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

QUALIFICATION WORK

on the topic: «**SEARCH FOR ANTIBACTERIAL AGENTS AMONG THE DERIVATIVES OF 3-(2-METHOXYETHYL)-2-(ALKYLTHIO)QUINAZOLIN-4(3*H*)-ONE»**

Prepared by: higher education graduate of group Фм19(4,10д)англ-03
specialty 226 Pharmacy, industrial pharmacy
educational program Pharmacy
Wafa IDOUMGHAR
Supervisor: professor of higher education institution of pharmaceutical chemistry department, DSc, professor Serhii VLASOV
Reviewer: professor of higher education institution of medicinal chemistry department, DSc, professor Illya PODOLSKY

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ANNOTATION

Effective methods of synthesis were developed and a number of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives were obtained. Optimization of the synthetic procedure made it possible to easily obtain the key intermediate product, namely 3-(2-methoxyethyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one. Since *M. tuberculosis* and *P. aeruginosa* are known to be resistant to antibiotics, these strains were used to predict binding to TrmD, which made it possible to identify the leading compounds with the best binding parameters.

The work consists of an introduction, three chapters, general conclusions and a list of references, which consists of 84 sources. The content of the work is placed on 45 pages and contains 2 tables, 5 figure, 2 schemes.

Key words: quinazoline, methoxy group, alkylation, molecular docking, antimicrobial activity.

АНОТАЦІЯ

Розроблено ефективні методики ситнтезу та отримано ряд похідних 3-(2-метокисетил)-2-(алкілтіо)хіназолін-4(3*H*)-ону. Оптимізація синтетичної процедури дозволила леко отримувати ключовий напівпродукт, а саме 3-(2-метоксиетил)-2-тіоксо-2,3-дигідрохіназолін-4(1*H*)-он. Оскільки для для *M. tuberculosis* та *P. aeruginosa* відомою є затність до формування резистентності до антибіотиків саме ці шатми було вкористано для прогнозування зв’язування з TrmD. Це дозволило вияити сполуки лідери із найкращіми параметрами зв’язування.

Робота складається із вступу, трьох розділів та загльних висновків, списка використаної літератури, який складає 84 джерело. Зміст роботи викладено на 45 сторінках і проілюстровано 2 таблицями, 5 рисунками, 2 схемами.

Ключові слова: хіназолін, метокси група, алкілування, молекулярний докінг, антимікробна активність.

CONTENTS

INTRODUCTION

Relevance of the topic. Infectious diseases of bacterial etiology have a significant impact on people because they can cause both temporary loss of work capacity and have more serious consequences. Infections can lead to inability and even death of a person, and therefore these types of diseases require timely diagnosis and appropriate treatment. Of course, the best ways to fight such diseases are sanitary and hygienic or immuno-prophylaxis. At the same time, antibiotics, chemotherapeutic agents, drugs of biotechnological origin, etc. exist in the arsenal of doctors to overcome diseases. On the other hand, a number of bacteria, such as mycobacterium tuberculosis, are difficult to treat due to their natural morphology. At the same time, bacteria such as *P. aeruginosa*, although they can be suppressed by the action of antibiotics, they quickly develop resistance to therapeutic agents. Therefore, the problem of developing new antimicrobial agents for the treatment of tuberculosis and infections caused by *P. aeruginosa* is an urgent issue. Moreover, a very interesting direction in this case is the development of antibiotics with fundamentally new mechanisms of action. It is the quinazoline derivatives, among which there are many antibacterial drugs, when modified with such an attractive fragment as 2-methoxyethyl from the point of view of medical chemistry, that can become new chemotherapeutic agents with fundamentally new mechanisms of action.

Purpose of the study. Synthesis of new 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives and prediction of their binding to active sites of TrmD inhibitors for enzymes isolated from *M. tuberculosis* and *P. aeruginosa*.

To achieve the goal, the following **tasks** were set:

1. To investigate modern approaches to the therapy of bacterial diseases and the creation of new antimicrobial drugs caused by bacteria capable of forming resistant cells using the example of tuberculosis.

2. On the basis of available reagents, synthesize 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives and confirm their structure using modern instrumental methods.

3. Perform computer prediction of the binding of the obtained 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives to the active sites of TrmD isolated from *M. tuberculosis* and *P. aeruginosa* and identify the leading compounds.

4. To formulate recommendations for modification of the structure to improve the parameters of binding to the active sites of TrmD data for both strains.

Object of the study. Derivatives of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one as potential antimicrobial agents with an innovative mechanism of action.

Subject of the study. Rational synthesis pathways of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one and their ability to bind to the active sites of TrmD emzimes isolated from *M. tuberculosis* and *P. aeruginosa*.

Methods of the study:

1. Analysis and systematization of scientific and patent literature. 2. Methods of synthetic organic chemistry. 3. Instrumental methods of analysis. 4. Molecular docking method.

The practical value of the results. The developed methods for the synthesis of new derivatives of quinazolin-4(3H)-one will enrich the arsenal of methods for obtaining substituted derivatives of this heterocyclic system, and the obtained samples of compounds will be useful for the study of antimicrobial activity by in vitro methods. The in silico prediction data and their analysis will provide opportunities for the rational design of antibacterial agents with innovative mechanisms of action (TrmD inhibition) in order to overcome bacterial resistance to antibiotic therapy.

Elements of scientific research. For a number of new derivatives of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one, effective synthesic methods were developed. For the first time, a study of the binding of the obtained derivatives to the

active sites of TrmD isolated from *M. tuberculosis* and *P. aeruginosa* was carried out, which made it possible to evaluate their potential as new antibacterial agents with innovative mechanisms of action.

Approbation of research results and publications. The results of the research were presented at XXX Scientific and Practical Conference of Young Scientists and Students "Topical issues of new medicines development": (17-19 April 2024 p., Kharkiv). The abstract was published:

Idoumghar W., Vlasov S.V. Synthesis of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)- ones as possible antibiotics with innovative mechanism of action. "Topical issues of new medicines development": materials of XXX Scientific and Practical Conference of Young Scientists and Students (17-19 April 2024 p., Kharkiv). – Kharkiv: NUPh, 2024. – P. 48.

Structure and scope of the qualification work. The work consists of an introduction, three chapters, general conclusions and a list of references, which consists of 84 sources. The content of the work is placed on 45 pages and contains 2 tables, 5 figure, 2 schemes.

CHAPTER 1. MODERN APPROACHES IN ANTITUBERCULOSIS DRUG DISCOVERY

(Literature review)

1.1. Tuberculosis drug discovery

German microbiologist Robert Koch identified the pathogen of tuberculosis as *Mycobacterium tuberculosis*. [1]. Tuberculosis (TB) is a serious concern as an infectious disease that poses a great risk to public health worldwide. Even with anti-tuberculosis drugs introduced over the years, tuberculosis remains one of the leading causes of death worldwide. [2]. According to the World Health Organization (WHO), this is the most common infection caused by a single bacterium. During 2017, about 10 million people were diagnosed with tuberculosis, and among them 558,000 developed resistances to the most effective first-line drug, rifampicin. According to another WHO study, approximately 1.5 million deaths were recorded in 2018. [3].

The infection of a third of the world's population and the death of approximately 1.7–1.8 million people annually from this pathogen indicate the difficulty in finding new antibiotics to combat this deadly disease. Thus, antimicrobial agents effective against *M. tuberculosis* are urgently needed to address this global epidemic, which is exacerbated by drug resistance, prolonged treatment, and co-infection, especially in the context of human immunodeficiency virus (HIV). For more than 40 years, not a single new antibiotic has been developed to treat tuberculosis. [4, 5].

Recently, efforts have been made to develop phenotypic screening using commercial libraries from suppliers to identify compounds that are capable of inhibiting the development of Mtb. [6, 7, 8]. This intervention brings a hope to the search for new therapeutic agents against *M. tuberculosis*. In connection with the need

to quickly stop the *M. tuberculosis* epidemic, it is important to improve diagnostic tools and increase the effectiveness of therapy used to treat tuberculosis in patients with a confirmed diagnosis. This intervention simplifies treatment regimens that usually require strict adherence to achieve effective treatment. Rapid and affordable diagnostic kits, which can be readily available to the public, facilitate early diagnosis, and multi-targeted drugs greatly improve treatment outcomes. [9]. Much attention is paid to the development of new potentially effective antimicrobial agents to reduce the resistance of tuberculosis strains. A variety of strategies and efforts have been employed, including structural design of pathogen-specific inhibitors using computational methods. [10, 11, 12, 13].

When starting the discovery of potential drugs, the first step involves identifying and studying the enzymes or proteins necessary for the growth and development of the pathogen. Researchers then test these proteins for their effectiveness and inhibitory effect against certain chemicals or compounds libraries. This process leads to the identification of potential drugs with the help of computer programs after careful study of the details of the target and lead molecule. This approach avoids following "false" leads and can be particularly useful for pharmaceutical companies, agencies and research laboratories. A new understanding of the quantitative relationship between structure and biological activity is contributing to the development of computer-aided drug design (CADD) programs, which greatly accelerates and simplifies the process of finding new therapeutic agents against tuberculosis, unlike the traditional approach,

The rapid pace of development of high-throughput screening (HTS) and computational chemistry technologies has created an environment that allows efficient screening and synthesis of huge libraries of compounds in a short period of time. This significantly speeds up the drug development process. [14]. CADD includes the storage, management, analysis and modeling of potential therapeutic compounds. It covers computer methods and techniques for storing, processing, analyzing and modeling chemical compounds. CADD also involves the use of computer programs to

design chemical compounds, tools to systematically evaluate potential candidates, and the creation of digital libraries to study chemical interactions between molecules, among other tasks. [15].

Advances in drug discovery include the use of computational analysis to identify and validate good molecular targets, leading to the development of new therapeutics; these methods are also used in preclinical trials, radically changing the drug development process. The use of computational methods can reduce drug production costs. [16, 17, 18]. It typically takes an average of 10 to 15 years and $500 to $800 million to bring a drug to market, with a significant portion of that cost going to analogue synthesis and testing. The use of computational methods during optimization significantly reduces drug development costs, as there are computational models that can screen thousands of compounds even before they are synthesized and tested *in vitro*.

New TB therapeutics have emerged through the use of high-throughput screening (HTS) and other related software developments. There is also increasing access to biological and chemical data related to *Mycobacterium tuberculosis* (Mtb) to facilitate the discovery of new molecular targets. Improvements in data storage, and parallel processing are advancing CADD (computer-aided drug design) as a necessary component of pharmaceutical research against tuberculosis. CADD makes the drug discovery process more comprehensive by covering different fields. The use of CADD computational tools enabled the assignment of more than 5,000 macromolecular structures from the Protein Data Bank (PDB) to Mtb. [19, 20]. This repository is fertile ground for the discovery of new compounds in the form of effective drug molecules to combat tuberculosis. [1, 19].

CADD can serve as structural drug design (SBDD) or ligand-based drug design (LBDD). These are the two most common approaches to drug discovery. Currently, none of the methods can fully satisfy all the requirements for drug discovery and production. As a result, a number of computational methods are widely and effectively used in combinatorial and system approaches [1]. The development of tolerance to

tuberculosis, topical treatments, and the development and introduction of new compounds as anti-tuberculosis therapies are all part of the evolution of the fight against the disease. Recent studies have also evaluated a wide range of anti-tuberculosis drug designs based on computer-aided drug design (CADD) techniques and identification of specific molecular targets using in silico approaches [21, 22].

1.2. TB Pathology, Management, and Control

Tuberculosis (TB) is a chronic infectious disease that is transmitted mainly by airborne droplets. A person becomes infected when he inhales air containing droplets of tuberculosis bacteria that enter the lungs. A newly infected person may develop symptoms due to a weakened immune system due to other infectious diseases, such as HIV. However, unless the immune system is compromised, Mtb remains dormant. Alveolar macrophages recognize bacteria as foreign bodies and assimilate them. The bacteria multiply and finally infect macrophages, spreading from this point through the bloodstream throughout the body. However, it is important to note that infections such as HIV, as well as diseases associated with alcoholism and smoking, contribute to a very high risk of developing tuberculosis [23, 24].

