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The synthesis of spiro[indole-3,1'-pyrrolo[3,4 -c]pyrrole]-2,4',6'-trione derivatives, the study of their antimicrobial activity and the molecular docking on staphylococcal dehydrosqualene synthase

Aim. To synthesize the series of new spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-trione derivatives, study their physicochemical characteristics, antibacterial activity and precision of the molecular docking on the model of staphylococcal dehydrosqualene synthase.

Materials and methods. The methods of organic synthesis, instrumental methods for analysis of organic compounds, as well as the molecular docking method i*n silico* and agar diffusion method *in vitro* were used.

Results and discussion. To synthesize new *bis*-derivatives of 3'a, 6'a-dihydro-3'H-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones the three-component reaction of 1,6-maleimidamidohexane with *L*-amino acids and isatin was studied. New *bis*-spiro derivatives were isolated with a double excess of the corresponding isatin and *L*-amino acids. With the equimolar ratio of three reagents 6-N-maleimidohexyl derivatives spiro[indole-3,1'-pyrrolo[3,4-c] pyrrole]-2,4',6'-triones were isolated with the yields of 30-90 %. To prove their reactivity two symmetrical bis-spirooxindoles were counter-synthesized by condensation of two 6-*N*-maleimidohexyl spiro-2-oxindole derivatives with isatin, *L*-phenylalanine or sarcosine with the yields of 35 and 38 %. In the microbiological screening it was found that some compounds revealed the activity against *S. aureus* at the level of cefalexin and against *C. albicans* fungi relative to fluconazole. The docking *in silico* identified a high ability of the compounds studied to interact with at least six key amino acid residues – Arg45, Asp48, Asp52, Gln165, Asn168 and Asp172 of the active center of *S. aureus* dehydrosqualene synthase (CrtM).

Conclusions. It has been found that the one-pot three-component reaction of isatin, *L*-amino acids and 1,6-maleimidohexane as a function of the mole ratio of the reagents leads to both *bis*-derivatives of spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-trione, and to the corresponding asymmetric 6-*N*-maleimidohexyl derivatives. The substances synthesized have predominantly shown the activity in relation to gram-positive bacteria and yeast-like fungi. For the first time it has been demonstrated by the molecular docking method that the compounds studied forming a complex with a high docking score are potential inhibitors of staphylococci CrtM.

Key words: spiro-2-oxindoles; bis-spirocyclic systems; 1,3-cycloaddition; antimicrobial activity; dehydrosqualene synthase; molecular docking

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Синтез похідних спіро[індол-3,1'-піроло[3,4-c]пірол]-2,4',6'-триону, вивчення їх антимікробної активності та молекулярний докінг на стафілококовій дегідроскваленсинтазі

Мета роботи – синтез ряду нових похідних спіро[індол-3,1'-піроло[3,4-c]пірол]-2,4',6'-трионів, дослідження їх фізико-хімічних характеристик, антибактеріальної активності та прецизійного молекулярного докінгу на моделі дегідроскваленсинтази стафілококів.

Матеріали та методи. Методи органічного синтезу, інструментальні методи встановлення будови органічних сполук, молекулярний докінг *in silico*, метод дифузії в агар *in vitro*.

Результати та їх обговорення. З метою синтезу нових *біс*-похідних 3'a,6'a-дигідро-3'H-спіро[індол-3,1'-піроло[3,4-c]пірол]-2,4',6'-трионів досліджено трикомпонентну реакцію 1,6-малеїнамідогексану з *L*-амінокислотами та ізатином. Нові біс-спіропохідні були виділені при двократному надлишку відповідних ізатинів та *L*-амінокислот. При еквімольному співвідношенні трьох реагентів нами були ізольовані 6-*N*-малеїнімідогексилпохідні спіро[індол-3,1'-піроло[3,4-c]пірол]-2,4',6'-трионів з виходами 30-90 %. Для доказу реакційної здатності здійснено зустрічний синтез двох симетричних *біс*-спіро-2-оксіндолів конденсацією двох 6-*N*-малеїнімідогексилпохідних з відповідними ізатинами, *L*-фенілаланіном або саркозином з виходами 35 та 38 % відповідно. У мікробіологічному скринінгу виявлено сполуки, які пригнічують ріст *S. aureus* на рівні цефалексину та грибів *C. albicans* у порівнянні з флуконазолом. У докінгу *in silico* показано високу здатність досліджених молекул взаємодіяти як мінімум із шістьма ключовими амінокислотними залишками Arg45, Asp48, Asp52, Gln165, Asn168 та Asp172 активного центру дегідроскаваленсинтази (CrtM) стафілококів.

Висновки. Встановлено, що однореакторна трикомпонентна реакція ізатинів, *L*-амінокислот та 1,6-малеїнамідогексану в залежності від мольного співвідношення реагентів призводить як до *біс*-3'a,6'a-дигідро-3'H-спіро[індол-3,1'-піроло[3,4-c]пірол]-2,4',6'-трионів, так і до відповідних несиметричних 6-*N*-малеїнімідогексилпохідних. Синтезовані речовини переважно проявили активність до грампозитивних бактерій та дріжджоподібних грибів. Методом молекулярного докінгу вперше показано, що досліджені сполуки є потенційними інгібіторами дегідроскваленсинтази (CrtM) стафілококів, утворюючи комплекс із високим виграшем енергії.

Ключові слова: спіро-2-оксіндоли; біс-спіроциклічні системи; 1,3-циклоприєднання; антимікробна активність; дегідроскваленсинтаза; молекулярний докінг

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Синтез производных спиро[индол-3,1'-пирроло[3,4-c]пиррол]-2,4',6'-триона, изучение их антимикробной активности и молекулярный докинг на стафилококковой дегидроскваленсинтазе

Цель работы – синтез ряда производных спиро[индол-3,1'-пирроло[3,4-c]пиррол]-2,4',6'-трионов, исследования их физико-химических характеристик, антибактериальной активности и прецизионного молекулярного докинга на модели дегидроскваленсинтазы стафилококков.

Материалы и методы. Методы органического синтеза, инструментальные методы установления строения органических соединений, молекулярный докинг *in silico*, метод диффузии в агар *in vitro*.

Результаты и их обсуждение. С целью синтеза новых *бис*-производных 3'a, 6'a-дигидро-3'H-спиро[индол-3,1'-пирроло[3,4-c]пиррол]-2,4',6'-трионов исследована трехкомпонентная реакция 1,6-малеинамидогексана с *L*-аминокислотами и изатином. Новые *бис*-спиропроизводные были выделены при двухкратном избытке соответствующих изатинов и *L*-аминокислот. При эквимольном соотношении трех реагентов нами были выделены 6-*N*-малеинимидогексилпроизводные 3'a,6'a-дигидро-3'H-спиро[индол-3,1'-пирроло[3,4-c]пиррол]-2,4',6'-трионов с выходами 30-90 %. Для доказательства их реакционной способности осуществлен встречный синтез двух симметричных *бис*-спирооксиндолов конденсацией двух 6-*N*-малеинимидогексилпроизводных с изатином, *L*-фенилаланином или саркозином с выходами 35 и 38 %. В микробиологическом скрининге найдены соединения, которые проявили активность в отношении *S. aureus* на уровне цефалексина и грибов *C. albicans* относительно флуконазола. В докинге *in silico* выявлена высокая способность исследованных соединений взаимодействовать как минимум с шестью ключевыми аминокислотными остатками Arg45, Asp48, Asp52, Gln165, Asn168 и Asp172 активного центра дегидроскаваленсинтазы (CrtM) стафилококков.

Выводы. Установлено, что однореакторная трехкомпонентная реакция изатина, *L*-аминокислот и 1,6-малеинамидогексана в зависимости от мольного соотношения реагентов приводит как к *бис*-производным 3'a,6'a-дигидро-3'H-спиро[индол-3,1'-пирроло[3,4-c]пиррол]-2,4',6'-триона, так и к соответствующим несимметричным 6-*N*-малеинимидогексилпроизводным. Синтезированные вещества преимущественно проявили активность к граммположительным бактериям и дрожжеподобным грибам. Методом молекулярного докинга впервые показано, что исследованные соединения являются потенциальными ингибиторами дегидроскваленсинтазы (CrtM) стафилококков, образуя комплекс с высоким выигрышем энергии.

