MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
faculty for foreign citizens' education
pharmaceutical chemistry department

QUALIFICATION WORK
on the topic: « **DEVELOPING NEW QUINOLONE-4-ONE DERIVATIVES TO COMBAT ANTIBACTERIAL RESISTANCE»**

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ANNOTATION

The paper presents the results of the search for new antimicrobial agents among 2-alkylquinoline-4(1H)-one derivatives. The reactivity of 2-methylquinoline-4(1H)-ones was studied for the targeted development of methods for the synthesis of compounds that can affect the processes of Quorum sensing. The application of the molecular docking method allowed us to select the most promising compounds for further research. The qualification work consists of an introduction, three chapters, general conclusions and a list of references. The content of the work is set out on 48 pages, illustrated with 10 figures, 2 tables, and contains 56 references.

Keywords: 2-alkylquinoline-4(1H)-one; virtual screening; molecular docking; quorum sensing; antibiotic resistance; AMR

АНОТАЦІЯ

В роботі представлені результати пошуку нових антимікробних агентів серед похідних 2-алкілхінолін-4(1H)-онів. Вивчено реакційну здатність 2-метилхінолін-4(1H)-онів для цілеспрямованої розробки методів синтезу сполук, які можуть впливати на процеси Quorum sensing. Застосування методу молекулярного докінгу дозволило відібрати найбільш перспективні сполуки для подальших досліджень. Кваліфікаційна робота складається зі вступу, трьох розділів, загальних висновків та списку використаних джерел. Зміст роботи викладено на 48 сторінках, ілюстровано 10 рисунками, 2 таблицями, містить 56 джерел літератури.

Ключові слова: 2-алкілхінолін-4(1H)-он; віртуальний скринінг; молекулярний докінг; відчуття кворуму; антибіотикорезистентність; AMP

CONTENTS

INTRODUCTION

Actuality of subject. Studies over the past decade have shown that most bacteria (more than 99%) exist in natural ecosystems as specifically organized biofilms attached to substrates, the formation of which is a complex, strictly regulated biological process. The study of biofilms is of great interest to researchers; it is primarily due to the fact that the ability of pathogenic bacteria to exist within biofilms creates great difficulties for medical practice, as it significantly increases the resistance of bacteria to antimicrobial drugs and host immune defense factors. The formation of bacterial biofilms is often the cause of severe, difficult-to-treat chronic diseases.

In the food industry, the formation of biofilms on food increases the risk of food contamination by pathogens; biofouling of pipelines, communications, equipment, oil platforms and, as a result, biocorrosion of these surfaces cause serious difficulties in the microbiological and oil refining industries. On the other hand, the formation of biofilms can be useful, for example, in biological water treatment. In addition, biofilms can provide increased resistance of producer bacteria to toxic substances present in the environment. The formation of biofilms by bacteria antagonists of phytopathogens promotes the competitive struggle of these bacteria with microorganisms - plant pathogens; this factor is important for the development of effective biocontrol methods. The above indicates the importance of studying the patterns of biofilm formation and the effect of various compounds on biofilms and their formation. Research on this problem is fundamentally relevant and important for medicine, biotechnology, and agriculture.

Purpose of work to study the reactivity of 2-methylquinoline-4(1H)-ions in the bromination reaction for the targeted development of methods for the synthesis of compounds that can affect the processes of quorum sensing of various bacterial communities and to conduct computational studies.

The object of the research development of potential drugs based on 2-alkylquinoline-4(1H)-one derivatives.

The subject of the research Methods of preparation, physicochemical and chemical properties of synthesized 2-alkylquinoline-4(1H)-one derivatives. Computer-aided drug design, appropriate preparation of both target and ligands, and then virtual docking screening.

Tasks of work for this objective the following tasks were supplied:

1. to study the peculiarities of the reactivity of 2-methylquinoline-4(1H)-ones in the bromination reaction;
2. to conduct a literature review on the presence of compounds that are inhibitors of Quorum Sensing and SAR analysis with further construction of a pharmacophore model;
3. conduct molecular docking at the binding sites of natural autoinducers of bacterial communication;
4. analyze the results and draw appropriate conclusions about how the length of the alkyl chain will affect binding to the active site of the target.

Methods of the research: Chemical synthesis, PC computer with OS Ubuntu 20.04 LTS. Non-commercial software OrenBabel, DataWarrior, QVina 2.0. Use these products to perform structure-based virtual screening.

The practical value of the results. New derivatives of 2-alkylquinolones were synthesized. A comprehensive analysis of the obtained virtual screening data was performed. Further analysis of this sample allowed us to identify two domains of compounds that have a fundamental difference in the type of binding to the PqsR (MvfR) of P. aeruginosa. The computer modeling is a valuable basis for further search for new compounds that can effectively counteract antimicrobial resistance.

The structure of the work. The work consists of an introduction, three chapters, general conclusions and list of references used, which is composed of 56 sources. Contents of work posted on 48 pages and contains 2 tables, 10 figures.

CHAPTER 1

Biofilms as a form of bacterial existence in nature

(Literature review)

AMR (Antimicrobial Resistance) refers to the ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to resist the effects of antimicrobial drugs. It occurs when these microorganisms evolve and develop mechanisms to withstand the drugs that were originally effective in treating infections caused by them. AMR is a global health concern as it poses a threat to the effective prevention and treatment of various infectious diseases. The misuse and overuse of antimicrobial drugs in humans, animals, and agriculture contribute to the emergence and spread of AMR. When microorganisms become resistant to commonly used antimicrobial drugs, infections caused by them become more difficult to treat, leading to prolonged illness, increased healthcare costs, and higher mortality rates. AMR can occur naturally over time, but the misuse of antimicrobial drugs accelerates the process. It is important to promote responsible and appropriate use of antimicrobial drugs, develop new antimicrobial agents, and strengthen surveillance and control measures to combat AMR effectively. Additionally, raising awareness, implementing infection prevention and control practices, and promoting research and development of new diagnostics and vaccines are crucial in addressing the AMR challenge.

AMR further exacerbates the challenge of treating biofilm-associated infections. The inherent resistance mechanisms possessed by biofilm-forming microorganisms, combined with acquired resistance due to AMR, make these infections particularly difficult to manage. Within biofilms, microorganisms can undergo genetic changes, exchange genetic material, and develop mechanisms that render them less susceptible to the effects of antimicrobial drugs. This can lead to persistent and recurrent infections that are refractory to standard antibiotic therapies.

The presence of biofilms can enhance the survival and growth of antimicrobial-resistant strains within a population, allowing them to persist and

spread in various environments. The protective matrix of the biofilm acts as a physical barrier that hinders the penetration of antimicrobial agents and shields the microorganisms from the host immune response. Moreover, the altered physiology and reduced metabolic activity of biofilm-associated microorganisms contribute to their decreased susceptibility to antimicrobial drugs.

Addressing the challenge of biofilm-associated infections with AMR requires a multidisciplinary approach. Strategies such as the development of novel antimicrobial agents that target biofilms, optimization of drug delivery methods, enhancement of host immune response, and improvement in infection prevention and control measures are crucial. Additionally, understanding the interplay between biofilms and AMR at a molecular level can aid in the design of effective treatment strategies and the development of new therapeutic interventions.

1.1. Stages of biofilm formation

Biofilms are a form of microbial communities fixed to various abiotic and biotic surfaces. Biofilms are composed of microbial cells and their associated extracellular matrix, which is made up of polysaccharides, proteins, and DNA. Biofilms are ubiquitous in nature. They also line oil pipelines, aquariums, indwelling catheters, internal implants, contact lenses, and prostheses. Everyone is familiar with such an example of a biofilm as plaque, a thin layer that forms on teeth. Bacterial biofilms are resistant to various stresses, including antibiotics and disinfectants. In biofilms, bacteria are protected from phagocytosis and other components of innate and acquired immunity.

In nature, several species of microorganisms usually coexist in biofilms as a single community. It has been shown that numerous physiological processes, including the production of metabolites and biologically active substances, occur in biofilms differently in comparison with pure planktonic bacterial cultures. The reaction of microorganisms to changes in environmental conditions in a biofilm

differs significantly from the reaction of each individual species in a monoculture. Such an organization ensures its physiological and functional stability and, therefore, is the key to competitive survival in an ecological niche.

Biofilms can also be very dangerous, as they are often formed in various infectious pathologies. The course of infectious diseases can be complicated precisely because of the formation of microbial biofilms in the body. Many chronic diseases are associated with biofilm infections, such as cystic fibrosis pneumonia, otitis media, dental and parotid tissue pathology, osteomyelitis, urinary tract infections, and others. It is believed that up to 80% of all human bacterial infections are associated with the formation of biofilms.

The study of biofilms is currently of great interest to researchers, mainly because this way of bacterial existence poses major problems in medical practice.

At the beginning of the 20th century, A. T. Henrici and later J. Costerton and colleagues pointed to the existence of populations of microorganisms living on surfaces. At the same time, it was first discovered that bacteria forming such surface fouling were capable of exhibiting new properties, such as resistance to antimicrobial agents.

The ability of microorganisms (fungi and bacteria) to switch between free-living and attached states under different conditions is now well known, using biofilm as a factor of colonization, pathogenicity, virulence and protection from environmental conditions.

Thus, the accumulation of microorganisms attached to a surface and the specific polymeric substances they release into the environment are called biofilms. The surface on which the biofilm is formed is conventionally called the substrate.

During its development, a biofilm goes through a number of stages (Fig. 1). The first stage involves the adhesion or sorption of microorganisms to the surface of a substrate from the environment (most often a liquid medium). This stage is reversible, as the adherent cells can return to the planktonic form of existence. The second stage is the final attachment of cells to the surface and is called fixation. At this stage, microbes secrete extracellular polymers that ensure strong adhesion. At

the third stage, microcolonies are formed - separate clusters of adherent cells. At this stage, the cells actively divide, and the released matrix holds the entire colony together. Finally, the microcolonies merge, and a mature biofilm with a complex three-dimensional structure is formed. It can change its size and shape.

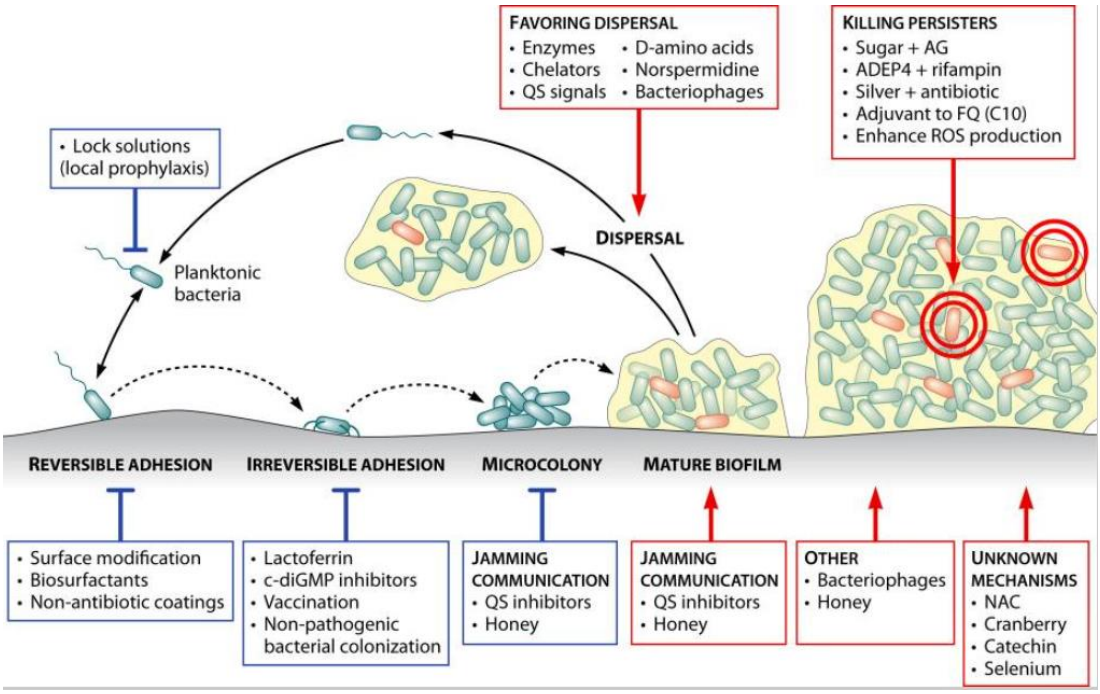


Fig. 1. Stages of biofilm formation [D. Lebeaux et al., 2014].

The extracellular matrix protects it from external threats. Also, for many bacteria with developed motility, the cell monolayer stage is distinguished. In this case, the cells are able to spread along the substrate, using various cell surface elements (saws, fimbriae) and forming schwermers - elongated cells with several chromosomes. Also, especially when the nutrient content is low, cells are able to leave the biofilm and turn into a planktonic form, which is called dispersion (bacterial release). As a result of dispersal, individual cells periodically detach from the biofilm, which can then attach to the surface and form a new colony. Inside mature biofilms, a population of persisters is isolated - cells with special resistance to antibiotics.

1.2. Structural organization of biofilms

Biofilms consist of microbial cells and a matrix. According to modern concepts, a living full-fledged biofilm is a formation formed by microcolonies of microorganisms in the form of towers or fungi (15-20% of the volume) and an exopolymer matrix (75-85% of the volume). Polymeric substances released by adherent cells into the external environment are called the matrix or extracellular polymeric substance (matrix, EPS). The matrix is highly hydrated, according to some estimates, 97% of it consists of bound water. Its structure resembles a "sponge," i.e., it has a porous structure that permeates low molecular weight compounds but retains large protein molecules and environmental particles. Among other things, the structure of biofilms often contains water channels through which substances dissolved in the medium enter the lower layers of cells.