Tuberculosis is usually considered either latent or active. Latent tuberculosis infection (LTBI) is not transmitted, and patients in this group do not have symptoms. However, patients with active TB can carry the bacteria, and common symptoms in such individuals include fever, weight loss, productive cough, and coughing up blood. [25, 26] It is estimated that about 1.7 billion people worldwide may have latent tuberculosis infection (LTI) and are at risk of developing active tuberculosis. A report by the World Health Organization (WHO) indicates that active tuberculosis affects 5.7 million men, 3.2 million women and 1.1 million children, of whom 9% were also infected with HIV in 2018 [27].

1.3. Treatment of tuberculosis and classification of antituberculosis drugs

The invention and development of the first line of therapy for tuberculosis in the 1960s marked the first successful treatment of this infectious disease. Antituberculosis drugs can be classified according to their origin (natural or synthetic), mode of action, and patient status (phase or regimen), dividing them into first-line and second-line regimens [28]. To date, there are approximately 20 drugs for the treatment of tuberculosis on the market. The use of these methods can be both individual and combined [29].

Untreated tuberculosis can cause death. During the period from 2000 to 2018, doctors saved about 58 million infected people using traditional methods of treatment. In 2017, an 85 percent global success rate for treating the first cases of tuberculosis was recorded. However, the same year was marked by a 56 percent success rate in treating drug-resistant tuberculosis worldwide [30].

Drugs of the first line

First-line treatment regimens for tuberculosis include drugs such as isoniazid, rifampicin, pyrazinamide, streptomycin, and ethambutol [29]. In the case of latent tuberculosis infection (LTBI), the World Health Organization (WHO) recommends the use of isoniazid in monotherapy or in combination with rifampicin for a period of 3 to 9 months [30].

For a long period of time, the treatment of tuberculosis involved the use of a conventional regimen that included first-line antibiotics for the first 2 months, followed by combination therapy with isoniazid and rifampicin for the next 4 months [2]. Despite this, this treatment plan shows a high level of effectiveness. However, prolonged treatment may cause various side effects such as skin rash, dizziness, and

gastrointestinal disturbances, among others, which may lead to patient noncompliance [31].

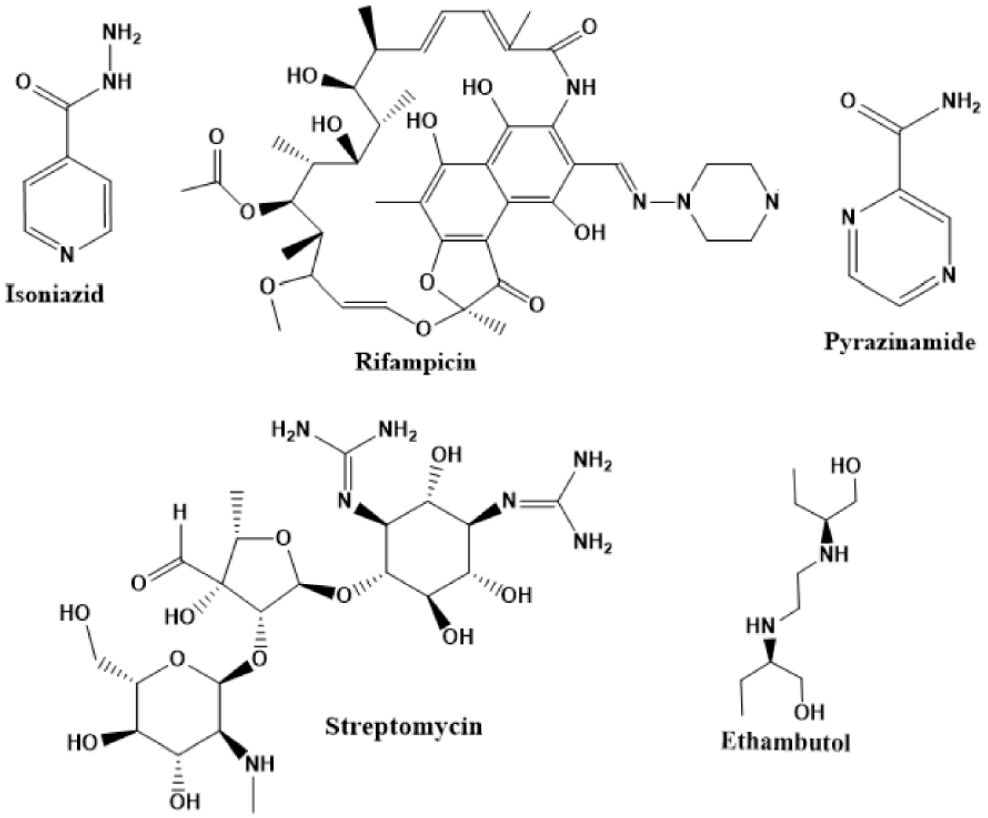


Fig. 1.1. Antituberculosis drugs of the first line

Second-Line Drugs

When first-line drugs fail or become less effective, medical professionals usually turn to second-line drugs. These drugs are often prescribed when a patient shows signs of resistance to one or more drugs [32]. A sudden decrease in the effectiveness of treatment, including incomplete tuberculosis treatment regimens, often leads to the recurrence of the disease and the formation of resistance. The management of resistance to tuberculosis involves the active development of several drugs to support global initiatives to combat the disease. In the second-line treatment regimen, drugs such as para-aminosalicylic acid (PAA), ethionamide, cycloserine, viomycin, and ciprofloxacin

are used. These methods of treatment are characterized by long-term action, questionable effectiveness and high toxicity, which can also lead to a decrease in the responsibility of patients and obtaining undesirable results.

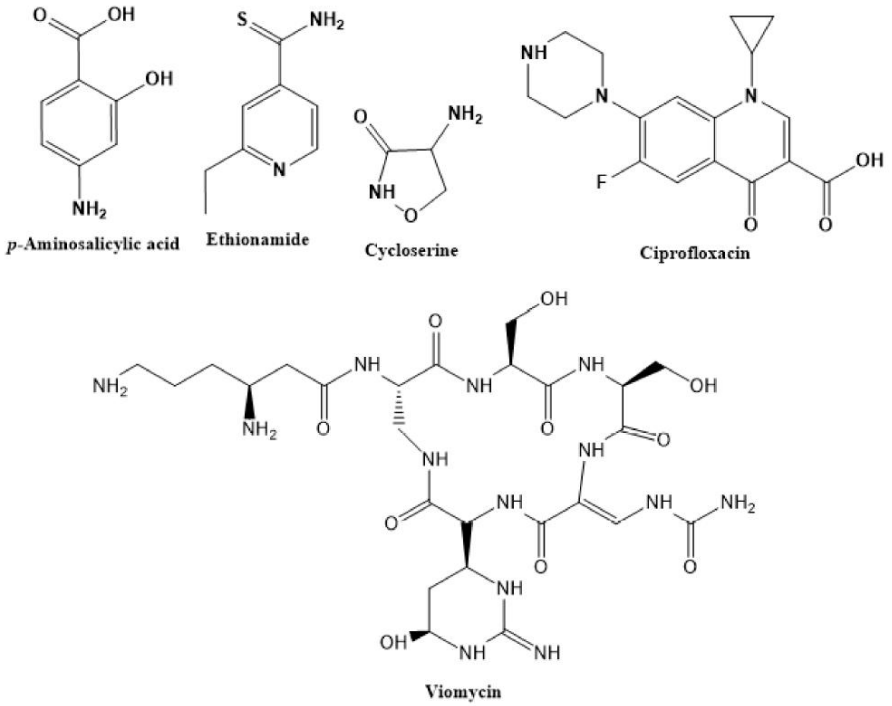


Fig. 1.2. Antituberculosis drugs of the second line

1.4. Emergence and treatment of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB)

The emergence of resistance is attributed to several factors, such as incorrect adherence to the prescribed course of treatment, inconsistent monitoring, drug abuse, and strain mutations. It is also possible to develop resistance due to changes in membrane pumps, changes in the interaction of the drug with the target, and chromosomal mutations [33].

Another reason may be the limited permeability of the mycobacterial cell wall due to its lipid-rich structure, which makes it difficult for compounds to reach their targets. MDR-TB was first identified in the 1990s because the bacteria did not respond to treatment with first-line drugs such as isoniazid and rifampicin [34]. In two years, from 2013 to 2014, about 500,000 new cases of MDR-TB were added to the global statistics, resulting in the loss of about 200,000 lives [35].

XDR-TB occurs when both first-line and second-line drugs are ineffective. Of the total volume of registration of cases of multidrug-resistant tuberculosis (MDR-TB) per year, which is 500,000, 5-7% are cases of XDR-TB [36]. In late 2012, the US Food and Drug Administration (FDA) approved the use of bedaquiline [1].

In 2014, the European Medicines Agency (EMA) granted conditional approval for the use of delamanid for the treatment of MDR-TB in adults. Recently, the US Food and Drug Administration (FDA) approved the use of pretomanid in combination with bedaquiline and linezolid for the treatment of drug-resistant tuberculosis [1]. However, this drug has shown high toxicity, and its use is associated with an increased risk of death. This negative effect was the reason for the outrage about its approval. In clinical trials, an approximately fourfold increase in the death rate was found among patients treated with bedaquiline compared with those treated with the alternative therapy placebo [37].

The danger of developing drug resistance is a real threat, so there is a need to create new therapeutic agents that do not cause cross-resistance to existing treatment methods.

1.5. The modern understanding of anti-tuberculosis drugs action

As for the first-line compound developed for tuberculosis, it includes isonicotinic acid hydrazide (ING) [38]. Even if the specific mechanism of action of isoniazid is not yet fully understood, researchers have put forward several possible mechanisms.

According to recent analyses, the hydroxyl radical oxidizes INH at the primary nitrogen of the hydrasil moiety, and further reduction of the hydrated electron occurs in the pyridine ring [39]. Another related study showed that bacteria penetrate the cell wall by passive diffusion. Further activation by an oxidative enzyme such as catalase peroxidase (KatG) leads to the formation of a reactive isonicotinylacyl radical intermediate. This step precedes the formation of INH-NAD and leads to the attachment and inhibition of InhA (2-trans-eniolacyl transfer protein reductase) in Mtb. In general, this process limits the formation of mycolic acid, an important component of the cell wall [38, 40, 41].

Similar studies also indicate that highly reactive oxygen species (ROS) can be generated by KatG-mediated activation of INH. Proposed ROS may include superoxide, nitric oxide, peroxide, and hydroxyl radicals, as well as isonicotinic acylanion. These reactive oxygen species attack different targets in Mtb cells, leading to increased oxidative stress and reduced Mtb resistance [42, 43, 44, 45]. This possibility, which was confirmed in another study, involved the use of a computational model to understand the mechanism of action of isoniazid as an anti-tuberculosis drug. The results of the study indicated the involvement of the KatG mutation at position 315 (S315T/S315N) in the formation of hydrogen bonds between INH and mutant Thr(T)/Asn(N) residues, which leads to the formation of the INH free radical [46].

Mutations in eight other genes (furA, inhA, kasA, rv0340, iniB, iniA, iniC, and efpA), as well as in two DNA regulatory regions (oxyR-ahpC and the mabA-inhA promoter), were also found to be associated with resistance to INH. [45].

Ethambutol (EMB) is a key component of antituberculosis therapy. It is used to treat Mtb infection in combination with other first-line drugs. The exact mechanism of action of EMB, as well as some other antituberculosis drugs, remains unclear. Studies have shown that EMB inhibits Mtb cell wall synthesis by disrupting arabinogalactan synthesis and inhibiting arabinosyltransferase [47].

Approximately 65% of strains that are resistant to INH are also resistant to EMB. [33]. Most cases of resistance to EMB are associated with mutations in the embB gene, in particular, in the intergenic region between embC and embA (IGR). In most cases, researchers established a connection between resistance to EMB and mutations in codon 306, embB406, embA(-16) and embB497 of the Mtb gene. There is also the possibility of EMB resistance associated with overexpression and mutations in the ubiA gene. [48, 49, 50].