Ключевые слова: спиро-2-оксиндолы; бис-спироциклические системы; 1,3-циклоприсоединения; антимикробная активность; дегидроскваленсинтаза; молекулярный докинг

The widespread use of β-lactam antibiotics in the twentieth century, in particular penicillin, led to formation of staphylococcal resistance due to mutations in virulence factors (β-lactamase, staphylokinase, exfoliatin, and others). To combat staphylococcal infections currently methicillin is used, in respect of which resistant strains are also recorded, which even appears in the division of strains of *S. aureus* into methicillin-sensitive and methicillin-resistant (MRSA), as well as more resistant to vancomycin-resistant (VRSA) and glycopeptide-resistant (GISA). Among patients infected with methicillin-resistant strains mortality is 31 % [1]. Therefore, the search for new chemotherapeutic agents, in particular with the anti-staphylococcal activity, is one of the urgent tasks of modern pharmaceutical science.

It is known that an important factor of virulence is the carotenoid pigment – stafiloxanthin in *S. aureus* cells [2]. This pigment acts as a powerful antioxidant with its nine conjugated double bonds, which allow to neutralize the reactive forms of oxygen [3]. The *S. aureus* bacteria without the carotenoid pigment die quickly. Thus, blocking of the stafiloxanthin biosynthesis is potentially attractive to the new therapeutic target to which resistance mechanisms have not been formed [4, 5]. The initial phase of stafiloxanthin synthesis is carried by the key enzyme – dehydrosqualene synthase (CrtM), which catalyzes the "head to head" condensation of two molecules of farnesyl pyrophosphate (FPP) in C_{30} carotenoid dehydrosqualene (4,4'-diapofitohen) [6].

Our attention was drawn to the derivatives of *bis*-spiro[indole-3,1'-pyrrolo [3,4-c]pyrrole synthesized for the first time as a promising antimicrobial agents; they appeared to have a significant antibacterial potential [7]. Spiro-2-oxindoles are widely represented in nature as hemiterpenic alkaloids – elakomin, chorsfillin, cerulusin from plants of the genus *Poaceae sp.*, antibiotics – paragerquamides and notamides from fungi of *Penicillium sp*. species, phytoncides from the genus *Brassicaceae sp*. [8], but spiro-2-oxindoles with a double spiro [indole-3,1'-pyrrolo[3,4-c]pyrrole] fragment are unknown in nature.

In this paper, we tried to consider the *bis* derivatives of spiro-2-oxindole synthesized as potent inhibitors of CrtM. The key idea of the design of new chemotherapeutic agents based on the spirocyc, non-planar synthetic platform is the possibility of introducing additional aliphatic substituents into new molecules located in the regions of the substrate-binding pocket of microbial cell enzymes. The applied "drug-design" approach by constructing a bis-fragment corresponds to the principle of the so-called "hybrid" or "double-drugs" first used successfully in the search for new antimalarial [9] and anti-HIV agents [10].

Materials and methods

Chemical part

All solvents and reagents were obtained from commercial sources. The ^{1}H, ^{13}C NMR-spectra were recorded on a Bruker Avance 170500 spectrometer at 500 MHz

for ^{1}H-NMR-spectra; the solvent was $DMSOd_6$; tetrametylsilan (TMS) was used as an internal standard for ^{1}H, ^{13}C. LC-MS were recorded using an Agilent 1100 HPLC device equipped with a diode array detector and mass spectrometer (Agilent LC-MSD SL); the column was Zorbax SB-C_{18} (4.6 × 15 mm) with the chemical ionization atmospheric pressure (APCI); for analysis TLC was performed on Silufol UV-254 aluminum plates in the solvent system of dichloromethane: methanol (9 : 1). The melting point was measured by Kofler device.

The derivatives of 1-R_1-3'-R_2-5'-(6-{3'-R_2-1-R_1-2.4',6'-trioxo-3'a, 6'-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)-3'a, 6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones (4.1-4.15) were obtained according to the one-pot method described earlier [7].

The general procedure for the synthesis of 1-R_1-3'-R_2-5 '(6-*N*-maleimido-hexyl)-3'a,6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones (5.1-5.10) was described in our previous paper [11]**.**

The cross synthesis of 1-R_1-3'-R_2-5'-(6-{3'-R_2-1-R_1-2.4'6'-trioxo-3'a,6'-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)-3'a, 6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones (4.6, 4.15). To the solution of 0.001 Mol of the corresponding 1-R_1-3'-R_2-5 '(6-*N*-maleimido-hexyl)-3'a,6'-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-trione (5.4 or 5.6) in ethanol-water solution (3 : 1, 10 ml) add 0.001 Mol of the corresponding isatin (1.1 or 1.4) and *L*-phenylalanine in the case of synthesis of 4.6, or 0.001 Mol of sarzosine in the case of synthesis 4.15, and reflux for 2 h; the reaction is monitored by TLC. Cool the solution and dilute with 50 ml of water, then extract with dichloromethane (3 × 20 ml), wash with water (100 ml), dry the resulting extract over magnesium sulfate and purify by column chromatography with 0.2-0.5 mm silica gel with the particle size of 40 Å (EMD Millipore®, 10181) in the dichloromethane : methanol (9 : 1) system.

Microbiological Experiment

The antimicrobial activity of drugs was studied *in vitro* commonly accepted in microbiological practice by the agar diffusion test in the "modification of wells" [12].

The molten agar nutrient medium was cooled to 45 °C, poured into the bottom layer in Petri dishes in the volume of 10 ml. After freezing agar six sterile cylinders of stainless steel with the height of 10 mm and the internal diameter of 8 mm were placed on it, around them the second layer was poured in the volume of 15 ml, and seeded with appropriate cultures of microorganisms. The microbial load was 500 thousand microbial cells per 1 ml of the dense nutrient medium. After clamping the top layer of agar on Petri dishes the cylinders were removed with sterile tweezers, and the drug was introduced into the wells formed.

Test strains of microorganisms. As test microorganisms the following reference strains from the American typical crop collection were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885-653. When they were grown, the appropriate nutrient media were used in the national part of the State Pharmacopeia of Ukraine – medium No. 1 and 2. The purity of each culture of the microorganism was confirmed by the typical morphological, tinctorial, culture and biochemical properties. The research was conducted in two stages. At the first stage, the antimicrobial activity was studied compared to Cefalexinum (for the cultures of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633) and Fluconazole (for the cultures of *Candida albicans* ATCC 885-653).

Preparation of samples of the substances. The accurate weight of each compound (1 mg) was thoroughly mixed with the solvent (1 ml) according to the rules of aseptics in sterile glass tubes. From the resulting suspension of the drug, 0.3 ml was taken and put into cubes on Petri dishes with microbial cultures. Petri dishes were kept for one hour at room temperature, then placed in a thermostat and incubated for 24 h at 37 °C with the meat-peptone agar and at 25 °C for 48 h with *Candida albicans* agar.

The level of the antimicrobial activity of the substances was recorded with the diameter of the growth inhibition zone of microorganisms around the well with the drug, and assessed compared to controls (the solvent and reference preparation).

The second phase of our research was to study the antimicrobial activity of drugs in relation to the cultures of *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 885-653 by two-fold serial dilutions in a liquid nutrient medium. The meat-peptone broth and Saburro broth were poured into 1 ml test tubes placed in a tray of 10 test tubes in row. Into the first test tube 1 ml of the chemotherapeutic solution in the concentration of (200 μg/ml) was introduced. After thorough mixing 1 ml from this test tube was transferred to the second one, mixed, then the quantity of the mixture from the second test tube was transferred to the third one, etc. In the ninth tube, from which 1 ml was poured out in all the test tubes, the volume of the liquid was identical. The tenth test-tube without the substances studied served as the control over the microorganism's culture growth. Subsequently, all test tubes containing the serially diluted drug and the same amount of the test-culture suspension were added to the control tube. For this, a 18-hour culture (*Staphylococcus aureus* ATCC 25923) and a 2-hour culture (*Candida albicans* ATCC 885-653) of the microbe tested on the oblique agar were washed with the saline solution, adjusted to a density of 5 *OU* according to the standard of turbidity, followed by dilution to the desired amount of microbial cells in 1 ml and were introduced into test tubes with a serially diluted drug. The results were taken into account, determining the presence or absence of the microbe growth in the medium containing various breeding chemotherapy. The last growth retort (a transparent broth) corresponded to the drug MIC in relation to the strain tested and indicated the degree of its sensitivity. If the medium is turbid in

all test tubes, then the microbe tested is resistant to the maximum concentration of chemotherapy that is taken in the test. The absence of growth in all test tubes, in addition to the control, indicates that MIC of the drug in relation to the microbe is lower than the concentration used.