The main components of the matrix are polysaccharides, proteins, and extracellular DNA (Figure 2).

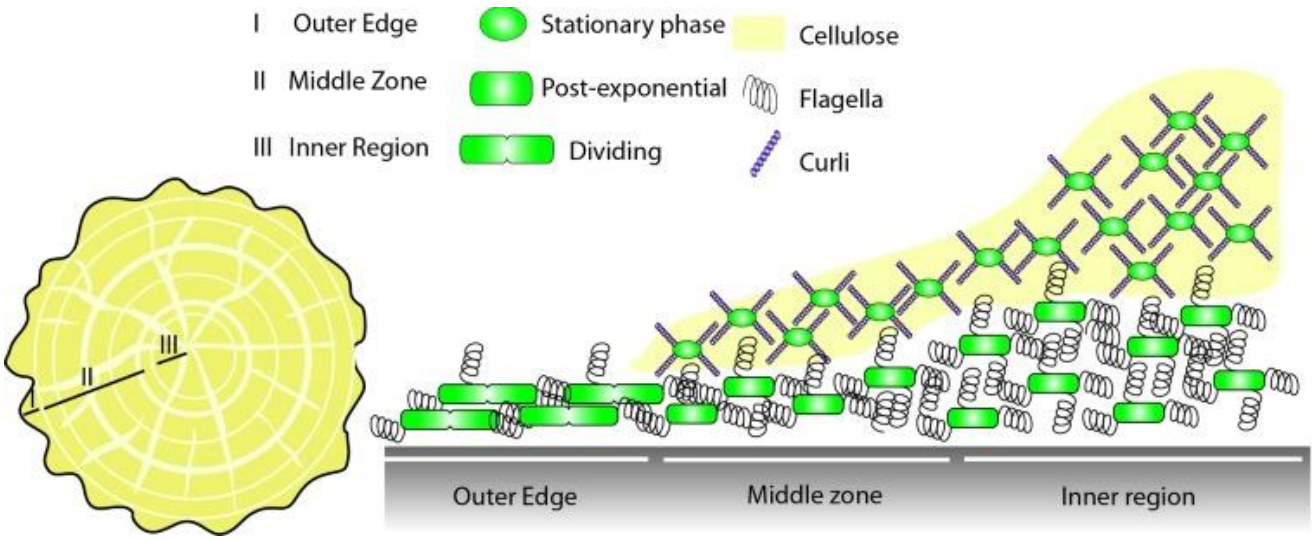
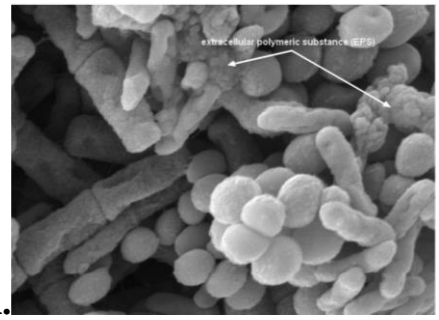


Fig. 2. Structural organization of biofilm [L. Hobley et al., 2015].

The composition of the matrix can vary greatly depending on the environmental conditions and the type of microorganisms. For example, in urinary catheters, the accumulation of inorganic calcium, magnesium, and phosphorus

compounds with the formation of crystallins is often observed on the surface of biofilms (Figure 3A).

А. 

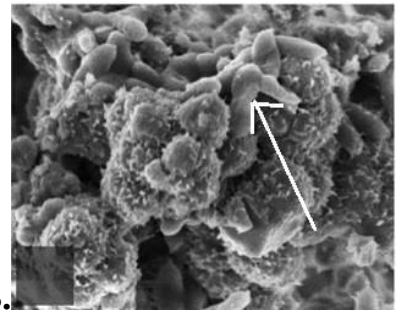
Б. 

Fig. 3. A - Crystallines (hydroxyapatites, struvites) on the surface of P. mirabilis biofilms in catheters [Holling et al., 2014]; B - Extracellular matrix (EPS) in a mixed biofilm formed by P. aeruginosa, S. aureus and Bacillus sp. [Otto, 2008].

Polysaccharide components of biofilms are commonly called exopolysaccharides. The most important condition for the appearance of a mature biofilm is the production of this component. Some researchers believe that the presence of a polysaccharide component is mandatory for all biofilms. Sugars can vary in their structure and composition; for the most part, they are represented by beta-1,6-N-acetyl-D-glucosamine and cellulose. The simplest method of visualizing them is immunofluorescent staining. Specific dyes for polysaccharide components and cellulose staining microscopy (e.g., calcofluor) are used. Exopolysaccharides can account for up to 80% of the total biofilm matrix.

Proteins account for up to 40% of the total matrix volume of biofilms. It has been found that the bulk of proteins in many biofilms are represented by amyloid-like proteins: curli fibers (CsgA/CsgB proteins) in Gram-negative bacteria and TasA/TapA proteins in bacilli. Amyloid-like structures of bacteria have attracted the attention of researchers as major components of bacterial biofilm matrices and simple models for studying infectious amyloids associated with neurodegenerative diseases such as prion diseases, Alzheimer's and Parkinson's. The amyloid groups in the protein determine its high thermal stability and adhesive ability. In addition, biofilms contain a number of specific proteins called bap-family proteins, lectins and

sugar-binding proteins, and autotransporters. These proteins play a role in intercellular contacts, cell-cell and cell-substrate adhesion, and also contribute to the binding of polysaccharides in the biofilm structure.

Extracellular DNA (eDNA) can be released by vesicular transport, but the main way it is released into the matrix is through cell lysis. This DNA is involved in horizontal gene transfer in films and signal transduction between cells. Also, extracellular DNA can play a structural role and serve as a target for exonucleases.

The total DNA content in the matrix is small. The ratio of matrix to cells in a biofilm can vary greatly. It is believed that in some cases, cells can occupy only 10% of the biofilm by volume. Cells in biofilms can be at different stages of the cell cycle (Fig. 1).

Thus, dead and destroyed cells are also part of the biofilm. Biofilms formed by several types of microorganisms are called multispecies. Biofilms formed by fungal filaments are called fungal. Most often in the natural environment, microorganisms coexist in mixed biofilms, jointly producing exopolysaccharides and proteins.

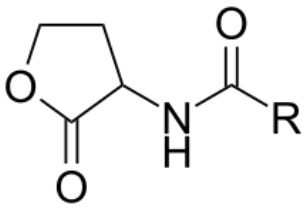
1.3. Regulation of biofilm formation

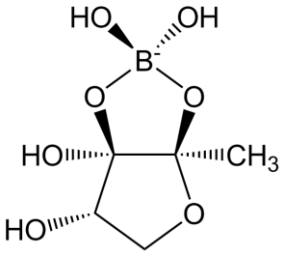
There are many genetically determined programs that regulate biofilm formation at different stages. The essential links in these processes at early stages are cell-environment and cell-surface interactions. At later stages, the regulation changes its nature somewhat. The driving force in the development of the bacterial community is self-organization and cooperation between cells, rather than the classical "competitive" natural selection of individual microorganisms.

The most important mechanism of such regulation in a cellular community is quorum sensing (QS). Quorum sensing is a special type of regulation of bacterial gene expression that depends on the population density. Specialized substances called auto-inducers can be secreted by cells and accumulate in the environment during the growth of the cell population. The response of the cell depends on the

concentration of the inducer in the medium, which, upon reaching a critical value, triggers a specific genetic program in each cell of the community. As a result, a high degree of coordination of gene expression and adaptation mechanisms is achieved.

N-acetylhomoserine lactones (AHL or AGL) play the role of autoinducer messengers in Gram-negative bacteria, and oligopeptides in Gram-positive bacteria. Messengers, also called type II autoinducers, are responsible for signal transduction between Gram-positive and Gram-negative microorganisms, most often represented by furanosyl borate-diefferent homologs.





The phenomenon of QS-regulation was first discovered in the early 70s when studying luminescence in the bacterium Vibrio fischeri. The system was named LuxI/LuxR. To date, it is the most well-studied model of quorum sensing in Gram-positive microorganisms. AGP molecules move freely between the medium and the cell with the help of membrane-bound proteins. The QS system in Gram-positive bacteria is considered to be more complex, since the autoinducer (oligopeptide) does not enter the cell directly, but is detected by an extracellular specific kinase.

Signals from the QS system can have both positive and negative effects on biofilm formation. In V. cholerae biofilms, cell density is negatively regulated by the QS system, i.e., the ability to form biofilms is reduced by a complex multilevel cascade of biochemical processes that start and stop the expression of genes for extracellular matrix production and cell division.

In S. aureus biofilms, a cascade related to quorum sensing also inhibits biofilm growth. The initially synthesized linear signal peptide undergoes maturation: the final products have a thiolactone ring and vary in size from seven to nine amino acid residues in length. The length of the signal peptide, as well as its amino acid composition, varies from strain to strain. The effects of signal peptides of similar type are summed up, while peptides that differ in structure can interfere with each

other. As a rule, the synthesis of polypeptides leads to the expression of proteolytic enzymes involved in the process of biofilm dispersal. High glucose content in the medium often leads to repression of the quorum sensing system, which directly leads to the formation of denser biofilms.

The role of quorum sensing in the formation of biofilms in P. aeruginosa has been clearly demonstrated. Two QSs have been described in this opportunistic pathogen: LasI-LasR and RhlI-RhlR. The LasI protein is responsible for the production of the autoinducer N-3(oxo-dodecanoyl) homoserine lactone (3OC12-HSL), and the RhlI protein is a synthase of N-butanoyl homoserine lactone (C4-HSL). QS in P. aeruginosa controls the expression of more than 600 genes. Extracellular DNA is also involved in the control of biofilm formation in pseudomonads. The signal positively stimulates biofilm growth, production of polysaccharides and DNA in the medium.

Since QS systems are involved in the control of bacterial virulence and biofilm formation, QS inhibitors may be of pharmaceutical importance, and drugs are developed on their basis to combat bacterial pathogenicity.

Other important regulators of biofilm formation include c-di-GMP (cyclic di-guanosine monophosphate), which is involved in the control of exopolysaccharide synthesis, and small RNAs. The role of these molecules in the regulation of biofilms is being actively studied.

1.4 The role of biofilms in nature and human infectious pathology

Under natural conditions, microorganisms can exist either as planktonic (free-floating) cultures or as biofilms. According to current knowledge, 95-99% of microorganisms in their natural habitats exist in the form of biofilms. Biofilms cover abiotic surfaces, organs and tissues of living organisms.

In nature, bacteria usually exist in the form of complex communities consisting of representatives of different species. Communities of microorganisms are able to realize normal metabolic cycles in natural conditions. For example, dense

biofilms - bacterial mats - form on the walls of caves, on the surface of mineral deposits, and around hot springs. The mats of photosynthetic, methanogenic and sulfate-reducing bacterial communities are well studied, as well as the community of microorganisms that develops in wastewater treatment plants. This type of biofilm can often reach a thickness of several tens of centimeters. Particularly important in this case is the ability to coordinate metabolism throughout the community through specific messenger compounds. The biofilm also binds water well, which is an essential component of most metabolic pathways involving inorganic compounds.

In biotechnology, the growth of microorganisms on abiotic surfaces is considered to be an extremely undesirable phenomenon. Cleaning bioreactors, turbines, and filters from biofilms is costly and, unfortunately, not always effective. The formation of biofilms on equipment often leads to contamination of media with undesirable microflora, low efficiency of biotechnology systems, corrosion and damage to equipment. Rhizosphere microorganisms and rhizo-plankton form biofilms on the surface of plant roots and roots. These microorganisms are able to stimulate plant growth by releasing a huge number of different biologically active compounds, and are also able to protect plants from pathogenic soil microflora.

In the human body, representatives of normal microflora form biofilms on the surface of various organs and tissues. The resident microflora of the mucous membranes of the oral cavity, intestines, and skin is present in the form of biofilms. For example, dental biofilms (plaques), formed by a complex community of many microorganisms, are very well studied. A strict sequence of colonization by different microorganisms, types and mechanisms of interaction between organisms have been described for dental plaques. Hundreds of species of microorganisms have been isolated from dental biofilms. The formed communities of microorganisms of the normal human microbiota protect the body from invasion and development of foreign and pathogenic microflora.

However, bacterial biofilms are of serious importance in clinical medicine. If the immune system is weakened in the course of a disease and medical intervention, secondary infections (i.e., concomitant infections) caused by bacteria from the

environment or normal human microflora may occur in the human body. In this case, biofilms can serve as a factor directly related to the penetration of infection and pathogenesis, as well as a reservoir for pathogenic microorganisms, a "transit point" for colonization of an organ or organ system. Biofilms are one of the pathogenetic factors in the formation of chronic infectious processes. According to various sources, 60-65% to 80% of all microbial infections are associated with the formation of biofilms. There are direct tissue infections and indirect device-related infections associated with the colonization, adhesion and persistence of pathogens on the surface of medical devices and catheters (Fig. 4).

