schroeder VA, Wolter WR, Mobashery S, Chang M. Structure-Activity Relationship for the 4(3H)-Quinazolinone Antibacterials. J Med Chem. 2016 May 26;59(10):5011–21. DOI: 10.1021/acs.jmedchem.6b00372.
25. Nakano S, Fujisawa T, Ito Y, Chang B, Matsumura Y, Yamamoto M, Suga S, Ohnishi M, Nagao M. Penicillin-Binding Protein Typing, Antibiotic Resistance Gene Identification, and Molecular Phylogenetic Analysis of Meropenem-Resistant Streptococcus pneumoniae Serotype 19A-CC3111 Strains in Japan. Antimicrob Agents Chemother. 2019 Aug 23;63(9):e00711–19. DOI: 10.1128/AAC.00711-19.
26. Brouwers R, Vass H, Dawson A, Squires T, Tavaddod S, Allen RJ. Stability of β-lactam antibiotics in bacterial growth media. PLoS One. 2020 Jul 20;15(7):e0236198. DOI: 10.1371/journal.pone.0236198.
27. Marco L, Liliana G, Anna B, Annarita M. Intrinsic role of coagulase negative staphylococci *norA*-like efflux system in fluoroquinolones resistance. AIMS Microbiol. 2017 Nov 14;3(4):908–914. DOI: 10.3934/microbiol.2017.4.908.
28. Ankireddy AR, Rambabu G, Balaraju T, Banothu V, Gundla PL, Addepally U, Mantipally M. Synthesis, characterization and antibacterial activity of some novel C-7-Substituted-2-morpholino-N-(pyridin-2-ylmethyl) quinazolin-4-amine derivatives. Der PharmaChemica. 2018;10(11):40–8.
29. Ghorab MM, Alqahtani AS, Soliman AM, Askar AA. Novel *N*-(Substituted) Thioacetamide Quinazolinone Benzenesulfonamides as Antimicrobial Agents. Int J Nanomedicine. 2020 May 5;15:3161–80. DOI: 10.2147/IJN.S241433.
30. De Rosa M, Verdino A, Soriente A, Marabotti A. The Odd Couple(s): An Overview of Beta-Lactam Antibiotics Bearing More Than One Pharmacophoric Group. Int J Mol Sci. 2021 Jan 9;22(2):617. DOI: 10.3390/ijms22020617.
31. Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, Rodloff A, Soussy CJ, Steinbakk M, Soriano F, Stetsiouk O. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. Clin Microbiol Infect. 2006 Jun;12(6):501–3. DOI: 10.1111/j.1469-0691.2006.01454.x.
32. Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin Microbiol Infect. 2006 May;12(5):418–25. DOI: 10.1111/j.1469-0691.2006.01377.x.
33. Luzhnova SA, Voronkov AV, Kodonidi IP, Gabitova NM, Hrapova AV, Billel Souda. ACTIVITY OF NEW DERIVATIVES OF 1,3-DIAZINON-4 AND THEIR NON-CYCLIC PRECURSORS AGAINST STAPHILOCOCCYS AUREUS. Astrakhan medical journal. 2017;12(2):56–63. Russian
34. Gajdács M. The Continuing Threat of Methicillin-Resistant *Staphylococcus aureus*. Antibiotics (Basel). 2019 May 2;8(2):52. DOI: 10.3390/antibiotics8020052.
35. Nandwana NK, Singh RP, Patel OPS, Dhiman S, Saini HK, Jha PN, Kumar A. Design and Synthesis of Imidazo/Benzimidazo[1,2-*c*]quinazoline Derivatives and Evaluation of Their Antimicrobial Activity. ACS Omega. 2018 Nov 30;3(11):16338–46. DOI: 10.1021/acsomega.8b01592.
36. Maruthamuthu D, Rajam S, Christina Ruby Stella P, BharathiDileepan AG, Ranjith R. The chemistry and biological significance of imidazole, benzimidazole, benzoxazole, tetrazole and quinazolinone nucleus. J. Chem. Pharm. Res. 2016;8(5):505–26.
37. Mahato A, Shrivastava B, Shanthi N. Synthesis, Molecular Docking and Biological Evaluation of Substituted Quinazolinones as Antibacterial Agents. Chemical Science Transactions. 2015;4(2):595–603. DOI: 10.7598/cst2015.995.
38. Fisher JF, Mobashery S. Constructing and deconstructing the bacterial cell wall. Protein Sci. 2020 Mar;29(3):629–46. DOI: 10.1002/pro.3737.
39. Ibrahim MAA, Abdeljawaad KAA, Abdelrahman AHM, Alzahrani OR, Alshabrmi FM, Khalaf E, Moustafa MF, Alrumaihi F, Allemailem KS, Soliman MES, Paré PW, Hegazy MF, Atia MAM. Non-*β*-Lactam Allosteric Inhibitors Target Methicillin-Resistant *Staphylococcus aureus*: An *In Silico* Drug Discovery Study. Antibiotics (Basel). 2021 Aug 1;10(8):934. DOI: 10.3390/antibiotics10080934.
40. Mahasenan KV, Molina R, Bouley R, Batuecas MT, Fisher JF, Hermoso JA, Chang M, Mobashery S. Conformational Dynamics in Penicillin-Binding Protein 2a of Methicillin-Resistant Staphylococcus aureus, Allosteric Communication Network and Enablement of Catalysis. J Am Chem Soc. 2017 Feb 8;139(5):2102–2110. DOI: 10.1021/jacs.6b12565.
41. Higgins DL, Chang R, Debabov DV, Leung J, Wu T, Krause KM, Sandvik E, Hubbard JM, Kaniga K, Schmidt DE Jr, Gao Q, Cass RT, Karr DE, Benton BM, Humphrey PP. Tela-

ОРИГИНАЛЬНАЯ СТАТЬЯ

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vancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2005 Mar;49(3):1127–34. DOI: 10.1128/AAC.49.3.1127-1134.2005.

42. Bayer AS, Schneider T, Sahl HG. Mechanisms of daptomycin resistance in Staphylococcus aureus: role of the cell membrane and cell wall. Ann N Y Acad Sci. 2013 Jan;1277(1):139–58. DOI: 10.1111/j.1749-6632.2012.06819.x.

43. Zubareva IV, Berenshtein TF, Fedyanin SD. Ob adgezii grampolozhitel'nyh kokkov [On the adhesion of gram-positive cocci]. Journal "Vestnik of Vitebsk State Medical University". 2010;9(1):6–15. Russian

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