To determine the bactericidal concentration (MBC) from 2-3 last samples with no apparent growth agar or broth cultures were used. After 24-48-72 hours of incubation in the thermostat the lowest concentration of the antibacterial chemotherapy in the test tube, which did not give rise to growth, was taken for MBC.

The molecular docking and screening *in silico* of the potential dehydrosqualene synthase of *Staphylococcus aureus (PDB ID 2zcq*) inhibitors.

The syllabus in silico consisted of four main steps: preparation of the library of compounds, docking, evaluation of the results and the procedure of filtering. The source of data on the structure of the complex enzyme-inhibitor is Protein Data Bank (PDB). The crystalline structure of the enzyme and its ligand coordinated with two cations of the Mg^{2+} ion exchanger of the bisphosphonate series – (1R)-4-(3-phenoxyphenyl)-1-phosphonobutane-1-sulfonic acid were selected as the model of staphylococcal dehydrosqualene synthase (CrtM) (**BPH-65**, SMILES code is c1ccc(cc1)Oc2cccc(c2)CCC[C@H](P(=O)(O)O)S(=O)(=O)O). The code structure of the ligand-enzyme complex according to the X-Ray is PDB ID 2zcq, resolution 2.38 Å, R-factor = 0.223, R-free = 0.270 [5]. The simulation was carried out to the chain A, the length of the amino acid sequence was 293 amino acids, the molecular weight – 34.79 KDa. The active center of the receptor was limited to a cube with a side of 2.38 Å, its location was determined by the coordinates of the ligand in the crystalline structure selected (X = 17.5902, Y = 52.3671, Z = 38.0749) for the construction of maps of energy potentials of atoms with an interval of 0.375 E. For the docking process the *Auto Dock Vina* program integrated on the on-line molecule platform (http://doc.mcule.com/doku.php?id=dockingvina#docking_vina) with the Vina docking algorithm was used [13]. For the docking procedure charges of the receptor and ligand molecules were calculated using the Gasteiger-Marsil method [14] using the *AutoDock Vina* program. The search for the optimal geometry of complexes was carried out using a non-moving active center and flexible ligands. The mobility of the latter was determined by rotation around single bonds that were not part of the cycle. Further, the energy gain in formation of the corresponding complex (E_{Doc}, kcal/mol) was calculated – the free binding energy of the corresponding site CrtM at T = 298.15 K. For the 3D visualization the GLmol browser based on WebGL/Javascript (http://webglmol.osdn.jp/index-en.html) was used. When forming the posture the ligand was placed in the binding site, selecting its rotational and translational degrees of freedom. Subsequently, affinity was estimated on the basis of the prevailing posture. For each structure 4 attempts were made, and the most active position considered was the location of the ligand corresponding to the lowest energy (the highest gain in energy).

Results and discussion

In order to synthesize a new series of the target 1-R_1-3'-R_2-5'-(6-{3'-R_2-1-R_1-2,4',6'-trioxo-3'a,6'-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)-3'a,6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones **4.1-4.15** the three-component condensation of isatines **1.1-1.4**, amino acids **2.1-2.11** and 1,6-*bis*-maleimidohexane **3** were studied. For example, in the previous work we first described the synthesis of compounds of this class containing the *bis*-spirocyclic system, in which two cores of spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-trione were linked by a 1,6-hexylidene radical [7]. Thus, the new *bis*-spiro derivaties **4.1-4.15** were synthesized according to the previous protocol when 1 mol of 1,6-*bis*-maleimidohexane was condensed with 2 Mol of the corresponding isatin and amino acids. When in the condensation with dipolarophile **3** the initial isatins (**1.1, 1.3, 1.4**) and amino acids (**2.3, 2.6-2.11**) were equilibrated, 1-R_1-3'-R_2-5'(6-*N*-maleimidohexyl)-3'a,6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-trions **5.1-5.10** were obtained with the yields of 30-90 % (Scheme).

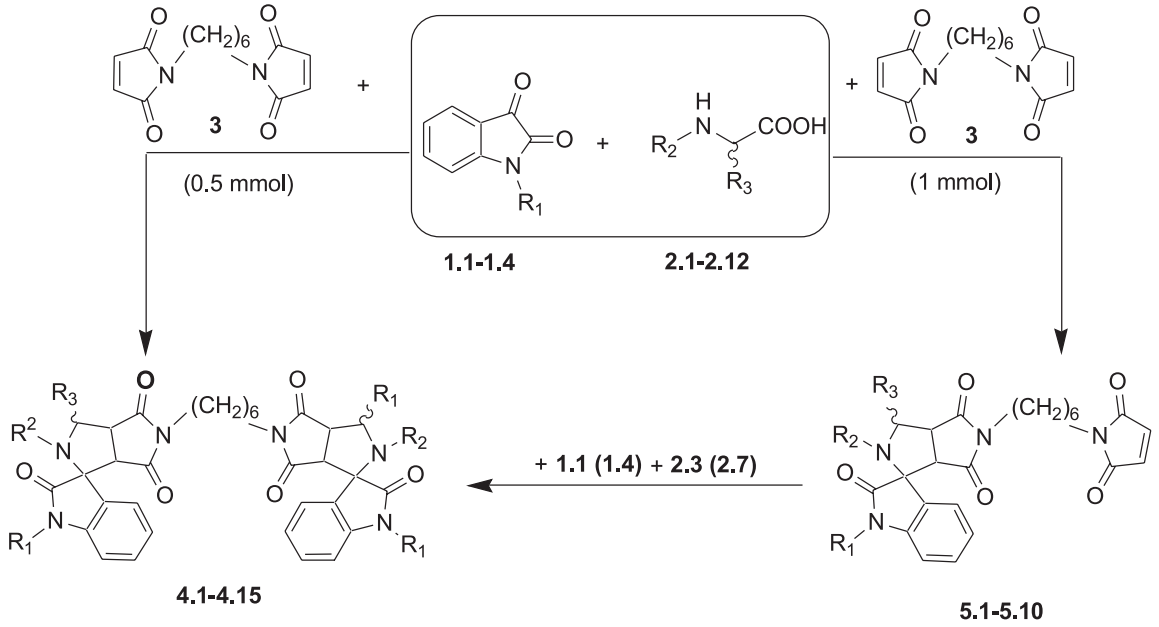
At the same time, the condensation of 6-*N*-maleimidohexyl derivatives of derivatives **5.4** and **5.6** with the corresponding isatins (**1.1, 1.4)** by *L*-phenylalanine or sarcosin were obtained by the counter-synthesis of *bis*-derivatives **4.6, 4.15** with the yields of 35 and 38 %, respectively.

The ^{1}H NMR and MC-spectral data for compounds **4** are listed in Tab.1. In the NMR-spectra of **4.1-4.15** there are signals from all protons of spacious fragments. The assignment of COOH and NH-groups signals were using the deutero-exchange with D_2O. The properties of **5.1-5.10** were published in the article [11].

The resonance of the methine protons of the pyrrolo[3,4-c]pyrrole system was displayed as a doublet at 3.40-3.50 ppm for the *H*-6a' proton, a triplet at 3.50-3.60 ppm for the *H*-3a' proton, and a multiplet for the *H*-3' proton located at 4.00-4.40 ppm in the spectra of compounds **4-5**. The values of the coupling constants of the *H*-3a' and *H*-6a' protons and of *H*-3' and *H*-3a' were 7-8 Hz, indicating their cis orientation. The 3'C-benzylic (3'-CH_2Ph) protons were detected as multiplets with the *H*-3' protons at δ 4.10-4.5 ppm for **4.6, 4.7, 4.8** and the N-benzylic ones (N-CH_2Ph) **4.2, 4.4, 4.7, 4.12, 4.15** were a singlet at δ 4.65-5 ppm. The sharp singlet at δ 3.01-3.10 due to the N-methyl protons was seen for compond **4.15**. The signals of aliphatic protons of the hexamethylene residue observed as a signal of CH_2CH_2 groups, two multiplets at δ 0.83-1.29 and δ 1.11-1.75 ppm, and the signal of 5'-NCH_2 two fragments were present in the region from δ 2.53 up to 3.52 ppm.

In this paper the antimicrobial potential of *bis*-spiro compounds (**4.1-4.15**) and derivatives with the free 6-maleimido-hexyl radical (**5.1-5.10**) was compared. The antimicrobial activity of all compounds tested is present in Fig. 1-4.