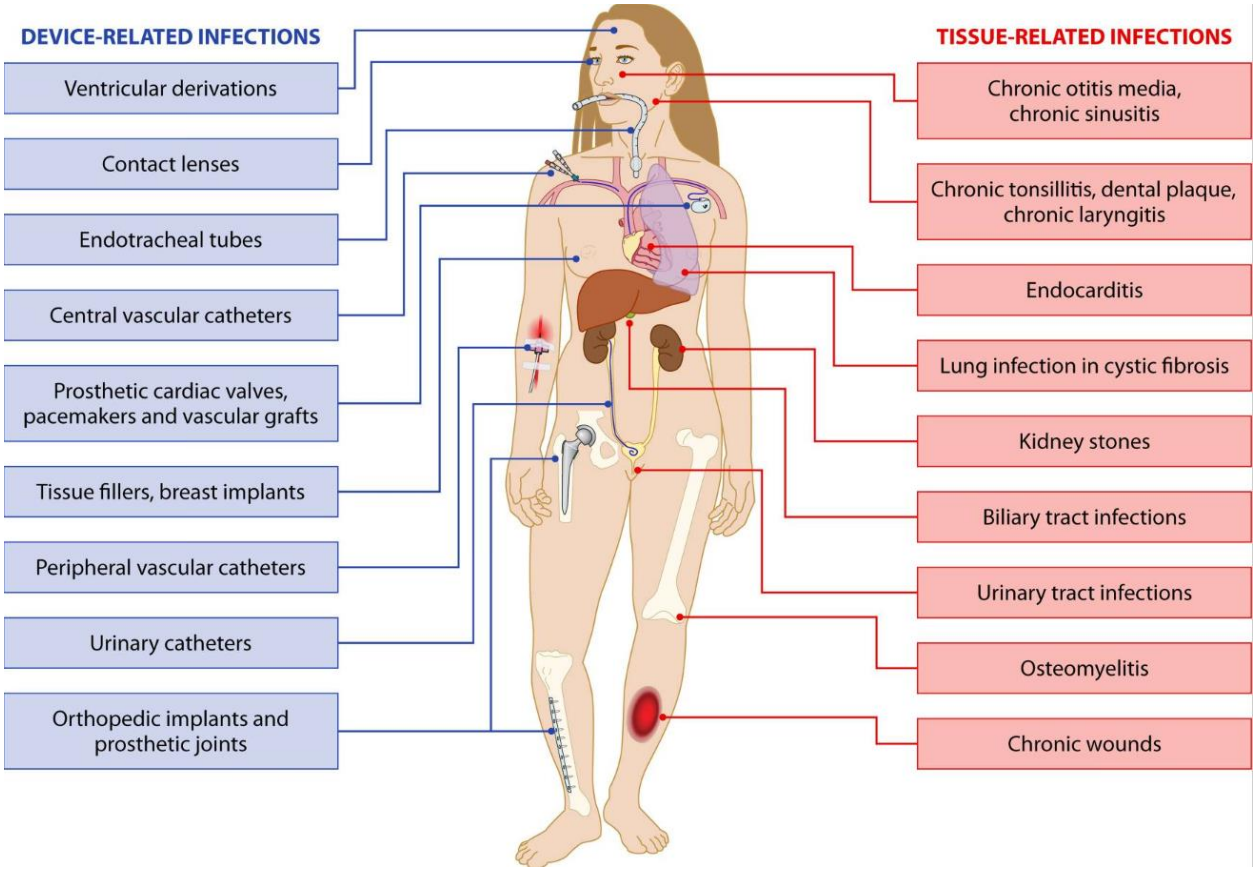


Figure 4. Infections associated with biofilm formation [Lebeaux et al., 2013].

It has been shown that both gram-positive (Enterococcus spp., Staphylococcus spp., Streptococcus viridans) and gram-negative (E. coli, K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter spp. and others) bacteria can form biofilms. Pathogenic, conditionally pathogenic and non-pathogenic microorganisms, as well as their associations, are capable of forming biofilms in the

human body (Table 1). The involvement of film-forming bacteria in various human pathologies has been established: chronic otitis media and sinusitis, pulmonary infections, exacerbation of gastric and duodenal ulcers, Crohn's disease, myocarditis, atherosclerosis, bronchial asthma, type I diabetes, vaginosis, caries, periodontal disease, etc. (Fig. 4).

Table 1

Microorganisms that form biofilms in medical practice

Microorganism	Medical equipment
Staphylococci Coagulase-neg.	• Artificial stem prosthesis • Artificial vocal prosthesis • Central venous catheter • Intra-ureteral devices • Prosthetic heart valve • Urinary catheter
Enterococcus spp.	• Artificial stem prosthesis • Central venous catheter • Intra-ureteral devices • Prosthetic heart valve
Klebsiella pneumoniae	• Central venous catheter • Urinary catheter
Pseudomonas aeruginosa	• Artificial stem prosthesis • Central venous catheter • Urinary catheter
Staphylococcus aureus	• Artificial stem prosthesis • Central venous catheter • Intra-ureteral devices • Prosthetic heart valve

The ability of bacteria to form biofilms on the surface of various medical instruments and equipment, catheters and implants creates major problems in medical practice (Table 2). Thus, currently, in surgery, the problems of microbial biofilms formed on medical implants, catheters, prostheses, heart valves and technical structures implanted during surgeries are coming to the fore. Chronic infections of

implanted medical devices can lead to sepsis and death, especially in immunocompromised patients, so the development of biofilms on synthetic implants is a major challenge for their successful implementation and efficient functioning.

Table 2

Medical devices on the surface of which biofilms can form

Catheters	Implants	Medical devices
Central venous catheter	Cardiac pacemaker	Biliary stent
Hemodialysis catheter	Arteriovenous shunt	Mechanical heart valve
Pulmonary artery catheter	Spinal implant	MOS structures
Arterial catheter	Breast implant	Prosthesis of the joints
Urinary catheter	Orthopedic prosthesis	Vascular transplant
Peritoneal dialysis catheter	Cochlear implant	Internal ureteral device
Enteral tubes	Neurosurgical stimulator	Coronary stent
Gastrostomy tube	Middle ear implant	Vascular shunt
Nasogastric tube	Dental implant	Intracranial devices
Endotracheal tube	Voice prosthesis	Intraocular lenses
Tracheostomy tube	Implanted electrode defibrillator	Suturing material
Umbilical catheter		Contact lenses

The formation of biofilms explains the peculiarities of catheter-associated infection in urological patients. Bacteria enter the urinary tract from the urethra during catheter insertion through the lumen of the catheter in an upward motion. In addition, large overgrowths can impede the flow of fluid through the catheter or even

disable the inserted medical device. Thus, the prevention of catheter-associated infection is to prevent catheterizations, bacteriuria, and its complications.

1.5. Resistance of bacteria in biofilms and methods of biofilm control

Bacteria living in biofilms differ significantly in their biological properties from planktonic forms. The interaction of biofilms with the human immune system and antibiotic-resistant bacteria in biofilms attracts the most attention. Bacteria in biofilms become difficult to attack by immune system factors. For example, it has been shown that leukocytes found even inside S. aureus biofilms are unable to phagocytize bacteria. It is assumed that the biofilm has mechanisms that can inhibit the normal functions of phagocytes.

The structure of biofilms and the peculiarities of the physiology of film bacteria provide for a multiple increase in the community's resistance to antimicrobial drugs compared to planktonic cultures. It turned out that the microorganisms that make up biofilms are 100-1000 times less sensitive to most antibiotics and other biocidal substances than planktonic cells. The nature of this resistance is being intensively studied. It is assumed that such resistance can be caused by various mechanisms: 1) difficulty or inability of antibiotics to penetrate deep into the matrix; 2) binding and inactivation of the antibiotic by polymers or matrix proteins; 3) slower rate of bacterial division in biofilms; 4) presence of metabolically inactive cells in biofilms that are insensitive to antibiotics.

The ability of biofilm bacteria to survive in the presence of antibiotics in concentrations many times higher than standard therapeutic concentrations creates difficulties in the treatment of many bacterial infections associated with the formation of biofilms in the body. Currently, the idea of the existence of special "persistent" cells in biofilms is popular. Persisters (from the English word persist - to persist, to persist) are a small part of the bacterial population (less than 0.1%) that have increased resistance to antimicrobial drugs. Different scientists put different meanings into this term. Some define persistent forms as all those that can survive the effects of antibiotics regardless of the mechanism. Others consider persisters to

be only those cells that enter a state of slow metabolism - dormant persisters, which are able to regulate their own stages of growth and proliferation, remaining tolerant to a dose of antibiotic that is lethal to most other bacteria in the population. While most planktonic persisters are attacked by cells of the immune system, persisters in the thickness of biofilms remain inaccessible. It is the persistent cells that will cause recurrent infections, and can also become potential producers of resistant (with specific programs to counteract the antimicrobial drug) clones. The nature of persister cells remains largely mysterious. It is believed that persistent cells express specific genes (in particular, the hipA gene) that act in a toxin-antitoxin model and block antibiotic targets, which limits growth (puts cells in a dormant state) but makes them resistant to these inhibitors.

The possibility of microorganisms "surviving" in biofilms through the formation of other forms (e.g., classical endospores), as well as through the formation of ultra-microbials (nanobacteria), should not be underestimated. However, the scale and significance of these processes have not yet been sufficiently studied. The problem of suppression or destruction of bacterial biofilms is an extremely urgent task, since in the clinic classical methods of antibiotic therapy of purulent-inflammatory infectious processes are often ineffective or unpredictable due to the high resistance of pathogens to biofilms. Currently, there are no means to ensure the direct and complete destruction of biofilms, but there is an understanding of how to create and develop approaches to prevent the formation, control the growth and destruction of biofilms. The search for substances that destroy biofilms and facilitate the access of antimicrobials to bacterial cells is actively underway. Materials and devices are being developed with antibacterial and anti-adhesive properties that prevent the formation of biofilms, for example, on implants.

Currently, there are several areas of promise in the fight against biofilms:

1) prevention of primary infection of implants;

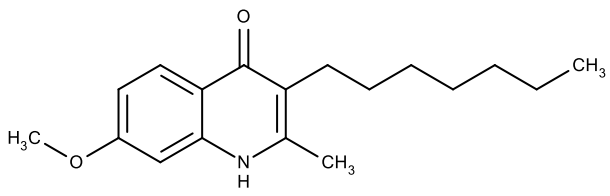
2) minimizing the initial adhesion of microbial cells;

3) development of methods for penetration of various biocides and antibiotics through the biofilm matrix to suppress the activity of cells inside the biofilm;
4) blocking the synthesis or destruction of the matrix;
5) disruption of intercellular information exchange (inhibition of QS regulation).

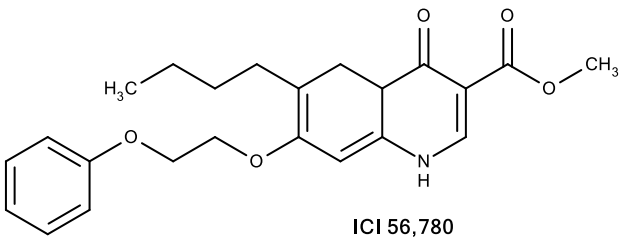
1.6. Current trends in the search for new biologically active substances among alkylquinolone derivatives

Since its discovery, quinoline and its derivatives have been used as anti-malarial drugs. In November 2007, the Bill & Melinda Gates Foundation, with the support of the World Health Organization (WHO), the Global Fund (GF) and the Affordable Medicine Foundation for Malaria (AMFm), announced the start of the global malaria eradication effort. WHO has adopted an ambitious plan to eliminate malaria by 2050.

The well-known antimalarial drugs of alkylquinolone derivatives are Endoquine (1946) and IP 56,780 (1968).



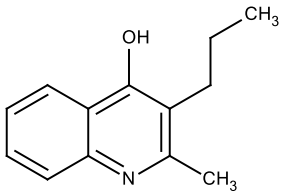
Endochin



ICI 56,780

After the announcement of the global malaria control program, researchers again turned their attention to the "old" drugs, and in the period 2009-2019, a number of fundamental articles appeared on the synthesis and pharmacological screening of new endoquinone-like alkylquinolones. Analyzing the new results, we can once again verify that the 4(1H) quinolone scaffold is a good basis for the design of new

antimalarial drugs. According to the ChEMBL database of the European Bioinformation Institute, as well as the published results of the Novartis screening program, both alkyl-substituted quinolone-4-ones and various halogen-, aryl-, alkene- and aminoalkyl-derivatives of quinolone-4-ones are considered active and confirmed hit compounds.