Rifampicin (RIF) is an antibiotic obtained from the gram-positive bacterium *Amycolatopsis rifamycinica* and belongs to the group of rifamycins. In addition to its activity against *Mycobacterium tuberculosis* (Mtb), it is effective against fungi and viruses, making it a broad-spectrum antibiotic. Other variants of rifamycin, such as rifamycin, rifamixin, rifabutin, and rifapentine, have been identified over time. The mechanism of action of rifampicin is inhibition of RNA synthesis, which depends on DNA. The use of rifampicin/rifampin has led to mutations in the b-subunit of RNA polymerase, which causes the development of resistance. The key mutant is located in the rpoB gene codons between 507 and 533, known as the rifampicin resistance region. Codons 516, 526, and 531 are frequently mutated in cases of rifampicin resistance [51].

Pyrazinamide (PZA is another effective drug that has been used in first-line therapy for about four decades. Its properties include the ability to penetrate the bacterial cell wall by passive diffusion. Under the action of pyrazinamidase, it is converted to pyrazinic acid, which in turn inhibits Mtb activity by several mechanisms, such as inhibition of the enzyme fatty acid synthase (FAS) I, binding to ribosomal protein S1 (RpsA), and membrane inhibition [52, 53]. In 1996, it was reported that a PZA-resistant strain of Mtb originated from mutations in the pncA gene [54]. The researchers found that mutations in the pncA gene are responsible for resistance to PZA [55]. Indeed, there is evidence for the existence of PZA-resistant strains that do not have mutations in the pncA gene. This suggests the possibility of PZA-mediated resistance through other genes and mechanisms [56]. Thus, according to studies,

mutated recombinant pncA can lead to a decrease in enzymatic activity, and this decrease can depend on the position and nature of the mutation. Any structural defect in the pncA gene can potentially affect the function of PZase (pyrazinamidase), which can affect sensitivity to the drug PZA (pyrazinamide) [57, 58].

Streptomycin (STR) is a natural product effective in the treatment of tuberculosis. It is obtained from *Streptomyces griseus*, a soil actinomycete. STR represses protein synthesis by interacting tightly with the 30S ribosomal subunit and 16S rRNA, which contains the rpsL and rrs genes [59]. In recent years, it has been established that mutations in the gidB gene, which encodes a conserved 7-methylguanosine methyltransferase specific for 16S rRNA, can cause streptomycin resistance (STR). These mutations can affect the sensitivity of microorganisms to STR by changing their ribosomal structures and interaction with the antibiotic [60]. The main mechanism of resistance to streptomycin (STR) is the presence of mutations in the genes rpsL and rrs, which combine more than 70% of cases of resistance. The most common of these mutations is a transition in codon 43, where lysine is replaced by arginine, which leads to the emergence of a high level of resistance to streptomycin in the rpsL gene. In particular, the most specific mutations in the rrs gene are found in the interval between nucleotides 530 and 915 [61]. Thus, there are strains resistant to streptomycin that do not have detectable mutations in the rpsL and rrs genes, which indicates the existence of other possible mechanisms of resistance. Ethionamide, which is an isonicotinic acid derivative and structurally similar to isoniazid, is another drug. These are prodrugs that require activation by the monooxygenase encoded by the ethA gene. The mechanism of action of ethionamide includes formation of an adduct with NAD and inhibition of the enzyme enoyl-acyl-coenzyme A-reductase, which leads to inhibition of the synthesis of mycolic acid [62]. Mutations in the etaA/ethA, ethR and inhA genes lead to resistance to ethionamide [63].

Also, experiments with spontaneous Mtb mutants that showed resistance to isoniazid and ethionamide showed that these mutations are associated with the mshA

gene. The mshA gene is responsible for encoding the mycothiol biosynthesis enzyme [64].

Para-aminosalicylic acid (PAS) was first discovered in 1948. Its mechanism of action is that it inhibits thymidylate synthase, interfering with iron absorption and folic acid synthesis. Recent studies indicate that resistance to PAS in Mtb isolates may be associated with multiple missense mutations in the folC gene, which encodes dihydrofolate synthase [65]. Mutations in the thyA gene found in clinical isolates were associated with para-aminosalicylic acid (PAS) resistance in a study using transposon mutagenesis [66].

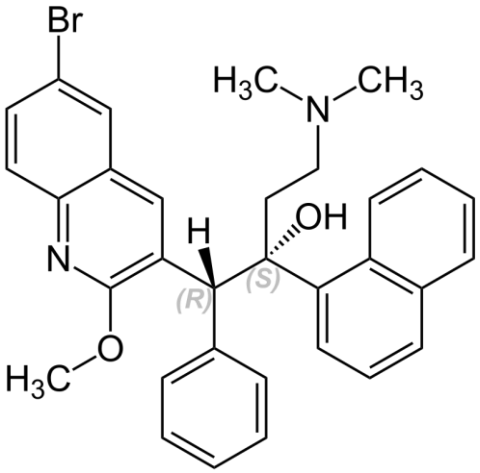
However, it is important to note that mutations in the thyA gene were found in less than 40% of PAS-resistant strains, indicating that other mechanisms of drug resistance may also be present. [66, 67].

Doctors often prescribe fluoroquinolones such as levofloxacin and melofloxacin as second-line drugs for the treatment of MDR-TB. The action of fluoroquinolones is based on blocking the action of topoisomerase II (DNA gyrase) and topoisomerase IV. These proteins are important for bacterial reproduction and survival, and their genes, including gyrA, gyrB, parC, and parE, encode these enzymes. [68].

Chromosomal mutations in the gyrA or gyrB region are the most common cause of fluoroquinolone resistance in *Mycobacterium tuberculosis*. Mutations in positions 90 and 94 of the GyrA gene are particularly frequent, but changes in other positions of this gene have also been found to cause resistance to these antibiotics. [69]. Indeed, there are other compounds used in the treatment of tuberculosis as second-line drugs and in combination therapy. These compounds include capreomycin, kanamycin, viomycin, amikacin, cycloserine, macrolides (especially clarithromycin), clofazimine, and linezolid. These drugs play an important role in the fight against tuberculosis, especially in cases where resistance to first-line drugs is found.

1.6. New anti-tuberculosis drugs discovered approaches

Researchers are discovering new anti-tuberculosis drugs through a variety of methods that allow them to review existing drugs and introduce new compounds into the TB treatment regimen. Such methods include drug repurposing, modification of drug scaffolds, revision of existing targets, target screening, and phenotypic screening. These approaches allow the discovery of new agents or the modification of existing ones to improve their effectiveness in the fight against tuberculosis. High-throughput screening is used to find drugs against Mycobacterium tuberculosis (Mtb), in which researchers analyze databases of compounds for their antibacterial activity against mycobacterial cells in culture. In most studies, it is rational to establish the effectiveness of identified compounds based on in vitro and *in vivo* experiments. The process of developing new anti-tuberculosis drugs and potential agents in clinical trials involves drug-to-target steps that include whole-cell screening.



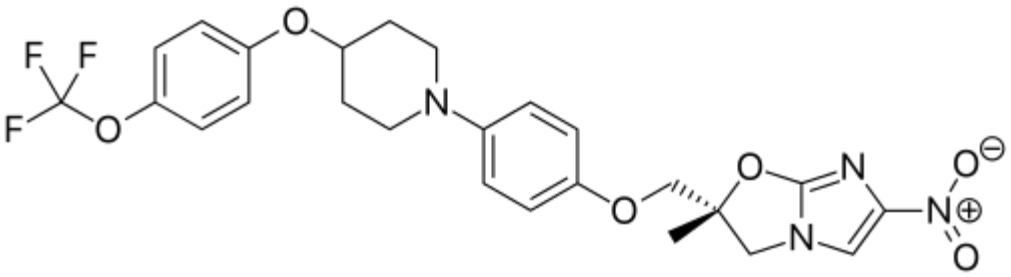
Bedaquiline

Johnson & Johnson discovered Bedaquiline by screening about 70,000 compounds against Mycobacterium smegmatis. They unveiled the compound in 2004

at the Inter-Science Conference on Antimicrobials and Chemotherapy (ICAAC) and later around 2012 it was approved by the FDA. Bedaquiline inhibits adenosine-50-triphosphate (ATP) synthase activity and interferes with energy supply, making it a unique targeting agent. Mtb ATP synthase became a recognized target after the discovery of bedaquiline [70].

Researchers including Pete et al discovered two series of imidazopyridinamides (IPAs) during high-throughput screening of a library of 121,156 chemical compounds at the Institut Pasteur in Korea. The study aimed to determine the ability of these compounds to inhibit the growth of *Mycobacterium tuberculosis* in mouse macrophages. [71,70]. Synthesis and evaluation of 477 derivatives of the hit compound led to the development of an optimized imidazopyridinamide (IPA) called telazebec (Q203). The primary target of Q203 is the bc1 cytochrome unit complex, a critical component of the electron transport system that is essential for ATP synthesis [72]. Currently, Qurient Co. Ltd. is conducting phase 2 clinical trials to evaluate the bactericidal efficacy, safety, tolerability, and pharmacokinetic properties of Q203 in repeated oral doses. Additionally, other compounds identified by high-throughput screening (HTS) include benzothiazinones, maconisone, azaindoles, and OPC-167832.

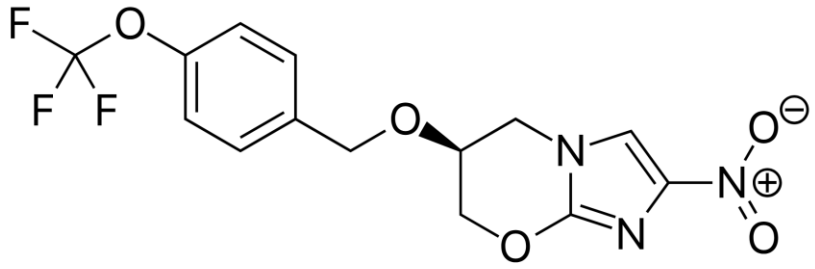
Further chemical studies of some existing antimicrobial molecules by modifying the drug frameworks led to the development of many analogues, among which the important antituberculosis drugs are pretomanid and delamanid. These compounds have recently been registered as agents for the treatment of tuberculosis and are part of a new treatment regimen for cases with multidrug resistance (MDR). Delamanid (OPC-67683) and pretomanid (PA-824), both belonging to the class of nitroimidazoles, were discovered in the microorganism Streptomyces eurocidicus [73].



Delamanid

In 2014, the European Medicines Agency (EMA) conditionally approved delamanid, a nitro-dihydro-imidazooxazole derivative developed by Otsuka Pharmaceutical, for the treatment of multidrug-resistant tuberculosis (MDR-TB) in adults. Delamanid and pretomanid have a similar multipurpose mode of action, affecting the biosynthesis of the cell wall due to disruption of the synthesis of methoxy- and ketomycolic acid. They also exhibit respiratory toxicity due to the release of nitric oxide during the metabolism of bacterial drugs [74].

Pretomanid is another analogue that shows activity against *Mycobacterium tuberculosis* (Mtb) [74].



Pretomanid

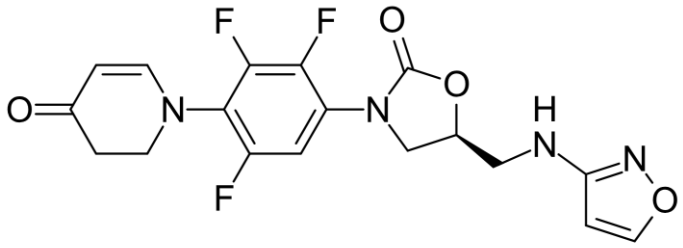
PathoGenesis Corporation, in collaboration with the Global Alliance for Tuberculosis Drug Development, developed pretomanid. In animal models, this relatively small molecule shows excellent activity both in vitro and in vivo, and shows

promise in terms of tolerability and safety. The mechanism of its action consists in the activation of nitroreductase, which leads to the inhibition of the synthesis of proteins and lipids of the Mtb cell wall. [75].