The results of the minimal bactericidal concentration (MBC) determination for the compounds tested are given in Tab. 2.



1.1 R_1 = H; **1.2** R_1 = All; **1.3** R_1 = Me; **1.4** R_1 = Bn; **2.1** R_2 = H , R_3 = H; **2.2** R_2 = H, R_3 = *i*-Butyl; **2.3** R_2 = H, R_3 = Bn; **2.4** R_2 = H, R_3 = CH_2CO_2H; **2.5** R_2 = H, R_3 = $CH_2CH_2CO_2H$; **2.6** R_2 = H, R_3 = CH_2OH; **2.7** R_2 = H, R_3 = Me; **2.8** R_2 = H, R_3 = *i*-Pr; **2.9** R_2 = H, R_3 = Ph; **2.10** R_2 = Me, R_3 = H; **2.11** R_2-R_3 = $(CH_2)_3$; **4.1** R_1 = All, R_2 = H, R_3 = H; **4.2** R_1 = Bn, R_2 = H, R_3 = H; **4.3** R_1 = Me, R_2 = H, R_3 = H; **4.4** R_1 = Bn, R_2 = H, R_3 = *i*-Butyl; **4.5** R_1 = Me, R_2 = H, R_3 = *i*-Butyl; **4.6** R_1 = H, R_2 = H, R_3 = Bn; **4.7** R_1 = Bn, R_2 = H, R_3 = Bn; **4.8** R_1 = Me, R_2 = H, R_3 = Bn; **4.9** R_1 = All, R_2 = H, R_3 = CH_2CO_2H; **4.10** R_1 = Bn, R_2 = H, R_3 = CH_2CO_2H; **4.11** R_1 = Me, R_2 = H, R_3 = CH_2CO_2H; **4.12** R_1 = Bn, R_2 = H, R_3 = $CH_2CH_2CO_2H$; **4.13** R_1 = Me, R_2 = H, R_3 = $CH_2CH_2CO_2H$; **4.14** R_1 = Me, R_2 = H, R_3 = CH_2OH; **4.15** R_1 = Bn, R_2 = Me, R_3 = H; **5.1** R_1 = H, R_2 = H, R_3 = Me; **5.2** R_1 = H, R_2 = H, R_3 = *i*-Pr; **5.3** R_1 = H, R_2 = H, R_3 = Ph; **5.4** R_1 = H, R_2 = H, R_3 = Bn; **5.5** R_1 = H, R_2 = Me, R_3 = H; **5.6** R_1 = Bn, R_2 = Me, R_3 = H; **5.7** R_1 = H, R_2-R_3 = $(CH_2)_3$; **5.8** R_1 = Bn, R_2-R_3 = $(CH_2)_3$; **5.9** R_1 = Bn, R_2 = H, R_3 = CH_2OH; **5.10** R_1 = Me, R_2 = H, R_3 = Me

Scheme

Table 1

The ^{1}H NMR, MS-spectral data and the melting points for 1-R_1-3'-R_2-5'-(6-{3'-R_2-1-R_1-2,4',6'-trioxo-3'a,6'-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)-3'a,6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones (**4.1**-**4.15**)

Compounds	^{1}H NMR, δ, ppm (J, Hz)	M.p.,°C	[M+H]
1	2	3	4
4.1	1.25-1.39 (m, 4H); 1.57-1.72 (m, 4H); 2.15 (dd, J = 14.4, 1.9, 2H); 2.30 (dd, J = 14.6, 7.1, 2H); 3.24 (d, J = 8.1, 2H); 3.81-3.90 (m, 4H); 4.12 (d, J = 8.1, 2H); 4.42 (d, J = 9.7; 4H); 5.05 (dd, J = 10.7, 4H); 6.01-6.26 (m, 2H); 6.91 (dd, J = 7.9, 2H); 7.14 (7.21 (dd, J = 8.4, 3H), 7.17 (dd, J = 7.9, 1H), 7.31 (dd, J = 8.4, 2H)	260	676.9
4.2	1.25-1.40 (m, 4H); 1.57-1.72 (m, 4H); 2.84 (dd, J = 14.8, 6.2, 2H); 3.09 (dd, J = 14.8, 7.6, 2H); 3.47 (d, J = 8.1, 2H); 3.73 (d, J = 8.1, 2H); 3.88 (t, J = 7.0, 4H); 4.77 (s, 4H); 6.91 (d, J = 8.0, 2H); 7.13-7.27 (m, 12H); 7.43 (d, J = 8.5, 2H), 7.80 (d, J = 8.0, 2H)	130	776.9
4.3	1.25-1.39 (m, 4H); 1.57-1.72 (m, 4H); 2.14 (dd, J = 14.6, 3H); 2.35 (dd, J = 14.6, 7.1 1H), 3.31 (dd, J = 8.1, 2H); 3.48 (s, 6H), 3.85 (t, J = 7.0, 4H); 4.12 (d, J = 8.1, 1H), 4.26 (d, J = 8.1,1H), 6.90 (dd, J = 7.9, 2H); 7.13-7.32 (m, 5H); 7.16 (d J = 7.9, 1H)	128-130	624.7
4.4	1.00-1.05 (m, 12H); 1.23-1.27 (m, 14H); 2.58 (dt, J = 7.8, 4.9 2H); 3.30 (dd, J = 8.1, 7.7, 2H); 3.88 (t, J = 7.0 4H); 4.29 (d, J = 8.1, 2H); 4.77 (s, 4H); 6.91 (dd, J = 7.9, 7.5, 2H); 7.13-7.31 (m, 12H); 7.39 (d, J = 8.5, 2H); 7.79 (d, J = 7.9, 1H), 7.96 (d, J = 7.9, 1H)	160	889.1
4.5	0.99-1.03 (m, 4H); 1.23-1.72 (m, 4H), 2.43 (dt, J = 6.0, 4.7, 4H), 2.66 (dt, J = 7.8, 4.8, 1H), 3.30 (dd, J = 8.1, 6.0, 2H); 3.49 (s, 6H); 3.88 (t, J = 7.0, 4H), 4.30 (d, J = 8.1, 2H); 7.01 (d, J = 7.9, 2H); 7.20 (dd, J = 8.4, 7.5, 2H); 7.40-7.43 (d, J = 8.4, 2H); 7.51 (d, J = 7.9, 2H)	180-182	736.9
4.6	1.40 (m, 4H); 1.72 (m, 4H); 2.58 (d, J = 6.6, 4H); 2.71 (dt, J = 7.8, 6.6, 2H); 3.31 (dd, J = 8.1, 7.7, 2H); 3.88 (t, J = 7.0, 4H); 4.29 (d, J = 8.1, 2H); 6.96 (dd, J = 7.9, 7.5, 2H); 7.28 (m, 14H); 7.79 (dd, J = 7.9, 1.4, 2H); 10.56 (s, 2H)	258-260	776.9
4.7	1.32 (m, 4H); 1.65 (m, 4H); 2.59 (dt, J = 6.6, 4H); 2.70 (dt, J = 7.8, 6.6, 2H); 3.32 (dd, J = 8.1, 7.7, 2H); 3.88 (t, J = 7.0 4H); 4.32 (d, J = 8.1, 2H); 4.77 (s, 4H); 6.91 (dd, J = 7.9, 7.5, 2H); 7.11-7.42 (m, 24H); 7.96 (d, J = 7.9, 2H)	120-122	957.1