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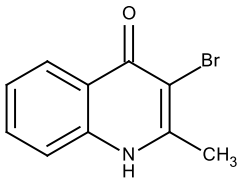

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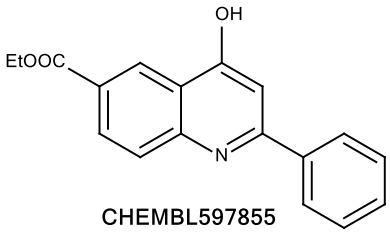
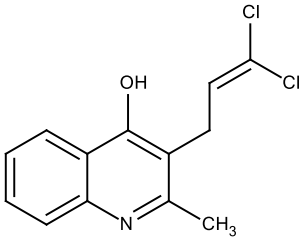


CHEMBL597855



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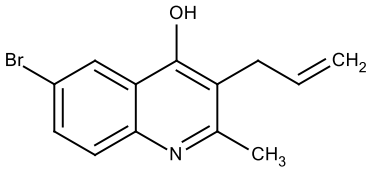

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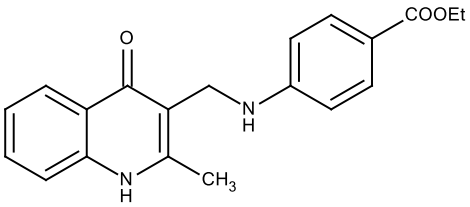

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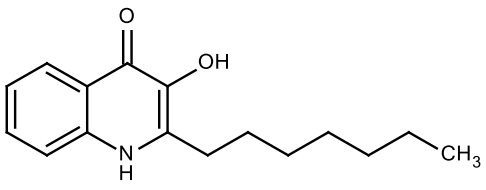
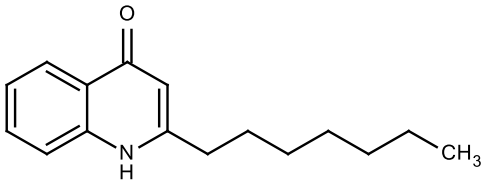
Even more interesting is the task of studying and precisely establishing the role and mechanism of 2-alkylquinoline-4-ones in the phenomenon of bacterial regulatory systems (QS). QS is a communication mechanism used by bacteria to coordinate gene expression with the help of low molecular weight signaling molecules called auto-inducers. The synchronization of gene expression allows bacterial populations to acquire new properties that are beneficial to bacterial colonies in the process of bacterial interaction with higher organisms. Such properties of colonies can include, for example, the regulation of bioluminescence, the formation of biofilms, and the release of virulence factors. The phenomenon of QS was discovered in 1970, but after a relative silence in the study of this phenomenon, the last decade, and especially since 2007, there has been a boom in the study of QS

From the point of view of practical pharmacy, the study and inhibition of QS systems can lead to the creation of new drugs, which are proposed to be called "pathogenicity poisons", since they, unlike classical antimicrobials (primarily antibiotics), do not have bactericidal or bacteriostatic effects on pathogenic bacteria, but act at the level of "communications" in bacterial colonies. At the same time, "pathogenicity poisons" will not create selective pressure that leads to the formation of bacterial forms resistant to antibacterial substances.

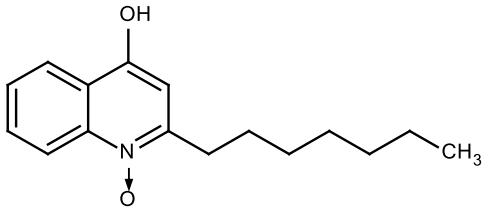
One of the main groups of QS signaling molecules is quinoline-4-ones/quinolines (4Qs), which were first discovered in the Gram-negative bacteria Pseudomonas aeruginosa.



2-heptyl-3-hydroxy-4(1H)-quinolone (PQS)



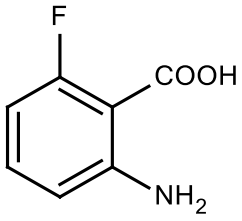
2-heptyl-4(1H)-quinolone (HHQ)



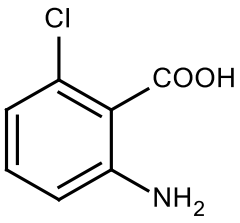
4-hydroxy-2-heptylquinoline-N-oxide (HQNO)

In addition to the presented autoinducers, P. aeruginosa produces more than 50 other 4Qs, the role of which is currently unclear.

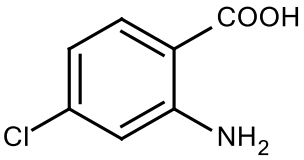
The development of inhibitors of the P. aeruginosa QS system (PQS) is currently in its infancy. Only in 2007, the first PQS inhibitors were proposed that could interfere with the biosynthesis of 4Qs and at the same time significantly reduce the mortality of mice from P. aeruginosa.



6FABA

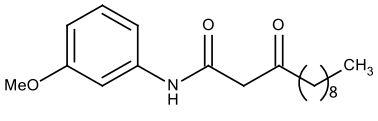


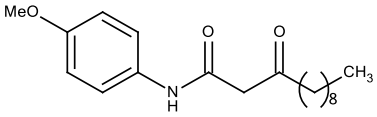
6CABA

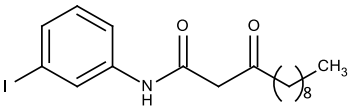


4CABA

Substituted acetanilides act on a similar principle of QS inhibition.







However, despite the small number of confirmed PQS inhibitors, it should be noted that a large number of studies are currently being conducted worldwide to develop new or improve existing methods for the synthesis of 2-alkylquinoline-4-ones, as shown in the literature review of this qualification work. Undoubtedly, this fact is also related to the study of the phenomenal phenomenon of nature - bacterial communication.

Conclusions to Chapter 1

1. Biofilms are complex microbial communities that form on surfaces, encapsulated within an extracellular matrix. They exhibit unique properties and behaviors, including increased resistance to antimicrobial agents and the ability to cause persistent infections. Further research and understanding of biofilms are essential for developing effective strategies to prevent and control biofilm-related issues in various fields, including medicine, industry, and environmental management.
2. The formation of biofilms involves a multistep process that includes initial attachment of microorganisms to a surface, subsequent growth and replication, production of an extracellular matrix, and the development of microcolonies within

the mature biofilm. This process is influenced by various factors, including adhesion mechanisms, microbial communication, and environmental conditions

3. To consider in detail the mechanisms of formation and interaction of Quorum Sensing proteins and inducers in P. Aeruginosa, as well as potential targets for the development of drugs against this bacterium and its biofilms. The role of the Quorum Sensing system is shown and potentional inhibitors of QS where described.

CHAPTER 2

BROMINE DERIVATIVES OF QUINOLINE-4-ONES

2.1. Study of the bromination reaction direction of quinoline-2-one and quinoline-4-one systems

One of the key steps in the WHO's plan to overcome the AMR crisis is to intensify efforts to develop new antimicrobial drugs. Over the past decade, scientists have begun to develop fundamentally new approaches to overcome the problem of rapid antibiotic resistance in microorganisms. Antibiotics work by killing bacteria or inhibiting their growth, and this has a significant impact on the selection of resistant variants in a population, whether resistance is pre-existing or developing as a new mechanism. As soon as resistance spreads among susceptible bacteria, new expensive drugs become ineffective almost immediately after they enter the market. New ideas focus on alternative principles of action on microorganisms, namely the development of a class of drugs designed to affect the virulence factors of microorganisms, resulting in neutralization rather than destruction of bacterial pathogens. Focusing on virulence offers several potential advantages, including:

- increasing the number of pharmacological targets;

- generation of antimicrobials with new mechanisms of action;

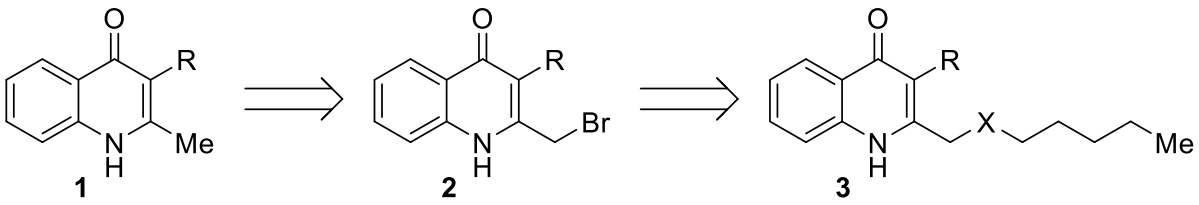
- reduction of resistance due to a decrease in selective pressure;

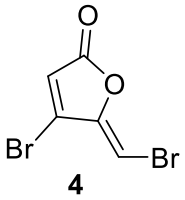
- potential possibility of preserving the intestinal microflora.

The reactions of halogenation of heterocycles are a powerful tool for their further modification and open up wide opportunities for expanding the chemical diversity of heterocyclic structures. In modern organic chemistry, aryl and hetero halides are among the main reagents of such named syntheses as amination by Buchwald-Hartwig and Ullmann; formation of C-C bonds in palladium-catalyzed

reactions by Heck, Negishi and Suzuki, as well as various syntheses involving Grignard reagents. Thus, in order to find possible ways to further diversify the classes of quinolone-based derivatives, the directions of bromination of these heterocycles were investigated.

The bromination of unsubstituted or N-substituted quinoline-4(1H)-ones is a well-studied reaction that results in the formation of the corresponding 3-bromo derivatives. The bromination of 2-methylquinolin-4(1H)-ones 1, since, in addition to the expected halogenation according to the heterocycle's aromatic system, it is possible to proceed with the reaction at another reaction center - the methyl group in the C(2) position of quinolone to form a bromomethyl derivative 2. This direction of bromination deserves attention in terms of its possible practical application. If this direction of halogenation is realized in practice, it will open up great prospects for the synthesis of 2-substituted quinoline-4(1H)-ones 3, which, in turn, are of interest as possible analogues of bacterial signaling molecules in QS processes. In addition, 2-(bromomethyl)-1H-quinoline-4(1H)-ones 2 may be interesting because they have similarities with the known QS inhibitor compound 4 (furanone C30, CID 10131246) based on the distance-based analysis of the topological structures of the molecules.





A promising method for the synthesis of novel P. aeruginosa QS signaling molecules.

It is known that there are studies on the bromination of 2-methylquinolin-4(1H)-one. In these works, the authors obtained 3-bromo-2-methylquinolin-4(1H)-one and used it as an intermediate for the following synthesis steps. No examples describing the halogenation at the methyl group in the C(2) position were found in the scientific literature.

The bromination of quinolines is a rather complicated process, and the formation of a particular product depends on the reagents used and the reaction conditions. In this work, we limited ourselves to a set of bromination reagents such as molecular bromine/acetic acid and N-bromosuccinimide (NBS)/chloroform. In some cases, benzoyl peroxide, a radical process initiator, was also used for bromination. Based on the literature data, we assumed that the priority direction of bromination of 2-methylquinoline-4(1H)-one would be electrophilic substitution via the aromatic system of the heterocycle. At the same time, we did not exclude the possibility of realizing the direction of radical bromination of the methyl group by the Wohl-Ziegler reaction.

First of all, in order to evaluate possible ways of bromination, it was interesting to analyze the electron density distribution in the molecule 2 methylquinoline-4(1H)-one (1, R = H). According to ab initio quantum chemical calculations (GAMESS-US, B3LYP, 31G(d)), the most probable centers in the electrophilic substitution reactions are carbon atoms in the C(3), C(6), and C(8) positions. According to the calculated charge values, they will be arranged in the following order by their reactivity: C(3) > C(6) > C(8) (Fig. 5).

The experimental results are in full agreement with theoretical considerations and literature data. The bromination of 2-methylquinolin-4(1H)-one (5) with molecular bromine in acetic acid or NBS in chloroform yielded the expected 3-bromo-2-methylquinolin-4(1H)-one (6) in 80 and 70% yields, respectively (Scheme 1). The addition of catalytic amounts of benzoyl peroxide to the NBS-chloroform system did not change the direction of bromination of quinolone 5 and as a result, compound 6 was isolated in 76% yield.

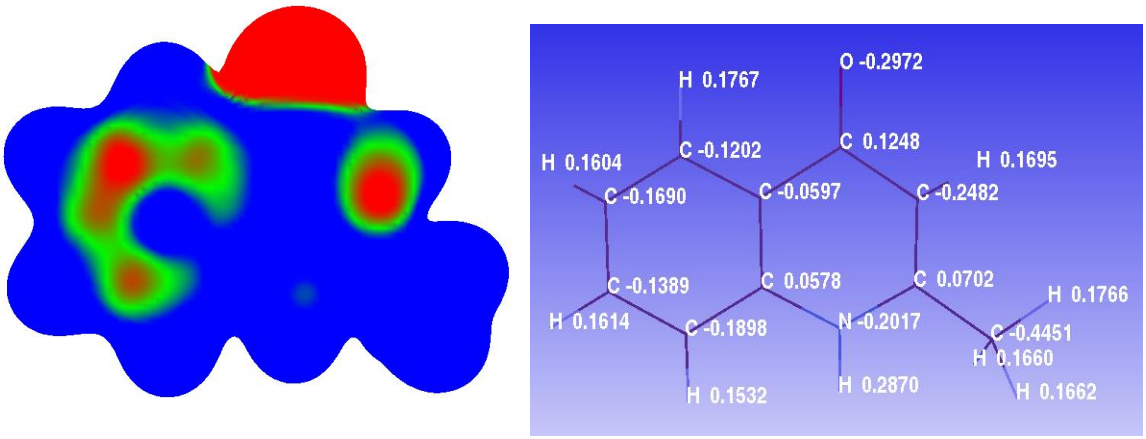
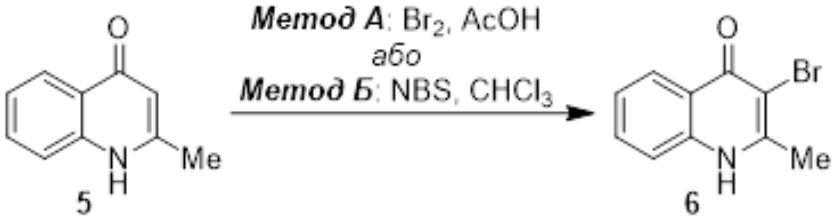
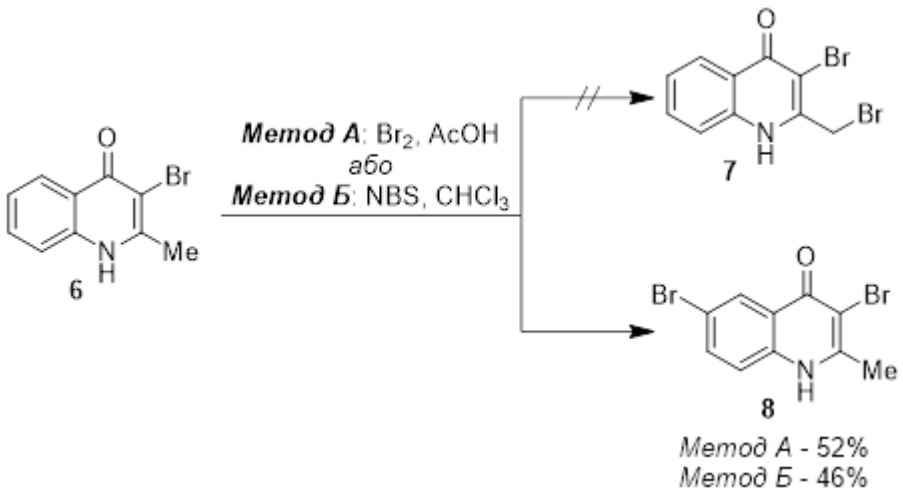