The FDA approved a new application for the drug pretomanide for the treatment of a broad spectrum of tuberculosis, including multidrug-resistant tuberculosis, in combination with bedaquiline and linezolid. This indicates the potential for the use of this combination in the treatment of tuberculosis in the future.

The search for a new drug acting as a second-generation drug compared to ethambutol led to the creation of a library based on the 1,2-ethylenediamine pharmacophore. These compounds, tested against Mycobacterium tuberculosis (Mtb), led to the discovery of the potent drug SQ109. This breakthrough was made possible by the joint efforts of scientists at Sequella, Inc. (Rockville, Maryland, USA) and the US National Institutes of Health [76]. The mechanism of action of the drug SQ109 involves the inhibition of MmpL3, which is a membrane transporter of trehalose monomycolate, which is important in the process of cell wall synthesis. In addition, SQ109 inhibits MenA and MenG enzymes, which play a key role in the biosynthesis of menaquinone. This drug acts as a decoupler, reducing the synthesis of adenosine triphosphate (ATP) [77].

Contezolid and contezolid acefosamil were developed through structural modification of linezolid to overcome limitations associated with its clinical use, such as myelosuppression and inhibition of serotonergic monoamine oxidase. Contezolid is currently in phase 3 clinical trials, and its intravenous administration is facilitated by a water-soluble prodrug known as contezolid acefosamil, which has no pronounced antimicrobial activity. The activity of contezolid in vitro against a resistant Mtb strain is related to the activity of linezolid. [78, 79, 80, 81].



Contezolid

Sanfethrinem and its oral prodrug, cilexetil, are a new carbapenem that was developed by GSK and is in clinical trials. The discovery of this drug occurred during the screening of nearly 2000 β-lactams for their activity against Mtb H37Rv. Research was also conducted to identify promising activity against MDR and XDR clinical isolates.

Recently, the development of new Mtb therapeutics involves the identification of compounds that effectively inhibit specific targets required for bacterial survival and proliferation in the host. It is important to note that Mtb secretes key proteins that play a role in providing access to nutrients, modifying the host's immune system, and generating resistance to therapeutic agents. [82, 83].

This approach is an important aspect of host-pathogen interaction. Inhibition of any of these key proteins disrupts the activity of pathogens in the host and limits their destructive effects on the body. Considering this, inhibition of proteins has become an important criterion in the development of new drugs. The application of computational methods allows diverse libraries of compounds to be screened for their effects on key proteins known to be involved in Mtb survival. Even with more than 500 known Mtb core proteins, only 73 targets and 10 potential targets for the development of new anti-tuberculosis drugs have been identified so far. Thus, there is a need to investigate the inhibition of a number of other key proteins to identify new effective antituberculosis agents [84]. Some scientists believe that an effective anti-tuberculosis drug must be able to inhibit multiple protein targets, limiting the ability of the microbe to develop resistance over a long period of time. This approach simplifies the Mtb treatment

regimen and avoids complications associated with the rapid development of drug resistance.

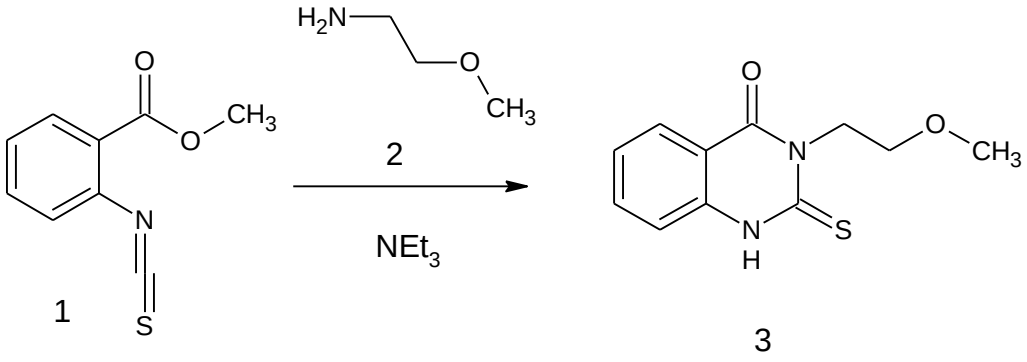
Conclusions to the Chapter 1

1. Using tuberculosis, which is a dangerous and difficult to treat disease, as an example the ways of treatment and mechanisms of antimicrobial drug resistance are analyzed.
2. Innovative anti-tuberculosis drugs and approaches to the development of new drugs were considered, as a result of which it was established that resistance can be overcome by creating antibacterial drugs with fundamentally new mechanisms of action.

CHAPTER 2. SYNTEHSIS OF 3-(2-METHOXYETHYL)-2-(ALKYLTHIO)QUINAZOLIN-4(3*H*)-ONE DERIVATIVES

The synthesis of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives was carried out on the basis of methyl 2-isothiocyanatobenzoate **1** by cyclization of 2-methoxyethanamine, which was stimulated by adding triethylamine to the reaction medium. The 2-propanol medium was used for the reaction, which proved to be effective from the point of view of toxicity and the ability to maintain the reaction temperature sufficient for cyclization.

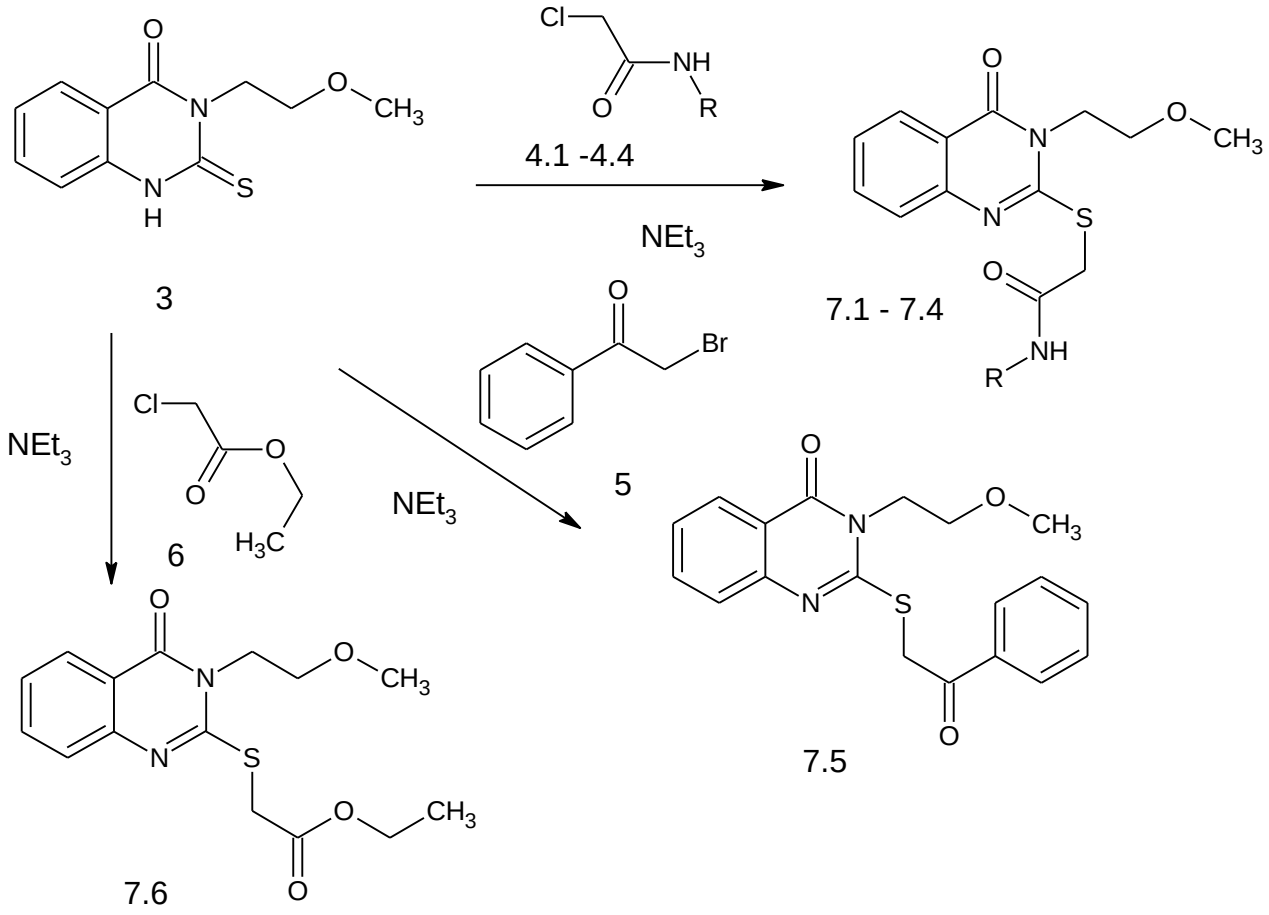
Scheme 2.1



Isolation of the reaction product **3** in the form of thione was carried out by acidifying the reaction mixture with orthophosphoric acid, and the precipitate formed was filtered and thoroughly washed with a large amount of water.

Subsequently, compound 3 was alkylated with amides of chloroacetic acid, phenacyl bromide, and ethyl chloroacetate (Scheme 2.2). As a result, various products 7 were obtained in the form of white crystalline substances. In order to establish the structure of the obtained substances, a key role was assigned to the application of nuclear magnetic resonance spectroscopy on protons.

Scheme 2.2



In the ^{1}H NMR spectra of the obtained compounds **7**, the signals of the methoxy group of the methoxy substituent are observed in the range of 3.26–3.28 ppm. Also, the clear signals of the methylene groups of the ethylene bridge, which are observed at 3.61 - 3.67 and 4.28 - 4.59 m.p.h., are characteristic for the majority of the compound. but in some cases they overlap with the signals of other groups of protons that resonate in the same region. Signals of methylene protons of the S-alkyl radical are observed at 4.20–4.22 ppm. for chloroacetamide alkylators and thioacetic acid ethyl ester

derivative. In the case of compound **7.5**, the signal of the methylene group of the 2-oxo-2-phenyl fragment is present at 4.86 ppm.

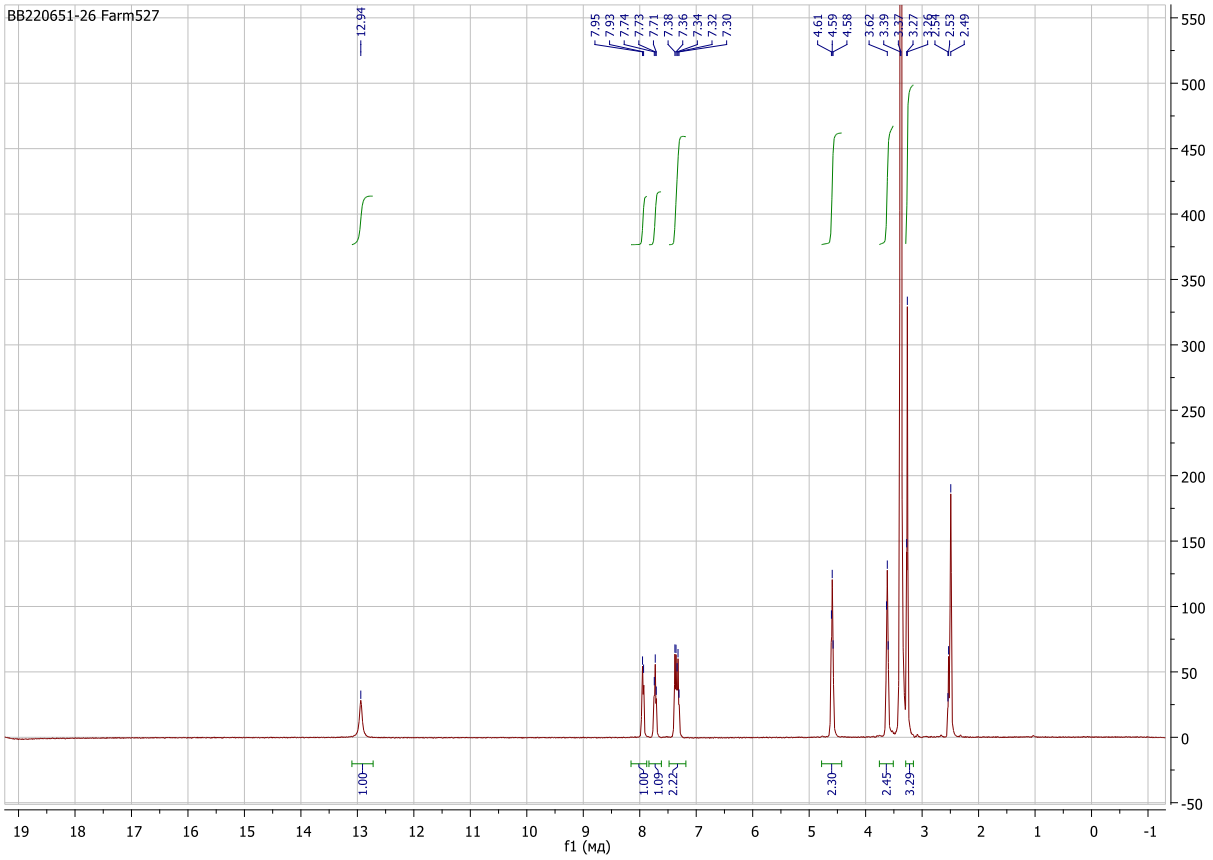


Fig. 2.1 ^{1}H NMR spectrum of 3-(2-methoxyethyl)-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one **3**

About the presence of ethyl radical in ester **7.6**. shows the characteristic signals in the form of a methyl group triplet at 1.19 ppm. and the component of the multiplet in the range of 4.06 – 4.20 ppm, which is formed by superposition of the methylene signals of the carbetooxy group with one of the signals of the ethylene fragment.