Continuation of Table 1

1	2	3	4
4.8	1.32 (m, 4H); 1.57 (m, 4H); 2.57 (dt, J = 6.6, 4H); 2.71 (dt, J = 7.7, 6.6, 2H), 3.32 (dd, J = 8.1, 7.7, 2H); 3.49 (s, 6H); 3.88 (t, J = 7.0, 4H); 4.13 (2H, d, J = 8.1 Hz); 6.98 (dd, J = 7.9, 7.5, 2H); 7.11-7.29 (m, 12H); 7.43 (d, J = 8.4, 2H); 7.51 (d, J = 7.9, 2H)	160-162	804.9
4.9	1.27 (m, 4H); 1.65 (m, 4H), 2.67 (dt, J = 6.6, 4H); 2.92 (dt, J = 7.7, 3.3, 4H); 3.38 (dd, J = 8.1, 7.7, 2H); 3.87 (t, J = 7.0, 4H); 4.29 (d, J = 8.1, 2H); 4.43 (d, J = 9.7, 4H); 5.05 (d, J = 16.6, 4H); 6.14 (dd, J = 16.6, 10.7, 2H); 6.91 (dd, J = 7.9, 7.5, 2H); 7.21 (dd, J = 8.5, 7.5, 2H), 7.45 (dd, J = 8.5, 1.3, 2H); 7.78 (d, J = 7.9, 2H); 12.43 (s, 2H)	114-115	792.9
4.10	1.28 (m, 4H); 1.59 (m, 4H); 2.66 (d, J = 6.3, 4H); 2.91 (dt, J = 7.7, 3.3, 4H), 3.39 (2H, dd, J = 8.1, 7.7 Hz); 4.30 (d, J = 8.1, 2H), 4.78 (s, 4H); 6.91 (dd, J = 7.9, 7.5, 2H); 7.19 (m, 12H); 7.39 (d, J = 8.5, 2H), 7.79 (d, J = 7.9, 2H); 12.43 (s, 2H)	148-150	892.9
4.11	1.32 (m, 4H); 1.65 (m, 4H); 2.66 (d, J = 6.6, 4H); 2.90 (dt, J = 7.6, 3.4, 4H), 3.38 (dd, J = 8.1, 7.7, 2H); 3.49 (s, 6H); 3.86 (t, J = 7.0, 4H); 4.29 (d, J = 8.1, 2H); 7.01 (dd, J = 7.9, 7.5, 2H); 7.20 (dd, J = 8.4, 7.5, 4H); 7.43 (d, J = 8.4, 2H); 12.39 (s, 2H)	158-160	740.8
4.12	1.32 (m, 5H); 1.61 (m, 4H); 2.42 (dt, J = 7.5, 3.4, 4H); 3.3 (d, J = 7.8, 2H); 3.9 (q, J = 7.0, 5H); 4.3 (dt, J = 8.1, 4.0, 2H); 4.8 (s, 5H); 7.0 (d, J = 7.8, 2H); 7.2 (m, 12H); 7.4 (s, 1H); 7.8 (m, 2H); 12.09 (s, 2H)	145-146	921.0
4.13	1.37 (m, 4H); 1.65 (m, 4H); 2.4 (dt, J = 7.7, 3.3, 4H); 3.3 (m, 3H); 3.5 (s, 6H); 3.8 (m, 4H); 4.3 (m, 2H) 7.0 (m, 2H); 7.2 (m, 4H); 7.4 (d, J = 11.8, 2H); 12.11 (s, 2H)	138-140	768.8
4.14	1.27 (m, 4H); 1.59 (m, 4H); 2.79 (dt, J = 7.7, 5.2, 4H), 3.49 (s, 6H), 3.79 (m, 8H); 4.29 (d, J = 8.1; 2H); 6.90 (dd, J = 7.9, 7.5; 2H), 7.25 (dd, J = 8.4, 7.5, 4H); 7.29 (d, J = 8.4, 2H)	158-160	684.7
4.15	1.26 (m, 4H); 1.62 (m, 4H); 2.56 (6H, s), 2.99 (dd, J = 15.1, 1.8, 4H); 3.51 (dd, J = 8.1, 7.0, 2H); 3.59 (d, J = 8.1, 2H); 3.85 (t, J = 7.0, 4H); 4.77 (s, 4H); 6.95 (2H, dd, J = 7.9, 7.5), 7.23 (m, 12H); 7.39 (dd, J = 8.5, 1.3, 2H), 7.81 (dd, J = 7.9, 1.4, 2H)	140*	804.9

According to the results of the microbiological study none of the compounds tested significantly exceeded the reference drug cefalexin by the ability to inhibit the growth of bacterial test cultures. It was found that *S. aureus* showed sensitivity to 10 substances (**4.1-4.4** and **4.6-4.11**) among the *bis*-spiro derivatives tested (**4.1-4.15**). Nevertheless, onty two compounds **4.1** and **4.9** had the greatest activity against *S. aureus* among these *bis*-spiro derivatives (**4.1-4.15**). However, compounds **5.1**, **5.2**, **5.5**, **5.6**, **5.9**, **5.10** showed the activity against *S. aureus* at the level of cefalexin (32.0 ± 0.1 mm), in particular compound **5.5** (33.0 ± 0.3 mm) demonstrated the greatest antibacterial effect. The compounds with a free maleimidine residue exhibited greater activity, in particular substances **5.1**, **5.2** and **5.5** among a number of 1-R_1-3'-R_2- 5'-(6-N-maleimidogexyl)-3'a,6'a-dihydro-3'*H*-spiro [indole-3,1'-pyrrolo [3,4c]pyrrole]-2,4',6'-triones **5.1-5.10** exhibited the bactericidal action (MBC = 12.5 mcg/mL) relative to *S. aureus* (Fig. 1).

The test culture of *B.subtilis* was sensitive and highly sensitive to most of the test substances, but none of them exceeded cefalexin by the diameter of the growth inhibition zones (Fig. 2).

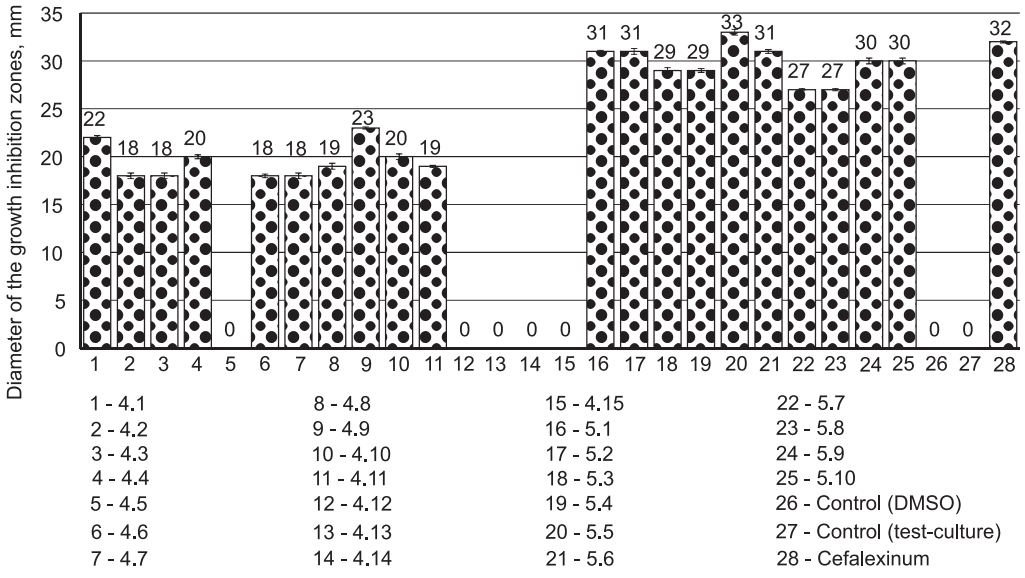


Fig. 1. The antibacterial activity against *S.aureus*

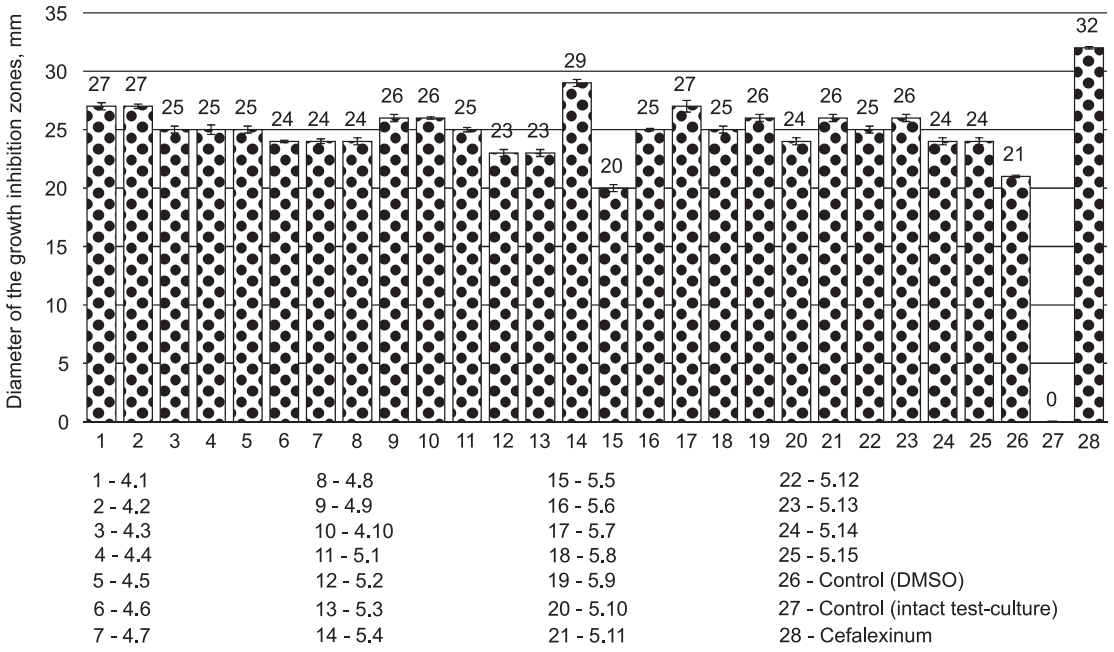


Fig. 2. The antibacterial activity against *B. subtilus*

The culture of *E. coli* was sensitive to compounds **5.7** and **5.8**. While the gram-negative test culture of *P. aeruginosa* was not sensitive to all compounds studied, except cefalexin (36.0 ± 0.1 mm), since the growth inhibition zones of *P. aeruginosa* were less than the solvent control in the study of samples.