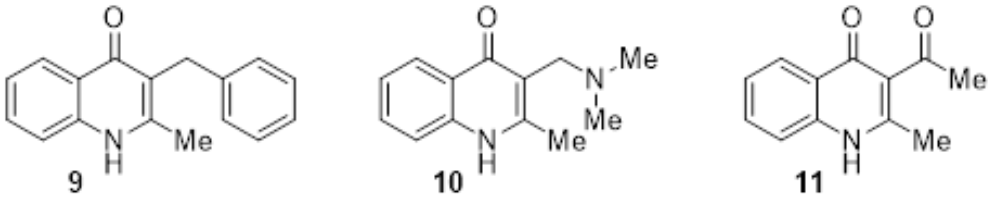
Fig. 5. Distribution of electron density and charges on atoms (according to Levdin) in the molecule of 2-methylquinoline-4(1H)-one (surface contour value - 0.01; molecular electrostatic potential - -0.01)



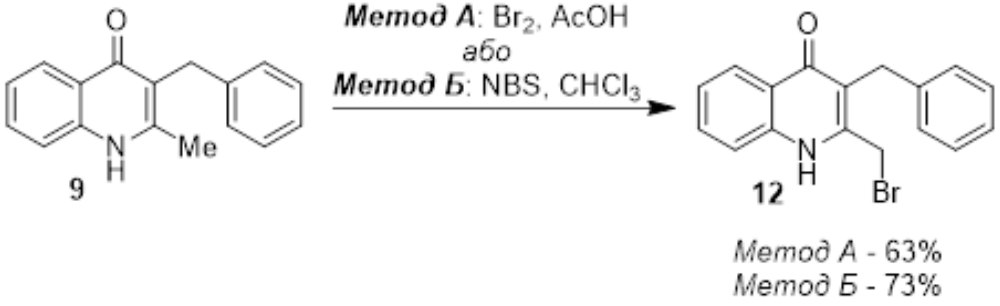
During the further bromination of 3-bromo-2-methylquinolin-4(1H)-one (6), the reaction proceeded at the C(6) position of the heterocycle's ring, resulting in the isolation of 3,6-dibromoproduct 8. At the same time, according to 1H NMR spectroscopy, the methyl group in position 2 was not subjected to bromination.



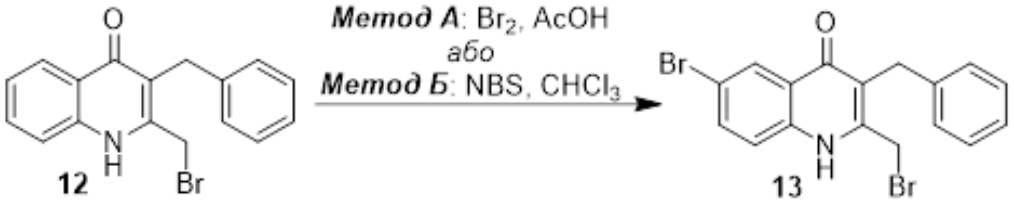
In order to search for possible ways to further modify the molecule 2 methylquinoline-4(1H)-one, the study of the direction of bromination of derivatives that already have substituents in the position 3 of the quinone ring was of particular interest. Compounds 9-11 were chosen to solve this problem.



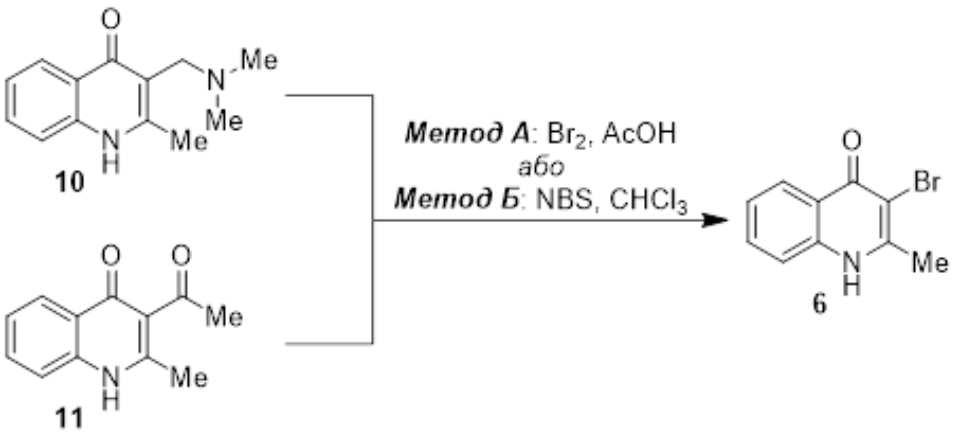
For the bromination of 3-substituted 2-methylquinolin-4(1H)-ones 9-11, the same set of conditions was used as in the case of compound 5. When the reaction conditions were changed, similar patterns of the final products were observed, with minor changes in yields. Based on the results of the syntheses, it was found that in the case of 3-benzyl-2-methylquinolin-4(1H)-one (9), bromination occurs at the C(2) methyl group of the quinolone position and 3-benzyl-2-bromomethylquinolin-4(1H)-one (12) was isolated as a product in 63% (Br2) and 73% (NBS) yields, respectively.



Upon further bromination of 3-benzyl-2-bromomethylquinolin-4(1H)-one (12), as in the case of 3-bromo-2-methylquinolin-4(1H)-one (6), the reaction proceeded at the C(6) position of the heterocycle's atomic ring in 56% (Br2) and 50% (NBS) yields, respectively.



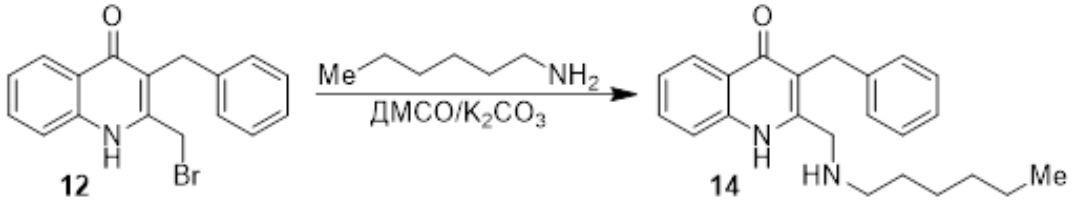
The results of the halogenation of 3-dimethylamino methyl-2-methylquinoline-4(1H)-one (10) and 3-acetyl-2-methylquinoline-4(1H)-one (11) were completely unexpected. In both cases, the final product was 3-bromo-2-methylquinolin-4(1H)-one (6). Its yield, depending on the method and the starting compound, was 58-72%.



After analyzing all the results obtained, it becomes clear that the presence of a substituent in the C(3) position of quinoline is of great importance in determining the direction of bromination of 3-substituted 2-methylquinoline-4(1H)-ones. However, additional research is certainly needed to identify certain regularities of this effect and the peculiarities of the bromination reaction mechanism

2.2 The using of 3-alkyl-2-bromomethylquinoline-4-one in the design of molecular diversity of 2-alkylquinoline-4-ones

The synthesized 3-benzyl-2-bromomethylquinolin-4(1H)-one (12) was used in the reaction with n-hexylamine to evaluate its reactivity as a promising building block for the construction of libraries of new QS molecules of P. aeruginosa.



The behavior of quinolone 12 as an alkylating agent was no different from that expected. During the interaction of the starting compounds in the DMSO/K2CO3 system, 3-benzyl-2-((hexylamino)methyl)quinolin-4(1H)-one (14) was isolated in 77% yield without any complications.

Experimental part

The solvents were preliminarily purified and dried according to standard procedures. 2-Methylquinolin-4(1H)-ones 5, 9-11 were synthesized according to previously described methods. The melting points of the synthesized substances were determined by the open capillary method and were not adjusted. The structures of the synthesized compounds were confirmed by 1H NMR spectroscopy and elemental analysis. The 1H NMR spectra were recorded in DMSO-d6 solution on a Varian Varian VXR-300 (300 MHz), internal TMS standard. Elemental analysis was performed using an Elementar Vario EI elemental analyzer.

Synthesis of 3-Bromo-2-methylquinolin-4(1H)-one (6).

Method A. To a mixture of quinolone 5 (1.59 g; 0.01 mol) and sodium acetate (0.82 g; 0.01 mol) in 20 mL of ice-cold acetic acid is added an equimolar amount of bromine (0.52 mL; 0.01 mol) under vigorous stirring in dropwise increments. After the addition of bromine is complete, the reaction mixture is stirred for 1 hour and

200 mL of water is added to it. The resulting precipitate is filtered and washed on the filter with 2-propanol. The yield is 80%.

Method B. A mixture of quinolone 5 (1.59 g; 0.01 mol) and 1.78 g (0.01 mol) of N-bromosuccinimide in 50 mL of crude oil is boiled for 6 hours. After cooling, the resulting precipitate is filtered, dried and crystallized from DMFA. The yield is 70%.

During the reaction according to Method B with the addition of 0.01 g of benzoyl peroxide, 3-bromo-2-methylquinolin-4(1H)-one (6) was isolated as a product. The yield was 76%.

M.p. > 260 °C (lit. m.p. > 260 °C [20]). 1H NMR (300 MHz, DMSO-d6), δ, ppm.: 2.54 (3H, s, CH3); 7.34 (1H, t, J = 7.5 Hz, ArH); 7.53 (1H, d, J = 8.2 Hz, ArH); 7.65 (1H, t, J = 7.6 Hz, ArH); 8.09 (1H, d, J = 8.1 Hz, ArH); 12.20 (1H, s, NH).

Synthesis of 3,6-dibromo-2-methylquinolin-4(1H)-one (8)

Method A. To a mixture of 3.15 g (0.01 mol) of 3-bromo-2-methylquinolin-4(1H)-one (6) and 0.82 g (0.01 mol) of sodium acetate in 20 mL of ice-cold acetic acid is added dropwise 0.52 mL (0.01 mol) of bromine. After the addition of bromine is complete, the reaction mixture is left at room temperature for 2 hours. To the resulting mixture is added a threefold amount of cold water; the resulting precipitate is filtered, dried and crystallized from DMFA. The yield is 52%.

Method B. A mixture of 3.15 g (0.01 mol) of 3-bromo-2-methylquinolin-4(1H)-one (6) and 1.78 g (0.01 mol) of N-bromosuccinimide in 50 mL of refluxing chloroform is boiled for 2-3 hours. After cooling, the resulting precipitate is filtered, dried and crystallized from DMFA. The yield is 46%.

During the reaction according to Method B with the addition of 0.01 g of benzoyl peroxide as a product, 3,6-dibromo-2-methylquinolin-4(1H)-one (8) was isolated in 48% yield.

Melting point >260^{0}C. Calculated for C10H7Br2NO, %: C 37.89; N 2.23; N 4.42. It is found, %: S 37.97; N 2.33; N 4.43. 1H NMR (300 MHz, DMSO-d6), δ, m.h: 2.56 (3H, s, CH3); 7.52 (1H, d, J = 8.8 Hz, ArH); 7.81 (1H, dd, J = 8.8 ta 2.2 Hz, ArH); 8.17 (1H, d, J = 1.8 Hz, ArH); 12.28 (1H, s, NH).

Synthesis of 3-benzyl-2-bromomethylquinoline-4(1H)-one (12).

This compound was obtained according to the synthesis of 3,6-dibromo-2-methylquinolin-4(1H)-one (8) (Methods A and B) using 3-benzyl-2-methylquinolin-4(1H)-one (9) as a starting compound. The precipitates formed were filtered and washed on the filter with 2-propanol. The yield by Method A was 63%, and by Method B - 73%. During the reaction according to Method B with the addition of 0.01 g of benzoyl peroxide, 3-benzyl-2-bromomethylquinolin-4(1H)-one (12) was isolated as a product in 78% yield.

Melting point. 240-242°C. Calculated for C17H14BrNO, %: C 62.21; N 4.30; N 4.27. The values are as follows: %: S 62.10; N 4.31; N 4.28. 1H NMR (300 MHz, DMSO-d6), δ, ppm: 3.99 (2H, s, CH2); 4.62 (2H, s, CH2); 7.27-7.10 (5H, m, ArH); 7.31 (1H, t, J = 7.5 Hz, ArH); 7.57 (1H, d, J = 8.3 Hz, ArH); 7.67 (1H, t, J = 7.6 Hz, ArH); 8.09 (1H, d, J = 8.8 Hz, ArH); 11.89 (1H, s, NH).

Synthesis of 3-benzyl-6-bromo-2-(bromomethyl)quinoline-4(1H)-one (13).

This compound was obtained by the synthesis of 3,6-dibromo-2-methylquinolin-4(1H)-one (8) (Methods A and B) using 3-benzyl-2-(bromomethyl)quinolin-4(1H)-one (12) as a starting compound. The yield by Method A was 56%, and by Method B - 50%. During the reaction according to Method B with the addition of 0.01 g of benzoyl peroxide, 3-benzyl-6-bromo-2-(bromomethyl)quinoline-4(1H)-one was isolated as a product (13). The yield was 46%.