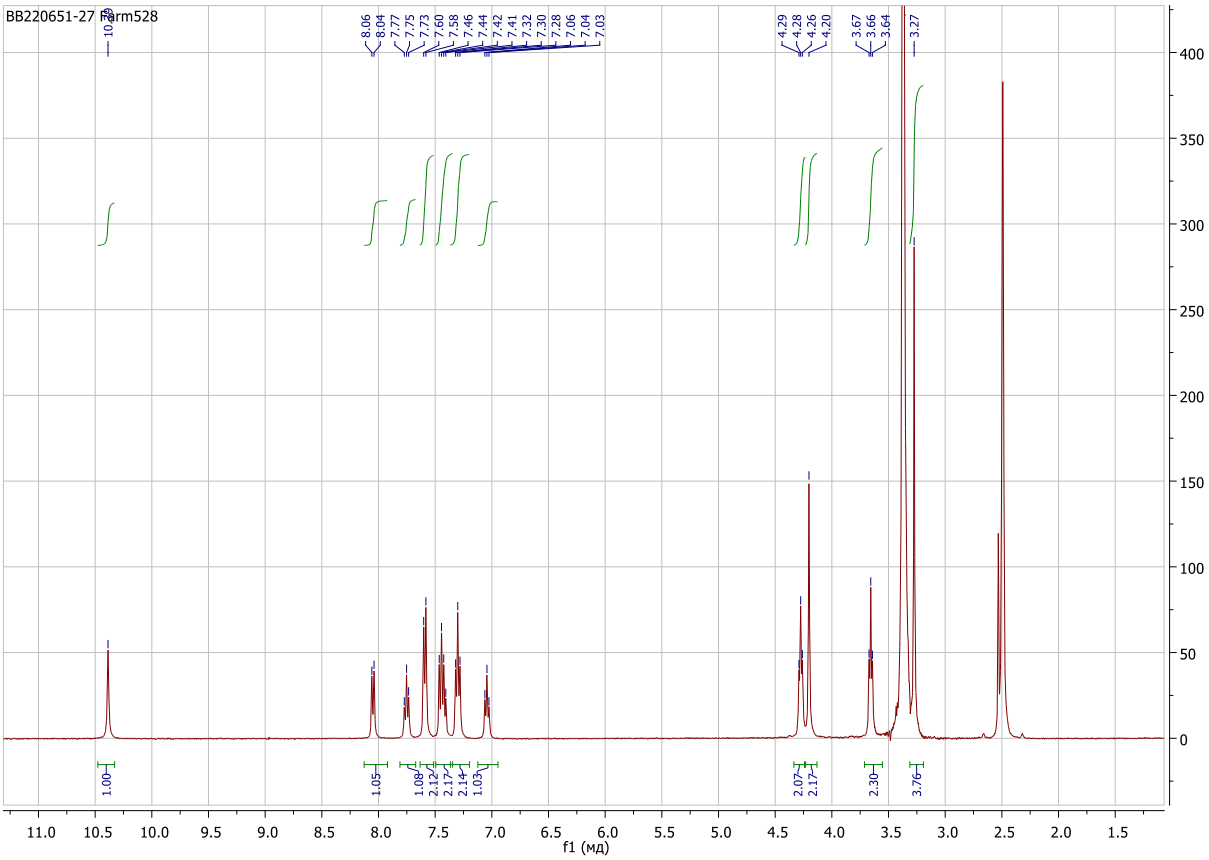


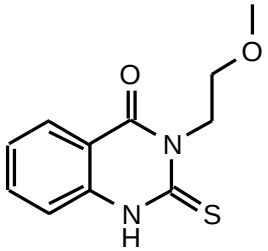
Fig. 2.2 ^{1}H NMR spectrum of 2-{[3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}-*N*-phenylacetamide **7.1**

Experimental part

All solvents and reagents were obtained from commercial sources. The melting points were determined in a capillary using an electrothermal IA9100X1 (Bibby Scientific Limited, Staffordshire, UK) digital melting point apparatus. The elemental analyses were performed on a Euro Vector EA-3000 (Eurovector SPA, Redavalle, Italy) microanalyzer and were within 0.4% of the theoretical values. ^{1}H NMR spectra for the compounds were recorded on Bruker Avance DRX 500 at 500 MHz and Varian 400 spectrometer at 400 MHz spectrometers, solvent - DMSO-d_6, internal standard TMS (^{1}H). LC/MS spectra were recorded on Agilent 1100 HPLC instrument equipped

with diode matrix and mass detectors (Agilent LC- MSD SL), column Zorbax SB-C18 (4.6 mm × 15 mm). Atmospheric Pressure Chemical Ionization (APCI) was used in the experiment.

Method for synthesis of 3-(2-Methoxyethyl)-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (3)



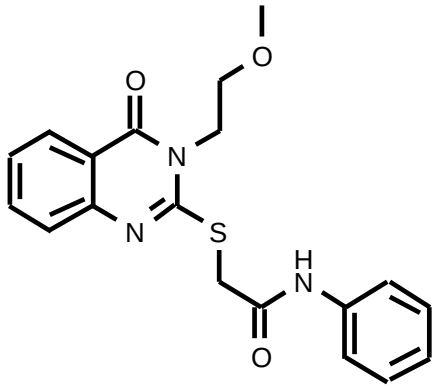
2-Methoxyethanamine (**2**) 4.3 ml (0.057 mol) was added to 10 g (0.052 mol) of methyl 2-isothiocyanatobenzoate (**1**) in 100 ml of 2-propanol, and the mixture was heated at 60ºC for 2 hours. Then 8.3 ml (0.06 mol) of triethylamine was added and the reaction mixture was heated for another 1 hour. After cooling, water was added to the reaction mixture and the resulting solution was neutralized with orthophosphoric acid in an amount equivalent to triethylamine (about 4 ml). The precipitate that formed was filtered and thoroughly washed from acid residues with a large amount of water.

Yield – 74 %, white crystals. ^{1}H NMR (400 MHz, DMSO) δ 12.94 (s, 1H), 7.94 (d, *J* = 7.4 Hz, 1H), 7.73 (t, *J* = 7.0 Hz, 1H), 7.48 – 7.18 (m, 2H), 4.59 (t, *J* = 5.8 Hz, 2H), 3.62 (t, *J* = 5.9 Hz, 2H), 3.27 (d, *J* = 4.8 Hz, 3H).

Загальна методика синтезу 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3*H*)-one

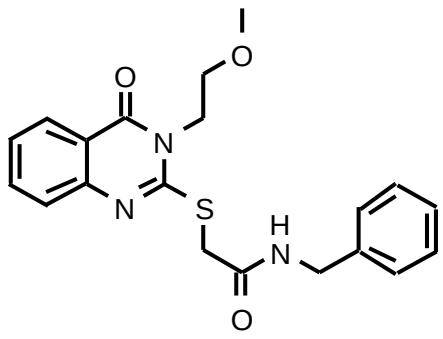
To 3-(2-Methoxyethyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 0.25 g (0.0011 mol) was added 3 ml of dimethylformamide, 0.15 ml (0.0011 mol) of triethylamine and (0.0011 mol) of the corresponding alkylating agent reagent and the mixture was heated at 75ºC with stirring for 5 hours. The reaction mixture was then cooled and diluted with water. The precipitate that formed was filtered, dried at 65ºC in an oven and recrystallized from ethanol.

2-{[3-(2-Methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}-*N*-phenylacetamide 7.1



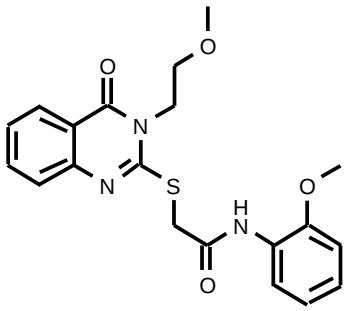
Yield – 82 %, white crystals. M. p. 179-180 °C. Anal. Calcd. for $C_{19}H_{19}N_3O_3S$, % (369,45): C, 61.77; H, 5.18; N, 11.37. Found, C, 61.80; H, 5.24; % N, 11.43. ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 2H), 7.43 (dd, *J* = 15.4, 7.7 Hz, 2H), 7.30 (t, *J* = 7.7 Hz, 2H), 7.04 (t, *J* = 7.3 Hz, 1H), 4.28 (t, *J* = 5.9 Hz, 2H), 4.20 (s, 2H), 3.66 (t, *J* = 5.9 Hz, 2H), 3.27 (s, 3H).

***N*-Benzyl-2-{[3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}acetamide 7.2**



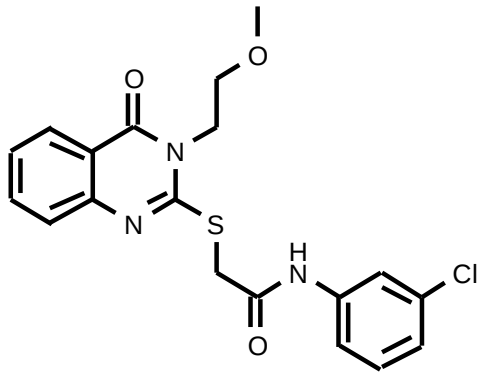
Yield – 59 %, white crystals. M. p. 167-168 °C. Anal. Calcd. for $C_{20}H_{21}N_3O_3S$, % (383,47): C, 62.64; H, 5.52; N, 10.96. Found, C, 62.85; H, 5.64; % N, 10.99. ^{1}H NMR (400 MHz, DMSO) δ 8.72 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 7.3 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.19 (d, *J* = 7.0 Hz, 5H), 4.40 – 4.16 (m, 4H), 4.06 (s, 2H), 3.64 (t, *J* = 5.7 Hz, 2H), 3.26 (s, 3H).

2-{[3-(2-Methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}-*N*-(2-methoxyphenyl)acetamide 7.3



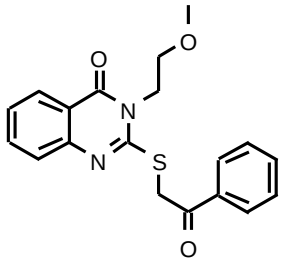
Yield – 93 %, white crystals. M. p. 133-135 °C. Anal. Calcd. for $C_{20}H_{21}N_3O_4S$, % (399,47): C, 60.14; H, 5.30; N, 10.52. Found, C, 60.32; H, 5.44; % N, 10.67. ^{1}H NMR (400 MHz, DMSO) δ 9.59 (s, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.81 (t, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.01 (dd, *J* = 15.3, 7.6 Hz, 2H), 6.87 (t, *J* = 7.3 Hz, 1H), 4.34 – 4.13 (m, 4H), 3.61 (d, *J* = 36.5 Hz, 5H), 3.26 (s, 3H).