Substances **4.1, 5.1, 5.2, 5.4, 5.6, 5.9, 5.10** exceeded fluconazole more than two times by the growth inhibition zones of the *C. albicans* culture. Only compound **4.1** (MBC = 12.5 mcg/mL) exhibited the fungicidal activity among the *bis*-derivatives (**4.1-4.15**) of spiro-2-oxindole, but it was surpassed by derivatives with the free maleimide residues **5.2** (MBC = 6.25 mcg/mL) and **5.1**, **5.5, 5.10** (MBC = 3.125 mcg/mL).

Thus, according to the results of the tests the substances synthesized showed predominantly the activity

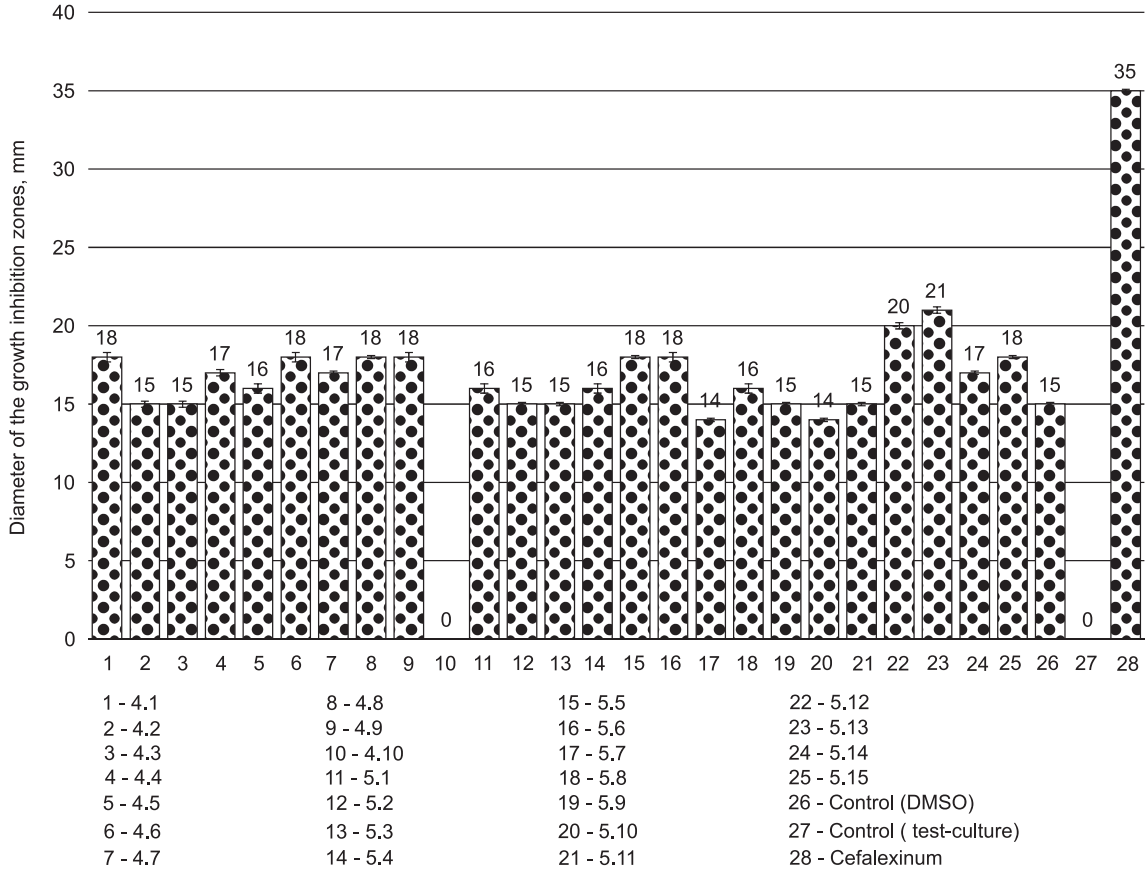


Fig. 3. The antibacterial activity against *E. coli*

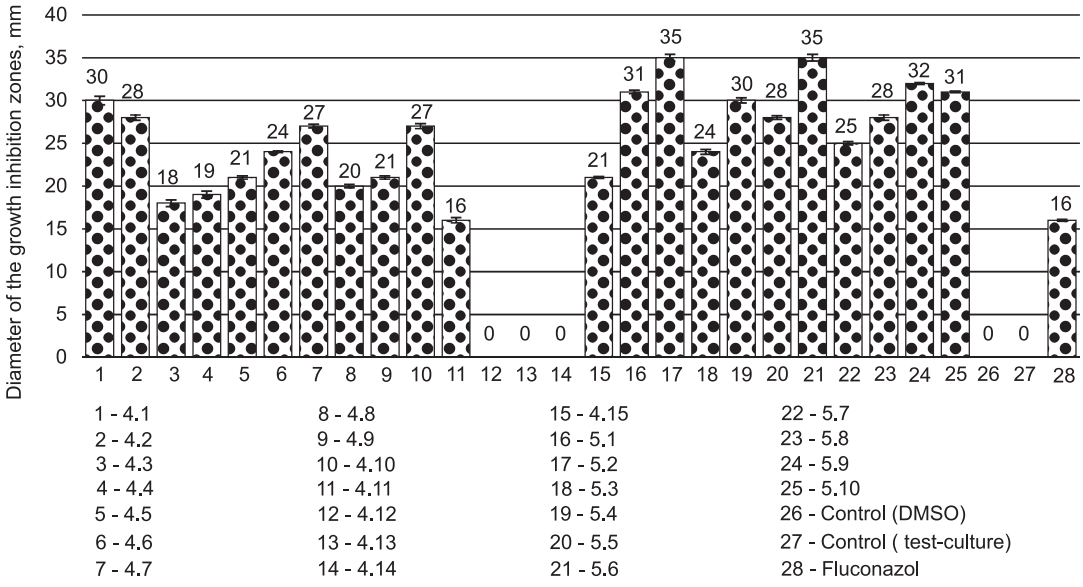


Fig. 4. The antibacterial activity against *C. albicans*

against gram-positive bacteria and yeast-like fungi. The following substances are promising for further research: in relation to *S. aureus* – **4.1, 4.9, 5.1, 5.2, 5.5, 5.10**, to *C. albicans* – **5.1, 5.5, 5.9, 5.6, 5.2, 5.10**.

Obviously, the more pronounced activity of compounds in the range of **5.1-5.10** compared to those among a number of bis-spiro-2-oxidol derivatives **4.1-4.15** can be explained by the presence of the first free maleimidic linker in the molecule, it provides irreversible fixation with the cysteine residues of the target protein bacterial cells.

The correlation of the high activity against *S. aureus* with their simultaneous antifungal action against *C. albicans* in the range of substances indicated in the experiment in vitro resulted in the possibility of their

Table 2

The MBC of the most active compounds selected

Compounds	MBC, mcg/mL*	
	S. aureus	*C.albicans*
4.1	–	12.5
5.1	12.5	3.125
5.2	12.5	6.25
5.3	50	-
5.4	50	-
5.5	12.5	3.125
5.6	50	–
5.9	50	–
5.10	–	3.125
Control (DMSO)	growth	growth
Control (intact test strains)	growth	growth
Cefalexin	2	n/t
Fluconazol	n/t	3.125

Notes: * – the average value for the experiments; n/t – not tested; "–" bacteriostatic activity.