Melting point >260^{0}C. Calculated for C17H13Br2NO, %: C 50.16; N 3.22; N 3.44. Calculated, %: S 50.03; N 3.23; N 3.78. 1H NMR (300 MHz, DMSO-d6), δ, ppm: 3.89 (2H, s, CH2); 4.61 (2H, s, CH2); 7.29-7.17 (5H, m, ArH); 7.48 (1H, d, J = 8.8 Hz, ArH); 7.75 (1H, dd, J = 8.9; 2.4 Hz, ArH); 8.18 (1H, d, J = 2.4 Hz, ArH); 11.72 (1H, s, NH). Melting point >260^{0}C. Calculated for C17H13Br2NO, %: S 50.16; N 3.22; N 3.44. Calculated, %: S 50.03; N 3.23; N 3.78. 1H NMR (300 MHz, DMSO-d6), δ, ppm: 3.89 (2H, s, CH2); 4.61 (2H, s, CH2); 7.29-7.17 (5H, m, ArH);

7.48 (1H, d, J = 8.8 Hz, ArH); 7.75 (1H, dd, J = 8.9; 2.4 Hz, ArH); 8.18 (1H, d, J = 2.4 Hz, ArH); 11.72 (1H, s, NH).

Bromination of 3-dimethylamino-2-methylquinolin-4(1H)-one (10) and 3-acetyl-2-methylquinolin-4(1H)-one (11) was carried out according to the methods for the synthesis of 3,6-dibromo-2-methyl-quinolin-4(1H)-one (8) (Methods A and B). In all cases, the bromination resulted in the isolation of compound 6 from the reaction mixture, which was crystallized from DMFA.

The yields of 3-dimethylamino-2-methylquinolin-4(1H)-one (10) were as follows: Method A - 72%, Method B - 58%.

1H NMR (300 MHz, DMSO-d6), δ, ppm: 2.55 (3H, s, CH3); 7.34 (1H, ddd, J = 8.0, 6.7, 1.2 Hz, ArH); 7.54 (1H, d, J = 8.1 Hz, ArH); 7.66 (1H, ddd, J = 8.3, 6.7, 1.6 Hz, ArH); 8.08 (1H, dd, J = 8.1, 1.4 Hz, ArH); 12.12 (1H, s, NH).

The bromination of 3-acetyl-2-methylquinolin-4(1H)-one (11) gave the following yields: Method A - 67%, Method B - 62%.

1H NMR (300 MHz, DMSO-d6), δ, ppm: 2.54 (3H, s, CH3); 7.34 (1H, t, J = 7.4 Hz, ArH); 7.54 (1H, d, J = 8.2 Hz, ArH); 7.65 (1H, t, J = 7.5 Hz, ArH); 8.08 (1H, d, J = 8.1 Hz, ArH); 12.10 (1H, s, NH).

Synthesis of 3-benzyl-2-((hexylamino)methyl)quinoline-4(1H)-one (14).
A mixture of 3-benzyl-2-bromomethylquinolin-4(1H)-one (12) (1.64 g; 0.005 mol), 0.56 g (0.0055 mol) of n-hexylamine and 0.76 g (0.0055 mol) of potassium carbonate in 15 mL of DMSO is stirred at 40 0C for 6 hours. After this time, 200 ml of water and 5 ml of acetic acid are added to the reaction mixture. The resulting precipitate is filtered, dried and crystallized from 2-propanol. The yield is 77%.
The melting point is 142-144 ^{0}C. Calculated for C23H28N2O, %: C 79.27; N 8.10; N 8.04. Determined, %: C 79.07; H 8.08; N 8.06. 1H NMR (300 MHz, DMSO-d6), δ, ppm: 0.82 (3H, t, J = 6.7 Hz, CH3); 1.38-0.95 (m, 8H, 4×CH2); 2.48-2.32 (m, 2H, CH2); 3.52-3.38 (1H, mq, CH2NH); 3.74 (2H, s, CH2Ph); 3.93 (2H, d, J = 7.0 Hz, CH2NH); 7.25-7.05 (5H, m, ArH); 7.29 (1H, t, J = 7.6 Hz, ArH); 7.60 (1H, t, J = 7.8 Hz, ArH); 7.75 (1H, d, J = 8.3 Hz, ArH); 8.12 (1H, d, J = 8.0 Hz, ArH); NH group

of quinolone heterocycle is in deutero exchange (with solvent) ((with water in solvent)).

Conclusions to Chapter 2

1. The peculiarities of bromination in the series of 2-methylquinolin-4(1H)-ones substituted at position 3 were investigated. It has been found that, depending on the nature of the substituent at the C(3) position of quinolone, bromination occurs at the methyl group at the C(2) position or at the C(3) and C(6) positions of the heterocycle.
2. In the case of 3-benzyl-2-methylquinolin-4(1H)-one, bromination occurs at the C(2) methyl group of the quinolone position to form 3-benzyl-2-(bromomethyl)quinolin-4(1H)-one, which can be used to develop a new class of drugs designed to affect the virulence factors of microorganisms.
3. The synthetic possibilities of 3-benzyl-2-bromomethylquinoline-4(1H)-one are demonstrated on the example of n-hexylamine alkylation.

CHAPTER 3

Computer-aided design of new quinolone-4-one derivatives

3.1. Chemoinformatic methods for the study of new QS inhibitors

In order to assess the prevalence of antibiofilm agents, we searched the ChemBL and PubChem databases, followed by clustering the obtained array of structures. The keyword "antibiofilm" resulted in 1143 compounds that have ever been tested for antibiofilm activity from the ChemBL database and 1622 from PubChem. The hierarchical clustering of the data was performed using the Jklustor program of the ChemAxon chemical information platform.

JKlustor is a tool for clustering and analyzing the diversity of chemical libraries. JKlustor is a clustering toolkit that performs clustering of composite libraries and focused sets based on similarity and structure - both in hierarchical and non-hierarchical style. It also performs diversity calculations and library comparisons based on molecular fingerprints and other descriptors. It is an important tool in combinatorial chemistry, virtual library design, and other areas where large numbers of compounds are analyzed.

Similarity-based clustering:

Hierarchical method:

Ward's method of minimum variance accelerated by the mutual nearest neighbor algorithm of Moore's creates dense and well-separated clusters. It is recommended to use it with smaller datasets, such as concentrated libraries with a structure of less than 100,000.

Non-hierarchical methods:

Sphere Exclusion clustering is based on fingerprints and/or other numerical data, it can easily handle millions of structures and is suitable for a variety of subset selection. The K-means cluster analysis method aims to find the center of natural clusters in the input data in a way that minimizes the variance within each cluster.

Finally, the *Jarvis-Patrick (Jarp) method* uses the nearest neighbor approach and performs clustering of variable-length chemical databases with hundreds of thousands of structures contained.

Structural clustering

Hierarchical methods:

LibraryMCS identifies the largest substructure common to several molecular structures.

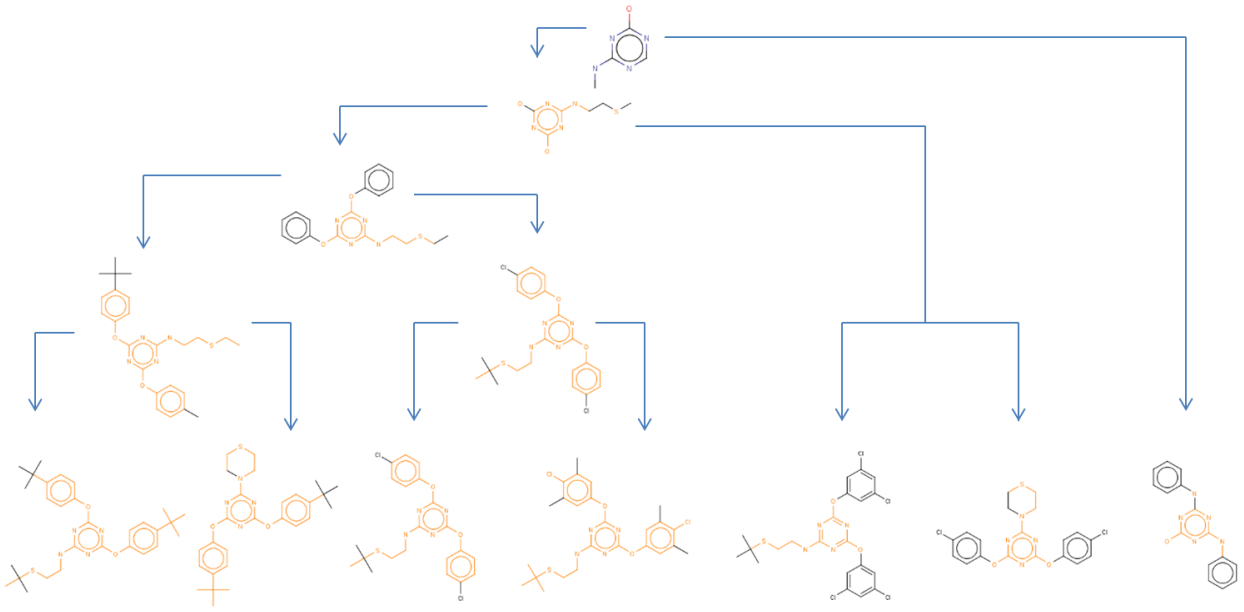


Figure 6. Hierarchical representation of clusters

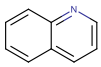
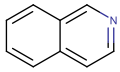
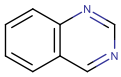
It uses a hierarchical representation of clusters (dendograms) and also visualizes an alternative tree and tabular view. MCS profiling helps scientists explore screening results to quickly identify new scaffolds and new examples of active compound families. The hierarchical SAR table allows you to view clusters and related non-structural data. R-group decomposition can also be performed using the MCS as the underlying structure for each cluster.

Non-hierarchical method:

JKlustor makes clustering available using pre-generated Bemis-Murcko framework structures, and therefore provides a convenient and fast way to analyze large databases with millions of compounds.

The results of the search indicate that the number of studies devoted to this topic is small. At the moment, there are few quinoline, isoquinoline and pyrimidine derivatives that have been tested for this type of activity (Table).

Table.

Scaffold	ChemBL	PubChem
	32	71
	5	-
	5	53

Based on the information provided, it appears that there is limited research on the use of quinoline, isoquinoline, and pyrimidine derivatives in the context of biofilm activity. As a result, further research in this area is indeed highly relevant. Exploring the potential of these derivatives as anti-biofilm agents could provide valuable insights into their effectiveness, mechanisms of action, and potential applications in combating biofilm-related issues. Conducting more studies to investigate the activity of these derivatives against biofilms would contribute to expanding the knowledge base and potentially lead to the development of new therapeutic strategies or preventive measures targeting biofilms in various fields, such as medicine, industry, and environmental management.

3.2. Molecular docking new quinolone-4-one derivatives

Computer-aided drug design (CADD) is an important tool in modern pharmaceutical research, especially in the fight against antimicrobial resistance. This method allows selecting potential drugs that are highly selective and effective against a wide range of microorganisms, including those that cause infections that are difficult to treat. The use of CADD in the pharmaceutical industry provides efficiency, cost reduction, and reduced time for the development of new drugs. Advances in CADD make it possible to discover new chemical compounds and find new targets for antimicrobial drugs that can effectively fight antimicrobial

resistance. Thus, CADD is an important tool in the development of new antimicrobial drugs that are critical for human health and the fight against the global exacerbation of the problem of antimicrobial resistance.

Target-oriented virtual screening is a virtual screening method that uses the structure of a protein target (target) to find potential drug compounds that can interact with this target. This method is based on knowledge of the target's structure and its interaction with drug compounds. The advantages of target-oriented virtual screening include:

- Reducing the number of compounds that need to be tested in experiments, which increases the speed and reduces the cost of finding new drugs;
- Reducing the time required to search for new drug compounds, which speeds up the process of developing new drugs;
- Increasing the accuracy of target structure determination, which allows for a more accurate prediction of the interaction of drug compounds with the target;
- Increase the chances of success in clinical trials, as drugs that have been selected using targeted virtual screening are more likely to show greater efficacy and less toxicity.

Selecting a target for computer research

To search for the molecular structures of P. aeruginosa PqsR/MvfR, the Protein Databank and Biofilms Structural Database were searched. A total of 12 X-ray structures were identified. Detailed information is shown in bellow

Available MvfR X-ray structures on PDB and BSD.

PDB Code	Protein	Resolution (Å)	Ligand	Strain
4JVC	Ligand-Binding Domain	2.50		UCBPP-PA14
4JVD	Ligand-Binding Domain	2.95	NNQ	
4JVI	Ligand-Binding Domain	2.90	QZN	
6B8A	Ligand-Binding Domain	2.65	M64	PAO1
6Q7U	Ligand-Binding Domain	3.15	HLH	PAO1
6Q7V	Ligand-Binding Domain	2.56	HLK	
6Q7W	Ligand-Binding Domain	2.82	HLQ	
6TPR	Ligand-Binding Domain	3.20	NV5	UCBPP-PA14
6Z07	Ligand-Binding Domain	2.95	Q4E	UCBPP-PA14
6Z17	Ligand-Binding Domain	3.15	Q4W	
6Z5K	Ligand-Binding Domain	3.20	QAE	
6YZ3	Ligand-Binding Domain	3.00	Q25	

Structures 4JVC, 4JVD, and 4JVI (2013) first presented the binding domain of the MvfR ligand in the apo form and in complex with its native agonist 2-nonyl-4hydroxyquinoline or NNQ (4JVC and 4JVD, respectively).