***N*-(3-Chlorophenyl)-2-{[3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}acetamide 7.4**



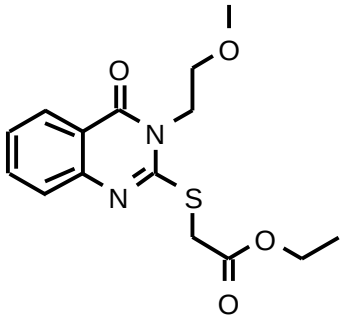
Yield – 78 %, white crystals. M. p. 159-160 °C. Anal. Calcd. for $C_{19}H_{18}ClN_3O_3S$, % (403,89): C, 56.50; H, 4.49; N, 10.40. Found, C, 56.70; H, 4.55; % N, 10.56. ^{1}H NMR (400 MHz, DMSO) δ 10.60 (s, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.80 (s, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.44 (dd, *J* = 20.7, 7.5 Hz, 3H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 4.27 (s, 2H), 4.20 (s, 2H), 3.66 (s, 2H), 3.27 (s, 3H).

3-(2-Methoxyethyl)-2-[(2-oxo-2-phenylethyl)thio]quinazolin-4(3*H*)-one 7.5



Yield – 85 %, white crystals. M. p. 163-165 °C. Anal. Calcd. for $C_{19}H_{18}N_2O_3S$, % (354,43): C, 64.39; H, 5.12; N, 7.90. Found, C, 64.65; H, 5.24; % N, 7.88. ^{1}H NMR (500 MHz, DMSO) δ 8.10 (d, *J* = 7.5 Hz, 2H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.3 Hz, 1H), 7.61 (dt, *J* = 15.0, 7.4 Hz, 3H), 7.38 (t, *J* = 7.3 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 4.86 (s, 2H), 4.29 (t, *J* = 5.7 Hz, 2H), 3.67 (t, *J* = 5.7 Hz, 2H), 3.28 (s, 3H).

Ethyl {[3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}acetate 7.6



Yield – 71 %, white crystals. M. p. 88-90 °C. Anal. Calcd. for $C_{15}H_{18}N_2O_4S$, % (322,37): C, 55.88; H, 5.62; N, 8.68. Found, C, 55.93; H, 5.68; % N, 8.79. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.84 – 7.72 (m, 1H), 7.50 – 7.34 (m, 2H), 4.24 (t, *J* = 5.9 Hz, 2H), 4.20 – 4.06 (m, 4H), 3.64 (q, *J* = 6.0 Hz, 2H), 3.26 (d, *J* = 1.9 Hz, 3H), 1.19 (t, *J* = 6.0 Hz, 3H). LC-MS m/z (ES+) 323 (MH^+).

Conclusions to the Chapter 2

1. An effective method for the synthesis of 3-(2-methoxyethyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one by single-reactor cyclization of 2-isothiocyanatobenzoate with the interaction of 2-methoxyethanamine was developed.
2. The synthesis of the target 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one using the alkylation reaction was proposed and carried out, and the structure of the obtained compounds was confirmed using modern instrumental methods.

CHAPTER 3. STUDY OF THE POTENTIAL ANTIMICROBIAL ACTIVITY OF DERIVATIVES OF [3-(2-METHOXYETHYL)-4-OXO-3,4-DIHYDROQUINAZOLIN-2-YL]THIOACETIC ACID BY MOLECULAR DOCKING

3.1. Anti-tuberculosis activity prediction for 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3*H*)-one derivatives

Tuberculosis is a problem of humanity, and currently there is an epidemic of this disease, which is associated with the low sensitivity of *M. tuberculosis* to most antibiotics. Despite the fact that therapy requires about six months even if it is successful, tuberculosis is a curable disease. The problem of therapy is significantly complicated for resistant strains of tuberculosis. Therefore, much attention is paid to the development of new potentially effective antimicrobial agents to reduce the resistance of tuberculosis strains. The aim of our work was to analyze the potential of antituberculosis activity of quinazolones modified in position 3 with an attractive ADME parameter methoxyethyl fragment through the mechanism of inhibition of TrmD, which is an important enzyme for protein synthesis by the bacterial cell and an innovative target for the development of antibiotics.

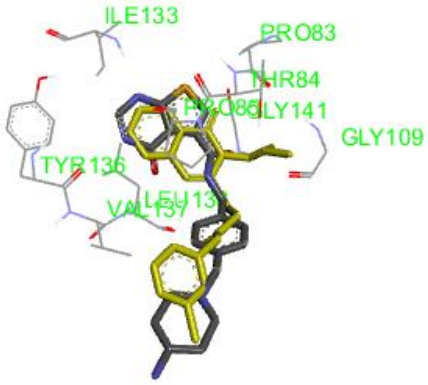


Fig. 3.1 Pose of **7.4** (yellow molecule) the in comparison with the native ligand in the active site of *M. tuberculosis* TrmD

As a result of docking studies in the active site of TrmD isolated from M. tuberculosis for [3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thioacetic acid derivatives, it was found that the best binding parameters are characteristic for 3-chloromethylphenyl amide and ethyl ester of this acid. However, the position of the 3-chlorophenyl derivative is closer to that of the native inhibitor (table 3.1).

Table 3.1

The results of the docking studies to micobacterial TrmD for 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3*H*)-one 7

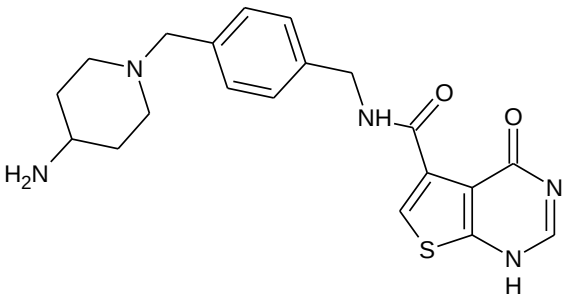
Code/Stucructure	**Interaction with the active site***	**Interacting amino acids**
1	2	3
Native inhibitor 		PRO83 THR84 PRO85 GLU112 SER132 ILE133 GLY134 TYR136 VAL137 LEU138 GLY140 GLY141 ALA144

Table 3.1 (continued)

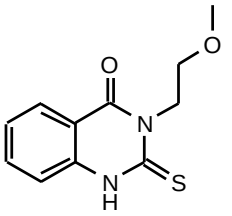
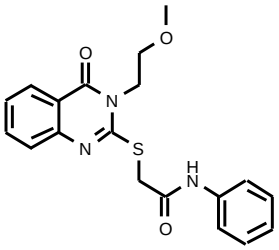
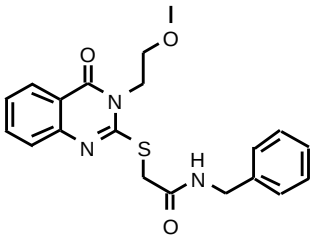
1	2	3
3 	+ (good docking results)	PRO83 THR84 PRO85 GLY109 TYR111 GLU112 GLY113 ILE133 TYR136 LEU138
7.1 	-	-
7.2 	-	-

Table 3.1 (continued)

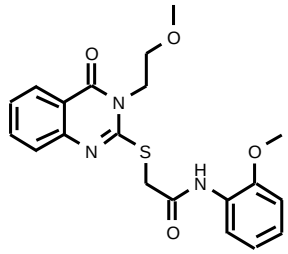
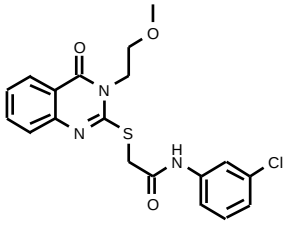
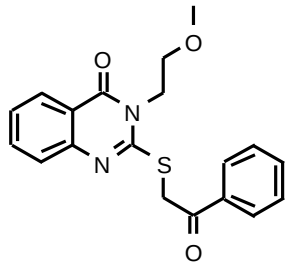
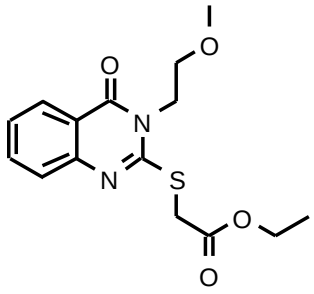
1	2	3
7.3 	-	-
7.4 	+ (very good matching native inhibitor)	PRO83 THR84 PRO85 GLY109 ILE133 TYR136 VAL137 LEU138 GLY141
7.5 	-	-

Table 3.1 (continued)

1	2	3
7.6 	**+ (**very good matching native inhibitor)	PRO83 THR84 PRO85 TYR111 GLU112 GLY113 ILE114 ILE133 GLY140 GLY141

* Interaction with the active site: + - ligand fits the active site cavity; +/- - ligand partially fits the active site cavity; - - ligand does not interact with the active site

3.2. Prediction of activity against *Pseudomonas aeruginosa* for 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3*H*)-one derivatives

Pseudomonas aeruginosa can infect patients with impaired barriers against bacterial invasion or immunodeficiency and is prone to the rapid formation of antibiotic resistance. Undoubtedly, interesting ways to overcome antibiotic resistance are exposure to new protein targets, effective inhibitors of which, if created, may even form a new class of antibiotics. Bacterial TrmD, which has critical differences from its ortholog in eukaryotes and archaea, is attractive in this regard and may serve as a target

for the development of inhibitors with antimicrobial properties. The aim of our work was to analyze the potential of antimicrobial activity as the new possible antibiotics for quinazolones modified at position 3 with methoxyethyl fragment through the mechanism of inhibition of TrmD.

As a result of docking studies in the active site of TrmD isolated from *P. aeruginosa* for [3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thioacetic acid derivatives, it was found that the best binding parameters were typical for benzyl amide of this acid. However, the pose in the active site of the benzyl derivative was the most similar of that to the native ligand.

Table 3.2

The results of the docking studies to *P. aeruginosa* TrmD for 53-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3*H*)-one drivatives 7

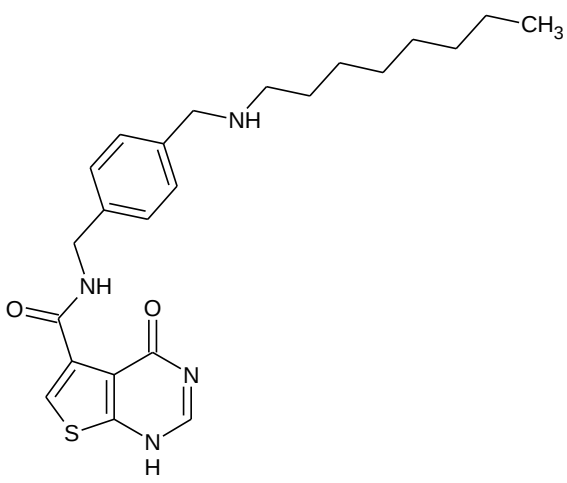
Code/Stucructure	**Interaction with the active site***	**Interacting amino acids**
1	2	3
Native inhibitor 		

Table 3.2 (continued)

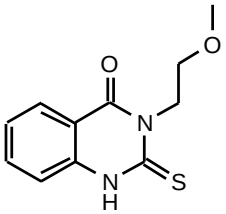
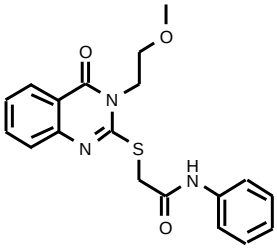
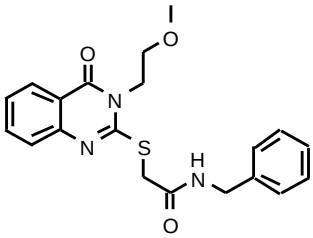
1	2	3
3 	+/- (moderate interaction with the active site)	GLN95 ARG119 TYR120 VAL142 LEU143 GLY145 ASP182
7.1 	-	-
7.2 	+ (perfect interaction with active site)	TYR91 LEU92 SER93 PRO94 GLN95 ARG119 TYR120 ILE138 TYR141 VAL142 LEU143