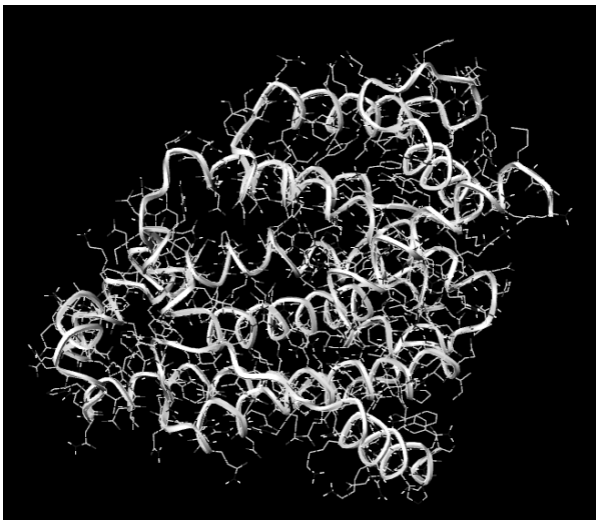


Fig. 5. The 3D-visualization of staphylococcal dehydrosqualen synthase CrtM (PDB ID 2zcq)

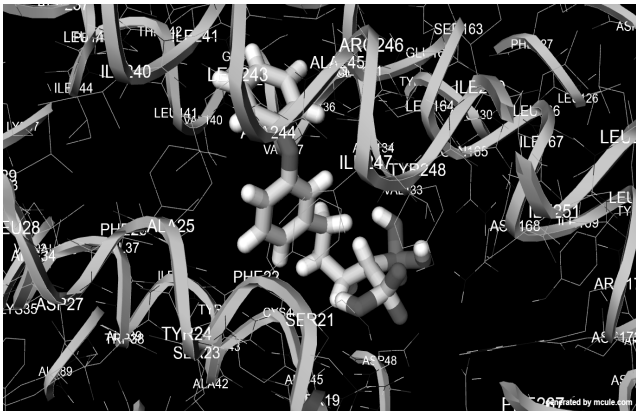
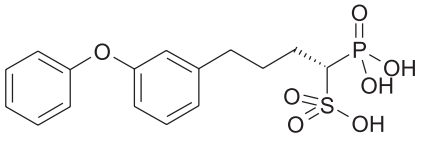


Fig. 6. The 3D-visualization of binding of the **BPH-65** (E_{Doc}=-9.2 kcal/mol) reference compound molecule to the active site of CrtM (PDB ID 2zcq) by the results of the molecular docking

Table 3

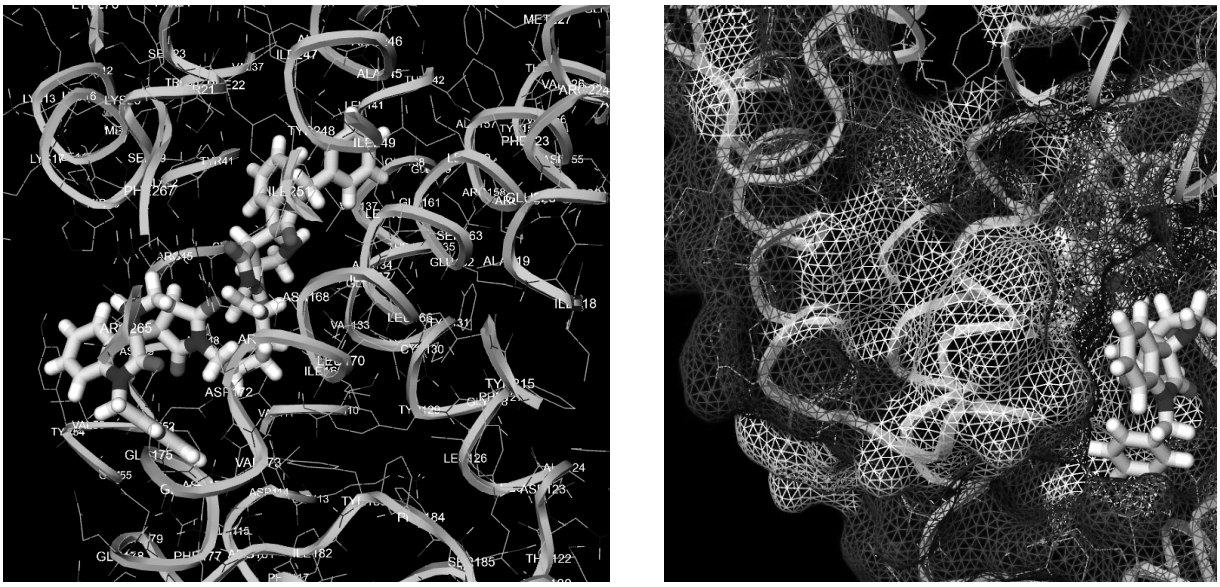
Molecular docking results for the compounds synthesized on dehydrosqualene synthase of *S. aureus* (CrtM, *PDB ID 2zcq*)

Compound	Docking score		Compound	Docking score		Compound	Docking score	
	Docking pose	E_{Doc}, kcal/mol		Docking pose	E_{Doc}, kcal/mol		Docking pose	E_{Doc}, kcal/mol
4.1	1	-12,7	**4.9**	1	-11,0	**5.2**	1	-10,8
	2	-12,5		2	-10,4		2	-10,6
	3	-11,9		3	-10,3		3	-9,0
	4	-11,8		4	-9,8		4	-8,4
4.2	1	-13,5	**4.10**	1	-12,0	**5.3**	1	-11,0
	2	-12,7		2	-12,0		2	-10,1
	3	-12,2		3	-11,6		3	-10,0
	4	-12,0		4	-11,2		4	-9,6
4.3	1	-12,9	**4.11**	1	-12,0	**5.4**	1	-10,5
	2	-12,7		2	-11,2		2	-10,2
	3	-12,7		3	-11,0		3	-10,0
	4	-12,4		4	-10,2		4	-9,7
4.4	1	-12,1	**4.12**	1	–	**5.5**	1	-10,3
	2	-12,0		2	–		2	-9,9
	3	-11,4		3	–		3	-9,6
	4	-11,3		4	–		4	-9,3
4.5	1	-12,2	**4.13**	1	-11,2	**5.6**	1	-11,1
	2	-10,9		2	-10,8		2	-10,2
	3	-10,3		3	-10,4		3	-9,9
	4	–		4	-10,2		4	-9,5
4.6	1	-12,6	**4.14**	1	-11,9	**5.7**	1	-10,5
	2	-12,5		2	-11,8		2	-10,2
	3	-11,6		3	-11,6		3	-10,1
	4	-11,3		4	-11,4		4	-9,7
4.7	1	-11,6	**4.15**	1	-12,9	**5.8**	1	-10,8
	2	-10,3		2	-12,4		2	-10,3
	3	-10,1		3	-11,7		3	-10,2
	4	–		4	-11,5		4	-8,8
4.8	1	-11,5	**5.1**	1	-10,6	**5.9**	1	-10,9
	2	-11,2		2	-10,6		2	-10,5
	3	-11,0		3	-10,4		3	-10,2
	4	-10,2		4	-10,2		4	-10,0

Compound	Docking score		Compound	Docking score	
	Docking pose	E_{Doc}, kcal/mol		Docking pose	E_{Doc}, kcal/mol
5.10	1	-11,0	**BPH-65(control)** 	1	-9,2
	2	-10,5		2	-9,0
	3	-10,2		3	-8,8
	4	-9,9		4	-8,3

effect on the synthesis of C_{30} isoprenoids of microbial cells. To substantiate this hypothetical mechanism of action of the compounds synthesized their precision docking on the 3D models of staphylococcal dehydrosqualen synthase CrtM was performed (Fig. 5).