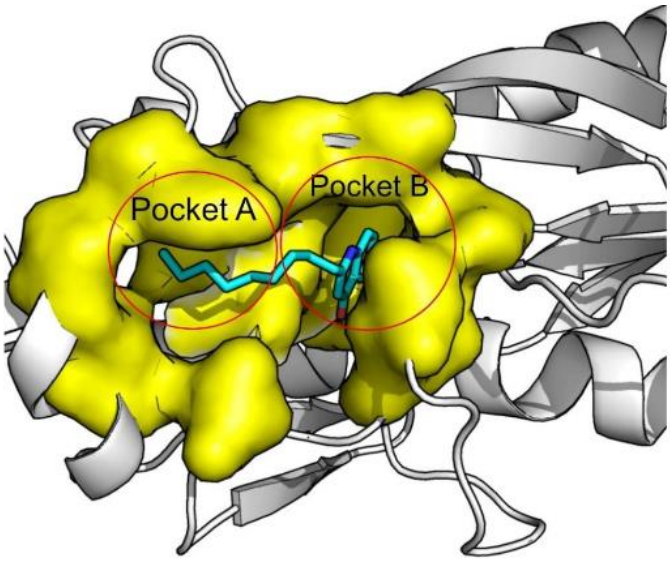


Figure. 7. Images of pocket A and pocket B of MvfR. Protein bound to its natural inducer NNQ. (PDB:4JVI)

The authors showed that the structure of this binding pocket is extremely large, in which the native agonist AQ is stabilized exclusively by hydrophobic interactions. They also presented a structure with an analog of NNQ in the binding pocket (4JVI), i.e., the compound 3-amino-7-chloro-2nonylquinazolin-4-one (QZN).

Of the 12 available X-ray structures of PqsR/MvfR available on PDB and BSD, the apoprotein structure (PDB: 4JVC, 2.50 Å) was chosen for the docking procedure. This choice was obvious, since the available cocrystallized structures (PDB 4JVD and 4JVI) have lower resolutions of 2.95 and 2.90 Å, respectively.

Creation of ligands for computer research

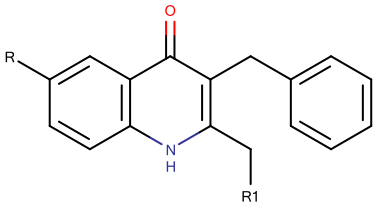
The chemical space is vast. There are estimates that the number of individual stable molecular structures with molecular weights in the range close to drugs is about 10^{60}. It will never be possible to calculate all of these structures to find among them the one with the most promising property profile for a particular purpose. Nevertheless, the search for yet unknown promising structures in this vast space of compounds can be successful if done with the right approach. This can lead to completely new ideas or starting points that would not be possible with traditional structurally limited virtual screening of existing or virtual combinatorial libraries.

Markush structures and Markush enumeration are an indispensable tool in the creation of chemical space. Markush structures and Markush enumeration are related concepts used in chemical and pharmaceutical industries to represent and explore the chemical space of a particular class of compounds. A Markush structure is a generalized chemical structure that represents a set of compounds sharing common features or substituent patterns. It is named after Eugene Markush, who developed this concept in the early 20th century. Markush structures are typically used to represent chemical libraries or patent claims, where a large number of related compounds are described by a single structure. In a Markush structure, certain parts of the structure are defined as variable positions or variable groups, represented by generic placeholders such as R, R', X, Y, etc. These placeholders can be replaced with specific atoms or groups to generate individual compounds within the Markush structure. The specific substitutions can vary within predefined sets or ranges.

Markush enumeration is the process of systematically generating and enumerating all possible combinations of substituents or variations within a Markush structure. It involves substituting the variable positions or groups in the

structure with specific atoms or groups from a predefined set or range. Enumeration algorithms can be used to generate the individual compounds represented by the Markush structure systematically. This process allows researchers to explore the chemical space and generate a large number of potential compounds with diverse properties. To perform molecular docking, a microspace of new quinolone derivatives was generated:

The generation was performed using the MarvinSketch program and the basic Markush structure is as follows:



The substituents in the benzene ring of the quinolone cycle at the sixth position were represented by H; NO2; SO2NH2 groups. R1 was represented by a set of 20 small molecular weight amines. Thus, the base set numbered 60 new virtual compounds To visualize the properties of the resulting library of virtual compounds, a graph of the distribution of the total number of compounds from the calculated parameter cLogP is shown below. As can be seen, the median compounds are in the range of cLogP values from 1.5 to 3.5, which fits into Lipinski's five rules.

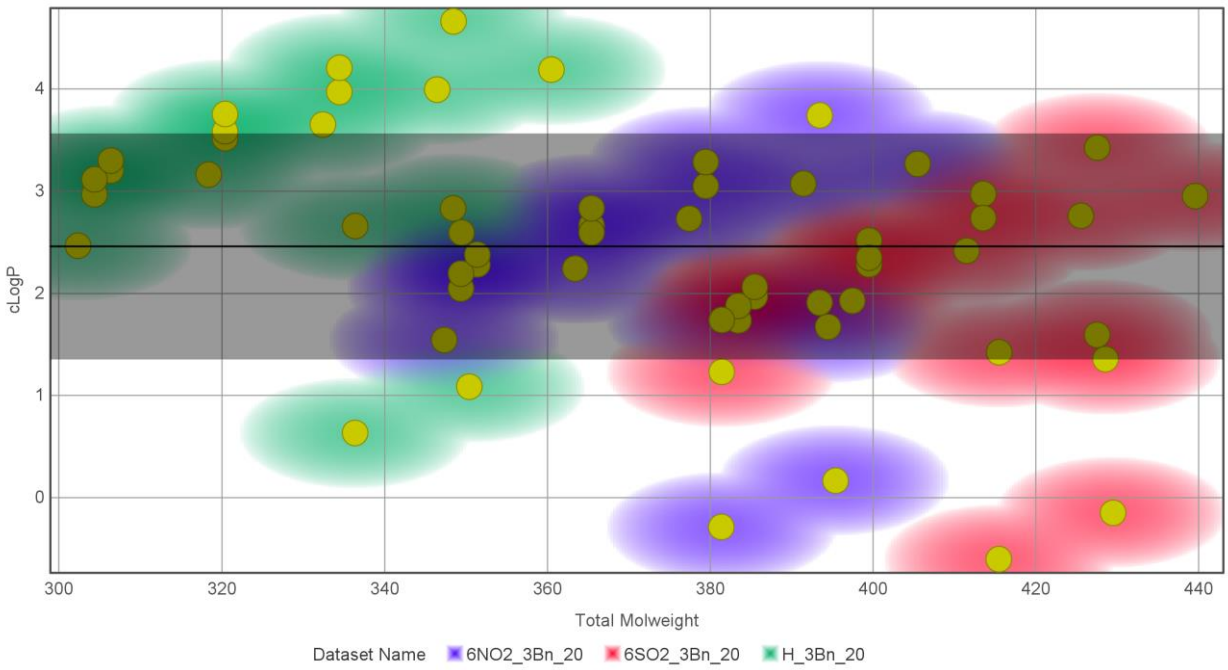


Figure. 8 Distribution of compounds in the virtual library by the cLogP parameter

Computer calculations of molecular docking were performed in the operating environment OS Ubuntu 20.04 LTS. Using the seeSAR package of BioSolveIT. The ligands were converted into 3D structures and minimized in the MMF94 force field using the OpenBabel program. As a result of docking studies, 10 conformers were

generated for each ligand, resulting in a base of 600 compounds. HYDE - scoring function was applied to them.

HYDE is patent protected and worldwide unique as it is not trained, calibrated, or fit on any particular data. Affinity predictions are generally possible for protein-ligand complexes, protein-protein interactions, as well as DNA & RNA binders. It is based on physical principles only by linking the two major driving forces, desolvation and interactions, in a sound scientific manner. HYDE is constantly improved and originated from a collaboration with BAYER, Hamburg University, and BioSolveIT.

Advantages of HYDE scoring function are:

•Interactive and iterative optimization of leads

• Compound classification: binders/weak binders/non binders

• Innovative approach compared to trained scoring functions

• Interpretable, visual feedback

• Implicit Hydrogen bonds and DEhydration

Interactive, desolvation-aware visual ΔG estimates. The values are estimated based on a difference calculation between the bound and unbound state, based on an atomic logP-based mathematical kernel. The system has NOT been trained to specific targets, instead H-bond contribution and dehydration ("desolvation") are intrinsically balanced without weighting parameters as seen in all other force fields. By design, HYDE allows the visualization of ΔG on atoms so that the user instantly gets a feedback on the computational details behind. HYDE will explain what can be improved here; some of these scenarios could be:

• Desolvation penalties for buried hydrophilic atoms

• Weak/questionable H-bonds

• Indifferent scaffold or linker, not contributing to ΔG.

The 6-sulfonamide derivatives were found to be the best in terms of potential affinity to the PqsR receptor pocket and the most promising structure is presented below.

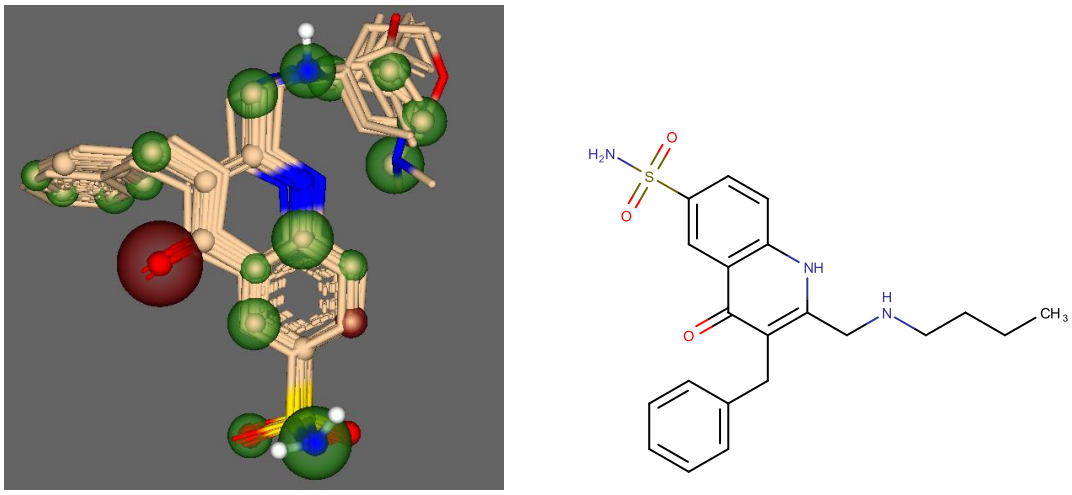


Fig. 9 potential affinity the 6-sulfonamide derivatives to the PqsR receptor pocket

However, the location of the 6-SO2NH2 ligands in the target pocket differs from such a natural 3-OH-HHQ autoinducer.

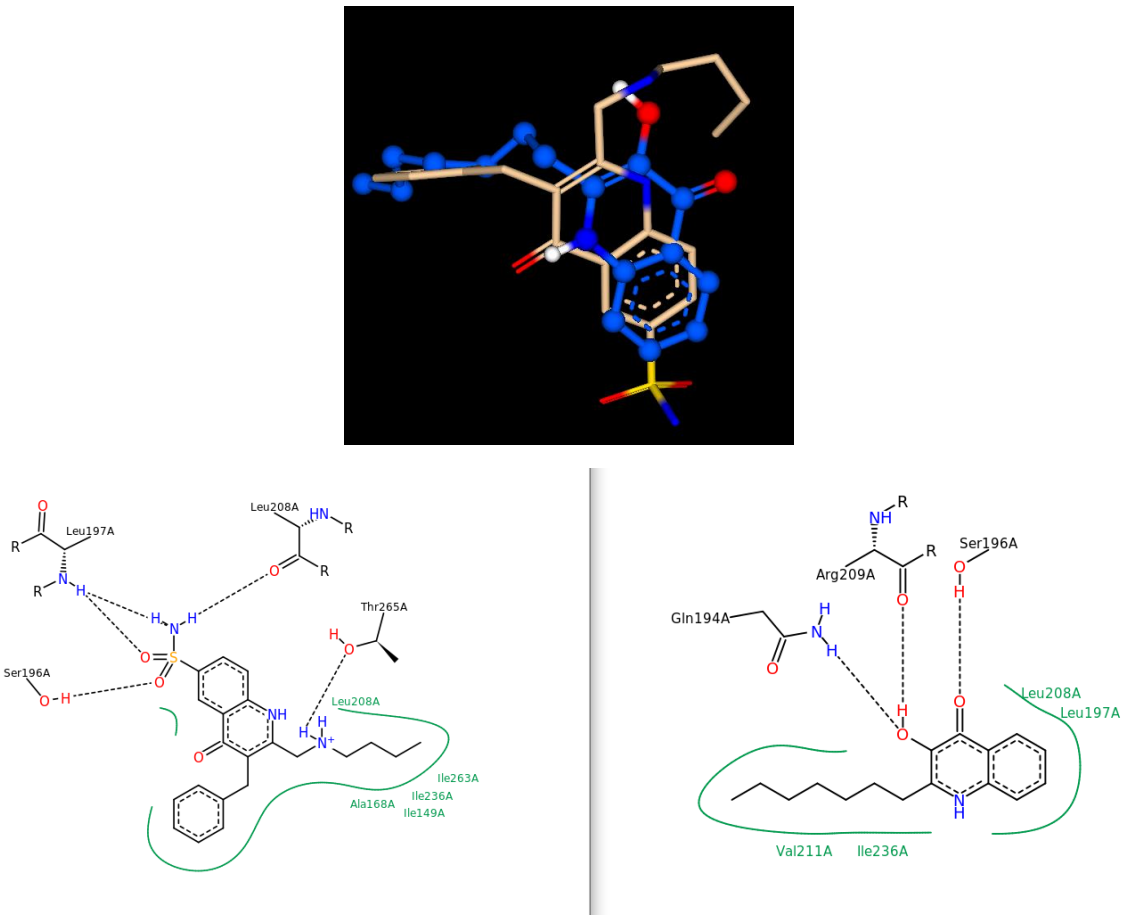


Fig. 10 The location of the ligands in the target pocket

Thus, the computer modeling allowed us to obtain theoretical data on the peculiarities of binding of quinoline-4-one derivative ligands to the multivariate virulence factor of P. aeruginosa, which is a valuable basis for further search for new compounds that can effectively counteract antimicrobial resistance.