Table 3.2 (continued)

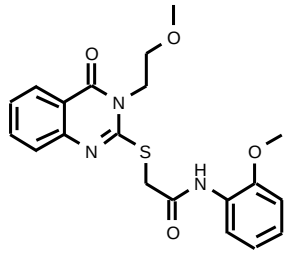
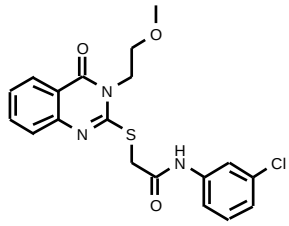
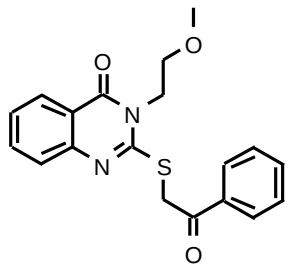
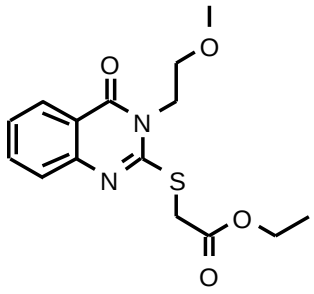
1	2	3
7.3 	-	-
7.4 	-	-
7.5 	+ (very good interaction with active site)	TYR91 LEU92 SER93 PRO94 GLN95 ARG119 TYR120 ILE138 TYR141 LEU143 PRO149 ASP182

Table 3.2 (continued)

1	2	3
7.6 	-	-

* with the active site: + - ligand fits the active site cavity; +/- - ligand partially fits the active site cavity; - - ligand does not interact with the active site

3.3. Experimental part

The structures of the compounds were drawn using ACD/ChemSketch (freeware) and saved in .pdb format using Discovery Studio Visualizer 2021. AutoDockTools-1.5.7 was used to convert .pdb files to .pdbqt, the number of active rotatory bonds was set by default. AutoDock Vina was used to calculate molecular docking. Discovery Studio 2021 was used for visualization. Biotarget macromolecules was selected from Protein Data Bank (Protein Data Bank): PDB ID – 6jof (*M. tuberculosis* TrmD model), PDB ID – 5zhn (*P. aeruginosa* TrmD model).

Conclusions to the Chapter 3

1. The docking simulation to TrmD isolated from *M. tuberculosis*, showed that 2-{[3-(3-chlorophenyl)-2-oxopropyl]thio}-3-(2-methoxyethyl)quinazolin-4(3H)-one an anti-tuberculosis agent with an innovative mechanism of action.
2. According to docking simulation to TrmD isolated from *P. aeruginosa*, it was revealed that N-benzyl-2-{[3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}acetamide can be the antibacterial agent (active agains *P. aeruginosa*) with an innovative mechanism of action.

GENERAL CONCLUSION

1. Using tuberculosis, which is a dangerous and difficult to treat disease, as an example the ways of treatment and mechanisms of antimicrobial drug resistance were analyzed; it was found that resistance can be overcome by creating antibacterial drugs with fundamentally new mechanisms of action.
2. An effective method for the synthesis of 3-(2-methoxyethyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one by single-reactor cyclization of 2-isothiocyanatobenzoate with the interaction of 2-methoxyethanamine was developed.
3. The synthesis of the target 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one using the alkylation reaction was proposed and carried out, and the structure of the obtained compounds was confirmed using modern instrumental methods.
4. The docking simulation to TrmD isolated from *M. tuberculosis*, showed that 2-{[3-(3-chlorophenyl)-2-oxopropyl]thio}-3-(2-methoxyethyl)quinazolin-4(3H)-one has anti-tuberculosisvproperties and according to docking simulation to TrmD isolated from *P. aeruginosa*, it was revealed that N-benzyl-2-{[3-(2-methoxyethyl)-4-oxo-3,4-dihydroquinazolin-2-yl]thio}acetamide can be the antibacterial agent (active against *P. aeruginosa*) with an innovative mechanism of action.

REFERENCES

1. Macalino S. J. Y. In silico strategies in tuberculosis drug discovery. *Molecules*. 2020. Vol. 25, № 3. P. 665.

2. Treatment of Tuberculosis: Guidelines. Geneva : World Health Organization, 2010. 147 p.

3. Global Tuberculosis Report 2018. Geneva : WHO, 2018. 231 p.

4. Balganesh T. S., Alzari P. M., Cole S. T. Rising standards for tuberculosis drug development. *Trends Pharmacol. Sci.* 2008. Vol. 29, № 11. P. 576–581.

5. New small-molecule synthetic antimycobacterials / L. Ballell et al. *Antimicrob. Agents* Chemother. 2005. Vol. 49, № 6. P. 2153–2163.

6. High-throughput screening for inhibitors of Mycobacterium tuberculosis H37Rv / S. Ananthan et al. *Tuberculosis*. 2009. Vol. 89, № 5. P. 334–353.

7. Antituberculosis activity of the molecular libraries screening center network library / J. A. Maddry et al. *Tuberculosis*. 2009. Vol. 89, № 5. P. 354–363.

8. High throughput screening of a library based on kinase inhibitor scaffolds against Mycobacterium tuberculosis H37Rv / R. C. Reynolds et al. *Tuberculosis*. 2012. Vol. 92, № 1. P. 72–83.

9. World TB day 2017: Advances, challenges and opportunities in the “end-TB” era / E. Petersen et al. *Int. J. Infect. Dis*. 2017. Vol. 56. P. 1–5.

10. Mechanism and inhibition of the FabI enoyl-ACP reductase from Burkholderia pseudomallei / N. Liu et al. *J. Antimicrob. Chemother.* 2011. Vol. 66, № 3. P. 564–573.

11. Substituted diphenyl ethers as a broad-spectrum platform for the development of chemotherapeutics for the treatment of tularaemia / K. J. England et al. *Antimicrob. Chemother*. 2009. Vol. 64, № 5. P. 1052–1061.

12. Mechanism and inhibition of saFabI, the enoyl reductase from Staphylococcus aureus / H. Xu et al. *Biochemistry*. 2008. Vol. 47, № 14. P. 4228–4236.

13. Design and Synthesis of Aryl Ether Inhibitors of the Bacillus Anthracis Enoyl–ACP Reductase / S. K. Tipparaju et al. *Chem. Med. Chem*. 2008. Vol. 3, № 8. P. 1250–1268.

14. Lobanov V. Using artificial neural networks to drive virtual screening of combinatorial libraries. *Drug Discov. Today BIOSILICO*. 2004. Vol. 2. P. 149–156.

15. Pliushcheuskaya P., Künze G. Recent Advances in Computer-Aided Structure-Based Drug Design on Ion Channels. *Int. J. Mol Sci*. 2023. Vol. 24, № 11. P. 9226.

16. Niazi S. K., Mariam Z. Computer-Aided Drug Design and Drug Discovery: A Prospective Analysis. *Pharmaceuticals*. 2024. Vol. 17, № 1. P. 22.

17. Computer-aided drug design: Lead discovery and optimization / M. Xiang et al. *Comb. Chem. High Throughput Screen*. 2012. Vol. 15, № 4. P. 328–337.

18. Zhang S. Computer-aided drug discovery and development. *Drug Des. Discov*. 2011. Vol. 716. P. 23–38.

19. The protein data bank / H. Berman et al. *Nucleic Acids Res*. 2000. Vol. 28. P. 235–242.

20. RSCB-PDB. A Structural View of Biology. 2021. URL: https://www.rcsb.org/ (Date of access: 26.04.2024)

21. Bruch E. M., Petrella S., Bellinzoni M. Structure-Based Drug Design for Tuberculosis: Challenges Still Ahead. *Appl. Sci*. 2020. Vol. 10, № 12. P. 4248.

22. Structure-based approaches to drug discovery against tuberculosis / S. J. Holton et al. *Curr. Protein Pept. Sci*. 2007. Vol. 8, № 4. P. 365–375.

23. Elkington P. T., Friedland J. S. Permutations of time and place in tuberculosis. *Lancet Infect. Dis*. 2015. Vol. 15. P. 1357–1360.

24. Microbiome changes during tuberculosis and antituberculous therapy / B.-Y. Hong et al. *Clin. Microbiol. Rev*. 2016. Vol. 29, № 4. P. 915–926.

25. Latent Mycobacterium tuberculosis infection / H. Getahun et al. *N. Engl. J. Med*. 2015. Vol. 372, № 22. P. 2127–2135.

26. A population-based survey of tuberculosis symptoms: How atypical are atypical presentations? / L. G. Miller et al. *Clin. Infect. Dis*. 2000. Vol. 30, № 2. P. 293–299.

27. Global Tuberculosis Report 2019. Geneva : World Health Organization, 2019. 283 p.

28. Green K., Garneau-Tsodikova S. Resistance in tuberculosis: What do we know and where can we go? *Front. Microbiol*. 2013. Vol. 4. P. 208.

29. Global Tuberculosis Report 2020 / World Health Organization. Geneva : World Health Organization, 2020. P. 115–127.

30. Guidelines on the Management of Latent Tuberculosis Infection / World Health Organization. Geneva : World Health Organization, 2015. 79 p.

31. Horsburgh C. R., Barry C. E., Lange C. Treatment of tuberculosis. *N. Engl. J. Med*. 2015. Vol. 373, № 22. P. 2149–2160.

32. Mitchison D., Davies G. The chemotherapy of tuberculosis: Past, present and future. *Int. J. Tuberc. Lung Dis*. 2012. Vol. 16, № 6. P. 724–732.

33. Tetrandrine reverses drug resistance in isoniazid and ethambutol dual drug-resistant Mycobacterium tuberculosis clinical isolates / Z. Zhang et al. *BMC Infect. Dis.* 2015. Vol. 15. P. 153.

34. Rich M., Jaramillo E. Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis. Geneva: World Health Organization, 2008. 96 p.

35. Developing and Strengthening the Global Supply Chain for Second-Line Drugs for Multidrug-Resistant Tuberculosis: Workshop Summary / A. B. Claiborne et al. Washington : National Academies Press, 2013. 179 p.

36. Wright A., Zignol M. Anti-Tuberculosis Drug Resistance in theWorld: Fourth Global Report: TheWorld Health Organization/International Union against Tuberculosis and Lung Disease (WHO/Union) Global Project on Anti-Tuberculosis

Drug Resistance Surveillance, 2002–2007. Geneva : World Health Organization, 2008. 237 p.

37. Mahajan R. Bedaquiline: First FDA-approved tuberculosis drug in 40 years. *Int. J. Appl. Basic Med. Res*. 2013. Vol. 3. P. 1–2.

38. Vilchèze C., Jacobs W. R. The mechanism of isoniazid killing: Clarity through the scope of genetics. *Annu. Rev. Microbiol*. 2007. Vol. 61. P. 35–50.

39. Metabolism of isoniazid by neutrophil myeloperoxidase leads to isoniazid-NAD+ adduct formation: A comparison of the reactivity of isoniazid with its known human metabolites / S. R. Khan et al. *Biochem. Pharmacol*. 2016. Vol. 106. P. 46–55.

40. Preziosi P. Isoniazid: Metabolic aspects and toxicological correlates. *Curr. Drug Metab*. 2007. Vol. 8, № 8. P. 839–851.

41. Isoniazid: Radical-induced oxidation and reduction chemistry / K. A. Rickman et al. *Bioorganic Med. Chem. Lett.* 2013. Vol. 23, № 10. P. 3096–3100.

42. Timmins G. S., Deretic V. Mechanisms of action of isoniazid. *Mol. Microbiol*. 2006. Vol. 62, № 5. P. 1220–1227.

43. Nitric oxide generated from isoniazid activation by KatG: Source of nitric oxide and activity against Mycobacterium tuberculosis / G. S. Timmins et al. *Antimicrob. Agents Chemother*. 2004. Vol. 48, № 8. P. 3006–3009.

44. Oxidative stress increases susceptibility of Mycobacterium tuberculosis to isoniazid / V. M. Bulatovic et al. *Antimicrob. Agents Chemother*. 2002. Vol. 46, № 9. P. 2765–2771.