The docking results of the corresponding structures are given in Tab. 3. In general, the values of the free energy of interaction (E_{Doc}) obtained for structures **4.1-4.15** and **5.1-5.10** characterized the ligands tested as highly active CrtM inhibitors. The maximum calculated energy gain in formation of the corresponding complex of ligand-enzyme E_{Doc} was -10.5 to -13.5 kcal/mol exceeding E_{Doc} for the CrtM complex with the reference structure of the known inhibitor **BPH-65**, which was -9.2 kcal/mol. Among the substances synthesized the structure **4.2** was the most active (E_{Doc} = -13.5 kcal/mol), some compounds such as **4.3, 4.15** (E_{Doc} = -12.9 kcal/mol) and **4.1** (E_{Doc} = -12.7 kcal/mol)), as well as **4.6, 4.5, 4.4, 4.10, 4.11**, showed the similar values of the docking score from -12.6 to -12.0 kcal/mol. Only for compound **4.12** any



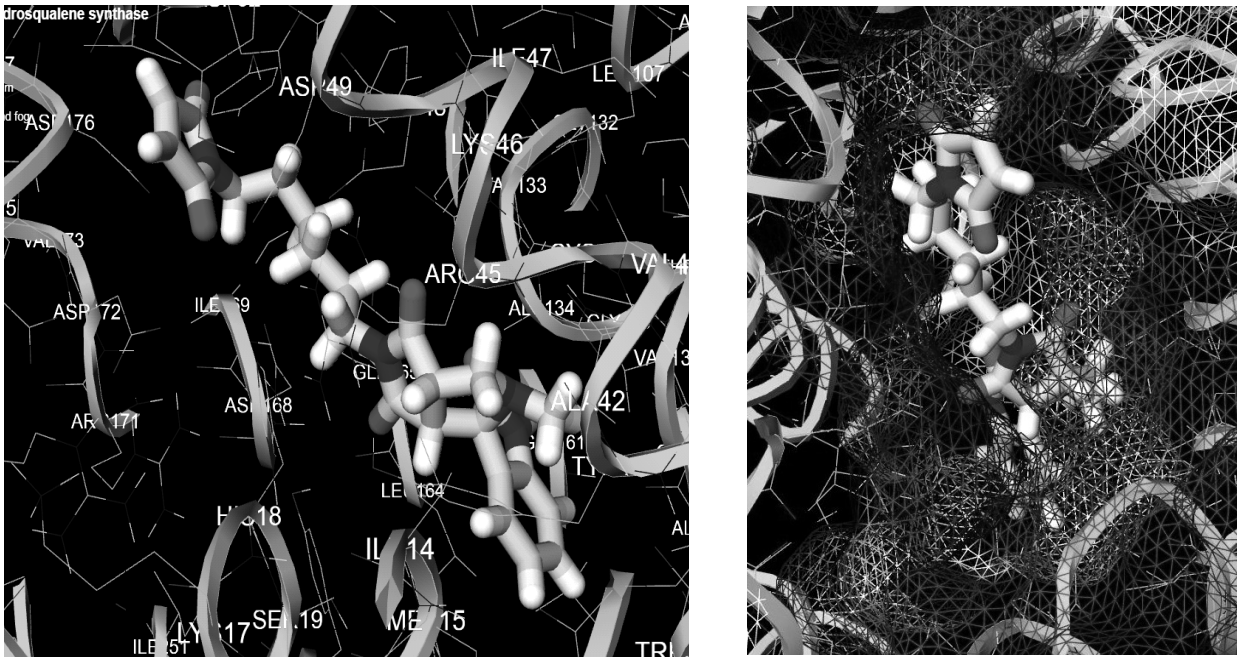
A B

Fig. 7. The 3D-visualization of binding of the molecule of compound **4.2** with the best docking score (E_{Doc} = -13.5 kcal/mol) with the active site CrtM (PDB ID 2zcq) according to the results of the molecular docking: **A.** Location of ligand **4.2** relative to the amino acid residues of the active enzyme center; **B.** Location of ligand **4.2** in the substrate-binding enzyme pocket

active position *in silico* was not generated. It also correlates with the absence of the antistaphylococcal and antifungal activity *in vitro*.

The validation of binding of the control structure of **BPH-65** to the amino acid residues of the active CrtM center after the results of the docking obtained by us *in silico* on the 3D CrtM model (Fig. 6) is close to the parameters of formation of the ligand-target complex obtained by the X-ray method for the co-crystal of the **BPH-65** compound with the CrtM enzyme in the literature [5].

In particular, as for location of the **BPH-65** ligand in the X-ray experiment, the π-π contact of the phenyl radical Phe22 with a nucleus of the biphenyl residue of the ligand, hydrophobic interactions with Val133, Val137 and formation of hydrogen bonds with Arg45, Asp48, Gln165, Asn168, Asp172, and its -P(O)$(OH)_2$ and -SO_3H polar residues reduced. Somewhat low docking energy when validating binding of the **BPH-65** ligand to the target can be explained by neglect of the *in silico* experiment to coordinate the ligand with two Mg^{2+} cations and water molecules.



A B

Fig. 8. The 3D-visualization of binding of the most active in vitro molecule compound **5.5** (E_{Doc} = -10.5 kcal/mol) with the active site of CrtM (PDB ID 2zcq) according to the results of the molecular docking: **A**. Location of ligand **5.5** relative to the amino acid residues of the active enzyme center; **B**. Location of ligand **5.5** in the substrate-binding enzyme pocket

As noted above, the highest gain in the docking energy was in *bis* derivative **4.2** most effectively filling the substrate-binding enzyme pocket (Fig. 7).

The binding of the ligand is provided by formation of hydrogen bonds with the key amino acid residues Arg45, Asp48, Asn168, Asp172, as well as π-π-contacts of the phenyl radical Phe22 with the benzene nucleus of the oxidol system of the ligand and the interaction of the hexamethylene chain of the molecule with Val133 and Val137. The oxo groups of 2-oxidol and imide cycles contribute to formation of intermolecular hydrogen bonds with Arg45, Arg265, and the secondary nitrogen atoms of the 3,1'-pyrrolo[3,4-c]pyrrole system contribute to formation of ligaments with the acid residues of Asp48, Asn168, Asp172. It is also possible to form π-π-contacts between the Tyr64 residue and the aromatic system of one of the 2-oxindol core of the molecule **4.2.** N-Benzyl radicals obviously contribute to the additional spatial overlapping of the substrate-binding pocket of the enzyme and the hydrophobic ligand contacts with the residues ILeu, Val, Leu.

Being the most active in the screening *in vitro* molecule **5.5** has the most effective position and forms hydrogen bonds with Arg45, Asp49, Asp52, Gln165, Asn168, Asp176, and the π-π contact of the phenyl radical Phe22 with the benzene nucleus of the 2-oxidol system of the ligand, orienting the maleimidine residue to the "entry" into a substrate-binding enzyme pocket (Fig. 8).

Moreover, an additional irreversible ligand binding of **5.5** may be hypothetically possible by formation of a covalent bond between its maleimide linker due to the nucleophilic attack of the sulfur atom of the Cys44 residue located in the same cavity substrate pockets of CrtM.

According to the literature, the active CrtM inhibitors are most likely to be coordinated at the active center of the enzyme with such amino acid residues as His18, Arg45, Asp48, Asp52, Tyr129, Gln165, Asn168 and Asp172 [16]. Thus, in our experiment the ability of molecules to form hydrogen bonds with at least six key amino acid residues – Arg45, Asp48, Asp52, Gln165, Asn168 and Asp172 has been found *in silico*.

CONCLUSIONS

It has been found that the one-pot three-component reaction of isatin, *L*-amino acids and 1,6-maleimidohexane depending on the molar ratio of reagents leads to *bis*-3'a,6'a-dihydro-3'*H*-spiro[indole-3,1'-pyrrolo [3,4-c]pyrrole]-2,4',6'-triones or to the corresponding asymmetric 6-*N*-maleimidohexyl derivatives.

The new substances synthesized have demonstrated a superior activity to gram-positive bacteria and yeast-like fungi. In particular, compounds **5.1, 5.2, 5.5, 5.6, 5.9, 5.10** have shown the activity against *S. aureus* at the level of cefalexin. The greatest antibacterial effect has been demonstrated by compound **5.5**. Substances **4.1, 5.1, 5.2, 5.4, 5.6, 5.9, 5.10** exceed fluconazole more than two times against *C. albicans*.

The molecular docking method has shown that the substances synthesized are potent highly active CrtM inhibitors. The greatest docking score was detected for *bis*-derivative **4.2**, which most effectively filled the substrate-binding enzyme pocket. The most active *in vitro* compound **5.5** by the docking score is superior to the known inhibitor **BPH-65**.

In the experiment in silico the ability of the molecules tested to form hydrogen bonds with at least six key amino acid residues – Arg45, Asp48, Asp52, Gln165, Asn168 and Asp172 of CrtM has been found. The 3D-model of binding of molecules of the compounds synthesized with the active center of staphylococci enzyme CrtM has been created. It can be used to effectively construct antimicrobial agents with a fundamentally new mechanism of the antimicrobial action.

Conflicts of Interest: authors have no conflict of interests to declare.

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