Conclusions to Chapter 3

1. A virtual database of new quinolones was created using Markush enumerations, and various chemical information descriptors were calculated for these compounds for virtual screening.
2. Based on the literature data, the structure of the apoprotein (PDB: 4JVC) was selected for the docking procedure, the ligands were prepared accordingly, and then a virtual docking screen was performed using the seeSAR program by BioSolveIT.
3. A comprehensive analysis of the obtained virtual screening data was carried out on the basis of which it was shown that 6-sulfamide derivatives of quinolones are promising compounds for biological tests.

GENERAL CONCLUSIONS

1. Knowledge of bacterial communication is important because it can be dangerous for humans. A detailed study of the mechanisms of formation and interaction of Quorum Sensing proteins and inducers in P. Aeruginosa can help to understand its behavior and develop new drugs to combat it and its biofilms.
2. The obtained results of SAR analysis of quinolone derivatives showed that for their effective action against biofilm formation and growth, the presence of heterocycles with electron-deficient groups at the 6th position is necessary.
3. The bromination features in the series of 3-substituted 2-methyl-1H-quinolin-4-ones have been investigated. In the case of 3-benzyl-2-methylquinoline-4(1H)-one, bromination occurs at the methyl group of the C(2) position of quinolone, with the formation of 3-benzyl-2-(bromomethyl)quinoline-4(1H)-one, which can be used to develop a new class of drugs designed to affect the virulence factors of microorganisms. The synthetic capabilities of 3-benzyl-2-bromomethylquinoline-4(1H)-one have been demonstrated on the example of alkylation of aliphatic amines.
4. A virtual library of quinoline-4-one derivatives was generated using the Markush enumeration. A virtual docking screening was performed using the seeSAR program. These steps may be important for the further development of new quinoline-4-one-based drugs.
5. A comprehensive analysis of the obtained virtual screening data was carried out using general approaches of chemoinformatics and statistics. A hypothesis was formulated about the binding of quinolone ligands to the PqsR (MvfR) protein. The computer modeling is a valuable basis for further search for new compounds that can effectively counteract antimicrobial resistance.

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Ф А 2.2.1–32-366

National University of Pharmacy

Faculty for foreign citizens' education
Department medicinal chemistry
Level of higher education master
Specialty 226 Pharmacy, industrial pharmacy
Educational program Pharmacy

APPROVED
The Head of Department
medicinal chemistry

Lina PEREKHODA
"_22nd_" of August 2022

ASSIGNMENT
FOR QUALIFICATION WORK
OF AN APPLICANT FOR HIGHER EDUCATION

Edwin Yuward MWINUKA

1. Topic of qualification work: «Developing new quinolone-4-one derivatives to combat antibacterial resistance», supervisor of qualification work: Vadim ZUBKOV, DSc
approved by order of NUPh from "6th" of February 2023 № 35

2. Deadline for submission of qualification work by the applicant for higher education: April 2023.

3. Outgoing data for qualification work: methods of synthesis, literature search, structure-activity relationship, antimicrobial activity, Quorum sensing processes, computer methods in the search for new drugs, chemoinformatics, molecular docking, chemical software, databases, scripts and command line work

4. Contents of the settlement and explanatory note (list of questions that need to be developed): chemical synthesis, to review the literature on the current state of the AMR problem and its situation in different countries. To understand the methods of chemoinformatics, bioinformatics and molecular docking used to optimize the selection of lead structures and further correlation between experimental pharmacological data and predicted activity models. Conduct a virtual screening based on molecular docking of ligands with PqsR protein

5. List of graphic material (with exact indication of the required drawings):

Tables – 2, pictures – 10

6. Consultants of chapters of qualification work

Chapters	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1	Vadim ZUBKOV, associate professor of department medicinal chemistry	September 2022	September 2022
2	Vadim ZUBKOV, associate professor of department medicinal chemistry	November 2022	November 2022
3	Vadim ZUBKOV, associate professor of department medicinal chemistry	January 2023	January 2023

7. Date of issue of the assignment: "_22nd_" of August 2022

CALENDAR PLAN

№ з/п	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1	Biofilms as a form of bacterial existence in nature (literature review) Writing 1 chapter.	Sept-Nov 2022	**done**
2	Study of the bromination reaction direction of quinoline-2-one and quinoline-4-one systems Writing 2 chapter.	Dec 2022 - Jan 2023	**done**
3	Cheminformatics methods for the study of compounds	Jan-Feb 2023	**done**
4	Molecular docking of ligands with PqsR protein and visualization of the results Writing 3 chapter.	March 2023	**done**
5	Summing up and submission to the Examination Board	April 2023	**done**

An applicant of higher education _____ Edwin Yuward MWINUKA

Supervisor of qualification work _____ Vadim ZUBKOV

ВИТЯГ З НАКАЗУ № 35
По Національному фармацевтичному університету
від 06 лютого 2023 року

нижченаведеним студентам 5-го курсу 2022-2023 навчального року, навчання за освітнім ступенем «магістр», галузь знань 22 охорона здоров'я, спеціальності 226 – фармація, промислова фармація, освітня програма – фармація, денна форма здобуття освіти (термін навчання 4 роки 10 місяців та 3 роки 10 місяців), які навчаються за контрактом, затвердити теми кваліфікаційних робіт:

Прізвище студента	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• **по кафедрі медичної хімії**				
Мвінука Едвін Ювард	Розробка нових похідних хінолон-4-ону для боротьби з антибактеріальною резистентністю	Developing new quinolone-4-one derivatives to combat antibacterial resistance	доц. Зубков В.О.	професор Георгіянц В.А.

Підстава: подання декана, згода ректора

Ректор

Вірно. Секретар

Ф А2.8-47-110

ВИСНОВОК

Комісії з академічної доброчесності про проведену експертизу

щодо академічного плагіату у кваліфікаційній роботі

здобувача вищої освіти

№ 114186 від « 28 » травня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Мвінука Едвін Ювард, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Розробка нових похідних хінолон-4-ону для боротьби з антибактеріальною резистентністю / Developing new quinolone-4-one derivatives to combat antibacterial resistance», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

Голова комісії,
професор **Інна ВЛАДИМИРОВА**

0%

33%

Ф А 2.2.1-32-353

REVIEW

of scientific supervisor for the qualification work of the master's level of higher education of the specialty 226 Pharmacy, industrial pharmacy

Edwin Yuward MWINUKA

on the topic: «Developing new quinolone-4-one derivatives to combat antibacterial resistance»

Relevance of the topic. Molecular modeling is a powerful tool used to study the chemical properties and behavior of molecules. Recently, there has been a growing interest in using molecular modeling to develop new quinolone derivatives that can act as potential inhibitors of Quorum Sensing processes. This approach has considerable potential for the development of new therapeutics for bacterial infections, making it an exciting area of research. Edwin MWINUKA's qualification work is devoted to computational studies of quinoline-4-one derivatives. The choice of the topic is relevant, as the use of computer technology is gaining momentum in modern medicinal and pharmaceutical chemistry.

Practical value of conclusions, recommendations and their validity. The practical value of the work is that the results of the virtual screening allowed us to develop a concept for further search for new quinolone derivatives as potential inhibitors of Pseudomonas aeruginosa biofilm formation.

Assessment of work. This work presents a number of original approaches to structure-based virtual screening and determination of ligand affinity for active binding sites in the PqsR pocket. It is suggested that ligands to PqsR may not have a long lipophilic residue, which will improve the drug-like properties of potential ligand compounds. The work is performed at a good scientific level, neatly designed, meets the requirements for qualifying works in terms of the amount of research conducted

General conclusion and recommendations on admission to defend. The qualification work of Edwin Yuward MWINUKA meets the requirements for

qualification works in terms of the relevance and scope of the performed research, the novelty of the obtained results, their theoretical and practical significance and can be recommended for defense at the Examination Commission.

Scientific supervisor _____ Vadim ZUBKOV

« 7th» of April 2023

REVIEW

for qualification work of the master's level of higher education, specialty 226 Pharmacy, industrial pharmacy

Edwin Yuward MWINUKA

on the topic: «Developing new quinolone-4-one derivatives to combat antibacterial resistance»

Relevance of the topic. Pseudomonas aeruginosa (PA) is one of the most dangerous bacteria that can cause serious infections in humans. Studies over the past decade have shown that most bacteria (more than 99%) exist in natural ecosystems as specifically organized biofilms attached to substrates. The study of biofilms is of great interest to researchers because the ability of pathogenic bacteria to exist within biofilms poses great challenges, as it significantly increases the resistance of bacteria to antimicrobial agents. In view of the growing resistance of microorganisms to antibiotics, the work of Edwin Yuward MWINUKA submitted for review is modern and relevant.

Theoretical level of work. The qualification work has a high theoretical level. The main feature of the work is an interesting and profound idea about the directions of modifications of 2-alkylquinoline-4-one derivatives in order to obtain wider improvements in the stages of preclinical and clinical research.

Author's suggestions on the research topic. In this work, based on molecular docking data, the authors proposed a new vision in the design of biologically active compounds aimed at reducing or terminating the communication of Pseudomonas aeruginosa

Practical value of conclusions, recommendations and their validity. The research results obtained by the author can be used for further search of promising molecules and optimization of the structures-leaders of new biologically active compounds among quinoline-4-one derivatives. An interesting hypothesis about the possibility of creating new antibacterial drugs based on a chemocentric strategy for the search

for biologically active compounds by modifying natural and already known inhibitors of the Quorum sensing process was proposed and tested.

Disadvantages of work. There are no fundamental comments regarding the content of the work, there are certain spelling errors that generally do not affect the content of the work.

General conclusion and assessment of the work. The qualification work of Edwin Yuward MWINUKA in terms of relevance, scientific novelty of the obtained results, methodological level, theoretical and practical significance, volume of performed research meets the requirements of the Regulation on the Procedure for the Preparation and Defense of Qualification Works at the National Pharmaceutical University and can be recommended for defense at the Examination Commission.

Reviewer _____ prof. Viktoriia GEORGIANTS

«14th» of April 2023

Ф А2.2.1-91-287

ВИТЯГ
з протоколу засідання кафедри медичної хімії
№ 10 від 21 квітня 2023 р.

ПРИСУТНІ:

проф. Ліна ПЕРЕХОДА, проф. Андрій ФЕДОСОВ, доц. Вадим ЗУБКОВ, доц. Ірина СИЧ, доц. Віталій ЯРЕМЕНКО, доц. Ілля ПОДОЛЬСЬКИЙ, доц. Наталія КОБЗАР, доц. Марина РАХІМОВА, доц. Маргарита СУЛЕЙМАН, ас. Олена БЕВЗ, ас. Ольга ВІСЛОУС

ПОРЯДОК ДЕННИЙ:

Звіт про стан виконання кваліфікаційної роботи здобувача вищої освіти факультету з підготовки іноземних громадян Фм18(5,0д)англ-04 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Едвін Ювард МВІНУКА на тему: «Розробка нових похідних хінолон-4-ону для боротьби з антибактеріальною резистентністю / Developing new quinolone-4-one derivatives to combat antibacterial resistance»

СЛУХАЛИ: доповідь здобувача вищої освіти факультету з підготовки іноземних громадян Фм18(5,0д)англ-04 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Едвін Ювард МВІНУКА на тему: «Розробка нових похідних хінолон-4-ону для боротьби з антибактеріальною резистентністю / Developing new quinolone-4-one derivatives to combat antibacterial resistance», керівник - доцент закладу вищої освіти кафедри медичної хімії, д.фарм.н., доцент Вадим ЗУБКОВ.

УХВАЛИЛИ: рекомендувати кваліфікаційну роботу Едвін Ювард МВІНУКІ до офіційного захисту в Екзаменаційній комісії.

Завідувачка кафедри медичної хімії,
професор **Ліна ПЕРЕХОДА**

Секретар кафедри медичної хімії,
доцент **Марина РАХІМОВА**

Ф А2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Едвін Ювард МВІНУКА до захисту кваліфікаційної роботи
за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Developing new quinolone-4-one derivatives to combat antibacterial resistance».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Едвін Ювард МВІНУКА у повному обсязі виконав кваліфікаційну роботу. За актуальністю, методичним рівнем, теоретичним та практичним значенням, об'ємом виконаних досліджень кваліфікаційна робота відповідає вимогам і допускається до захисту в Екзаменаційній комісії.

Керівник кваліфікаційної роботи

_____ Вадим ЗУБКОВ

«07» квітня 2023 р.

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувач вищої освіти Едвін Ювард МВІНУКА допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувачка кафедри
медичної хімії

_____ Ліна ПЕРЕХОДА

«21» квітня 2023 р.

Qualification work was defended

of Examination commission on

« » of June 2022

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Oleh SHPYCHAK /