45. Quantitative proteomics reveals novel insights into isoniazid susceptibility in mycobacteria mediated by a universal stress protein / X. Hu et al. *J. Proteome Res*. 2015. Vol. 14, № 3. P. 1445–1454.

46. Computational approach to understanding the mechanism of action of isoniazid, an anti-TB drug / L. Jena et al. *Int. J. Mycobacteriol*. 2014. Vol. 3, № 3. P. 276–282.

47. Laurenzo D., Mousa S. A. Mechanisms of drug resistance in Mycobacterium tuberculosis and current status of rapid molecular diagnostic testing. *Acta Trop*. 2011. Vol. 119, № 1. P. 5–10.

48. Analysis of embCAB mutations associated with ethambutol resistance in multidrug-resistant Mycobacterium tuberculosis isolates from China / L.–L. Zhao et al. *Antimicrob. Agents Chemother*. 2015. Vol. 59, № 4. P. 2045–2050.

49. Transfer of embB codon 306 mutations into clinical Mycobacterium tuberculosis strains alters susceptibility to ethambutol, isoniazid, and rifampin / H. Safi et al. *Antimicrob. Agents Chemother*. 2008. Vol. 52, № 6. P. 2027–2034.

50. UbiA (Rv3806c) encoding DPPR synthase involved in cell wall synthesis is associated with ethambutol resistance in Mycobacterium tuberculosis / L. He et al. *Tuberculosis.* 2015. Vol. 95, № 2. P. 149–154.

51. Almeida Da Silva P. E., Palomino J. C. Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: Classical and new drugs. *J. Antimicrob. Chemother*. 2011. Vol. 66, № 7. P. 1417–1430.

52. Zhang Y., Mitchison D. The curious characteristics of pyrazinamide: A review. *Int. J. Tuberc. Lung Dis*. 2003. Vol. 7, № 1. P. 6–21.

53. Mode of action of pyrazinamide: Disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid / Y. Zhang et al. *J. Antimicrob. Chemother*. 2003. Vol. 52, № 5. P. 790–795.

54. Scorpio A., Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nat. Med*. 1996. Vol. 2, № 6. P. 662–667.

55. Mutation in pncA is a major mechanism of pyrazinamide resistance in Mycobacterium tuberculosis / K. Hirano et al. *Tuber. Lung Dis*. 1997. Vol. 78, № 2. P. 117–122.

56. Mechanisms of pyrazinamide action and resistance / Y. Zhang et al. *Microbiol. Spectr*. 2014. Vol. 2, № 4. P. 0023–2013.

57. Study of the structure–activity relationships for the pyrazinamidase (PncA) from Mycobacterium tuberculosis / N. Lemaitre et al. *Biochem. J.* 2001. Vol. 353. P. 453–458.

58. Effect of pyrazinamidase activity on pyrazinamide resistance in Mycobacterium tuberculosis / P. Sheen et al. *Tuberculosis*. 2009. Vol. 89, № 2. P. 109–113.

59. Zumla A., Nahid P., Cole S. T. Advances in the development of new tuberculosis drugs and treatment regimens. *Nat. Rev. Drug Discov*. 2013. Vol. 12, № 5. P. 388–404.

60. Gillespie S. H. Evolution of drug resistance in Mycobacterium tuberculosis: Clinical and molecular perspective. *Antimicrob. Agents Chemother*. 2002. Vol. 46, № 2. P. 267–274.

61. Okamoto S. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. *Mol. Microbiol*. 2007. Vol. 63, № 4. P. 1096–1106.

62. Structural activation of the transcriptional repressor EthR from Mycobacterium tuberculosis by single amino acid change mimicking natural and synthetic ligands / X. Carette et al. *Nucleic Acids Res*. 2012. Vol. 40, № 7. P. 3018–3030.

63. Ethionamide activation and sensitivity in multidrug-resistant Mycobacterium tuberculosis / A. E. DeBarber et al. *Proc. Natl. Acad. Sci. USA*. 2000. Vol. 97, № 17. P. 9677–9682.

64. Mycothiol biosynthesis is essential for ethionamide susceptibility in Mycobacterium tuberculosis / C. Vilchèze et al. *Mol. Microbiol*. 2008. Vol. 69, № 5. P. 1316–1329.

65. Binding pocket alterations in dihydrofolate synthase confer resistance to para-aminosalicylic acid in clinical isolates of Mycobacterium tuberculosis / F. Zhao et al. *Antimicrob. Agents Chemother*. 2014. Vol. 58, № 3. P. 1479–1487.

66. Molecular genetics of para-aminosalicylic acid resistance in clinical isolates and spontaneous mutants of Mycobacterium tuberculosis / V. Mathys et al. *Antimicrob. Agents Chemother*. 2009. Vol. 53, № 5. P. 2100–2109.

67. Role of Whole-Genome Sequencing in Characterizing the Mechanism of Action of para-Aminosalicylic Acid and Its Resistance / G. Satta et al. *Antimicrob. Agents Chemother*. 2020. Vol. 64, № 9. P. e00675-20.

68. Mechanism of action of and resistance to quinolones / A. Fàbrega et al. *Microb. Biotechnol*. 2009. Vol. 2, № 1. P. 40–61.

69. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates / A. F. Cheng et al. *Antimicrob. Agents Chemother*. 2004. Vol. 48, № 2. P. 596–601.

70. Diarylquinolines target subunit c of mycobacterial ATP synthase / A. Koul et al. *Nat. Chem. Biol*. 2007. Vol. 3, № 6. P. 323–324.

71. Working Group on New TB Drugs StopTBPartnership. Clinical Pipeline. 2021. URL: https://www.newtbdrugs.org/pipeline/clinical (Date of access 29.04.2024).

72. Lead optimization of a novel series of imidazo [1, 2-a] pyridine amides leading to a clinical candidate (Q203) as a multi-and extensively-drug-resistant anti-tuberculosis agent / S. Kang et al. *J. Med. Chem*. 2014. Vol. 57, № 12. P. 5293–5305.

73. Maeda K. A new antibiotic, azomycin. *J. Antibiot*. 1953. Vol. 6, № 4. P. 182.

74. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis / C. K. Stover et al. *Nature*. 2000. Vol. 405. P. 962–966.

75. Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects / A. M. Ginsberg et al. *Antimicrob. Agents Chemother*. 2009. Vol. 53, № 9. P. 3720–3725.

76. Discovery and development of SQ109: A new antitubercular drug with a novel mechanism of action / K. A. Sacksteder et al. *Future Microbiol*. 2012. Vol. 7, № 7. P. 823–837.

77. SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of Mycobacterium tuberculosis / K. Tahlan et al. *Antimicrob. Agents Chemother*. 2012. Vol. 56, № 4. P. 1797–1809.

78. Water-Soluble O-Carbonyl Phosphoramidate Prodrugs for Therapeutic Administration : U.S. Patent 9382276. Publ. 5 July 2016.

79. Gordeev M. F., Yuan Z. Y. New potent antibacterial oxazolidinone (MRX-I) with an improved class safety profile. *J. Med. Chem*. 2014. Vol. 57, № 11. P. 4487–4497.

80. Nonclinical Evaluation of Antibacterial Oxazolidinones Contezolid and Contezolid Acefosamil with Low Serotonergic Neurotoxicity / W. Wang et al. *Chem. Res. Toxicol*. 2021. Vol. 34, № 5. P. 1348–1354.

81. In vitro and in vivo activities of contezolid (MRX-I) against Mycobacterium tuberculosis/ C. Shoen et al. *Antimicrob. Agents Chemother*. 2018. Vol. 62, № 8. P. e00493-18.

82. Chiliza T. E., Pillay M., Pillay B. Identification of unique essential proteins from a Mycobacterium tuberculosis F15/Lam4/KZN phage secretome library. *Pathog. Dis*. 2017. Vol. 75, № 1. P. 123–144.

83. Lamichhane G. Novel targets in M. tuberculosis: Search for new drugs. *Trends Mol. Med*. 2011. Vol. 17, № 1. P. 25–33.

84. Computational Deorphaning of Mycobacterium Tuberculosis Targets. In Drug Discovery and Development-New Advances / L. Y. Bishi et al. London, 2019. 19 p.

Appendices

Appendix A

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ГРАМОТА

за участь

отримав(ла)

Idoumghar Wafa

у секційному засіданні студентського наукового товариства кафедри фармацевтичної хімії

XXX Міжнародна науково-практична конференція молодих вчених та студентів **"Актуальні питання створення нових лікарських засобів"**

В.о. ректора
Національного фармацевтичного університету

Алла КОТВІЦЬКА

17-19 квітня 2024 р.
м. Харків

Cont. of the appendix A

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

АКТУАЛЬНІ ПИТАННЯ СТВОРЕННЯ НОВИХ ЛІКАРСЬКИХ ЗАСОБІВ

МАТЕРІАЛИ
XXX МІЖНАРОДНОЇ НАУКОВО-ПРАКТИЧНОЇ
КОНФЕРЕНЦІЇ МОЛОДИХ ВЧЕНИХ ТА СТУДЕНТІВ

17-19 квітня 2024 року
м. Харків

Харків
НФаУ
2024

Cont. of the appendix A

Секція 1
«СИНТЕЗ ФІЗІОЛОГІЧНО АКТИВНИХ РЕЧОВИН»

SYNTHESIS OF 3-(2-METHOXYETHYL)-2-(ALKYLTHIO)QUINAZOLIN-4(3*H*)-ONES AS POSSIBLE ANTIBIOTICS WITH INNOVATIVE MECHANISM OF ACTION

Idoumghar W., Vlasov S.V.
National University of Pharmacy, Kharkiv, Ukraine
2024pharmchem.vlasov@gmail.com

Introduction. Quinazoline derivatives have proven themselves as a privileged scaffold for the creation of biologically active substances. Among them, compounds with an anticancer effect, antimicrobial agents were found, a large number of representatives of these heterocyclic systems can affect the work of the central nervous system and have useful pharmacological properties. Obviously, it is important to increase the bioavailability of these compounds, which can be achieved by introducing functional groups that improve the ADME parameters of the molecules. It is clear that the 2-methoxyethyl group is one that has good parameters from the point of view of medicinal chemistry, and therefore its modification of quinazoline can be promising for the design of new drugs.

Aim. Development and implementation of methods of organic synthesis in order to effectively obtain a number of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives, which may be interesting due to the implementation of innovative mechanisms of antimicrobial action.

Materials and methods. Methods of organic synthesis and instrumental methods of confirmation of the structure (^{1}H NMR, etc.), the method of molecular docking.

Results and discussion. The synthesis of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives was carried out on the basis of methyl 2-isothiocyanatobenzoate and the cyclization of 2-methoxyethanamine, which was stimulated by adding triethylamine to the reaction medium. Subsequently, thione was alkylated with amides of chloroacetic acid, phenacyl bromide, and ethyl chloroacetate. As a result, various target products of alkylation by the sulfur atom were obtained in the form of white crystalline substances.

Conclusions. Methods have been developed that allowed obtaining a number of 3-(2-methoxyethyl)-2-(alkylthio)quinazolin-4(3H)-one derivatives, which may be interesting due to the implementation of innovative mechanisms of antimicrobial activity.

DETERMINATION OF ADMET PARAMETERS OF NEW 8-THIA/OXA-1,3-DIAZASPIRO[4.5]DECAN-2,4-DIONE DERIVATIVES AS AGENTS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Lagunets T.[1], Saifudinova R.P.[2]
Scientific supervisor: prof. Severina H.I.[1]
[1]National University of Pharmacy, Kharkiv, Ukraine
[2]Cherkasy Medical Academy, Cherkasy, Ukraine
lagunchika@gmail.com

Introduction. Currently, more than 55 million people worldwide suffer from dementia. Almost 10 million new cases are registered every year. Dementia is the result of a variety of diseases and injuries that affect the brain. Alzheimer's disease is the most common form of dementia and can cause 60-70% of cases. Alzheimer's disease is one of the top ten diseases for which patients need palliative care. Although the overall mortality rate from nervous system dysfunction in Ukraine is