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MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
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QUALIFICATION WORK

on the topic: «**CONSTRUCTION OF 5,6-DIMETHYL-2-(ALKYLTHIO)-3-PHENYLTHIENO[2,3-d]PYRIMIDIN-4(3H)-ONE DERIVATIVES AND THE POTENTIAL OF THEIR ANTIMICROBIAL ACTIVITY»**

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ANNOTATION

The synthesis of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives was planned and carried out, among which promising antimicrobial agents with an innovative mechanism of action on TrmD were selected using the molecular docking study. The structure of the obtained compounds was confirmed by instrumental methods of analysis. Predictions were made for *M. tuberculosis* and *P. aeruginosa*, which are known to have a high tendency to develop resistance to antibiotics.

The work consists of an introduction, three chapters, general conclusions and a list of references, which consists of 73 sources. The content of the work is placed on 46 pages and contains 2 tables, 3 figure, 2 schemes.

Key words: pyrimidine, thiophene, alkylation, molecular docking, antimicrobial activity.

АНОТАЦІЯ

Сплановано та проведено синтез похідних 5,6-диметил-2-(алкілтіо)-3-фенілтієно[2,3-d]піримідин-4(3H)-ону, серед яких методом молекулярного докінгу відібрано перспективні протимікробні засоби із інноваційним механізмом дії на TrmD. Структура отриманих сполук підтверджена інструментальними методами аналізу. Прогнозування проводили для *M. tuberculosis* та *P. aeruginosa* для ких відомою є висока схильність до розвитку резистентності до антиібіотиків.

Робота складається із вступу, трьох розділів та загльних висновків, списка використаної літератури, який складає 73 джерело. Зміст роботи викладено на 46 сторінках і проілюстровано 2 таблицями, 3 рисунками, 2 схемами.

Ключові слова: піримідин, тіофен, алкілування, молекулярний докінг, антимікробна активність.

CONTENTS

Page

INTRODUCTION

Relevance of the topic. Derivatives of thieno[2,3-d]pyrimidin-4(3H)-one are widely available compounds due to the discovery of the reaction of thiophenes synthesis by the German chemist Kahl Gewald on the basis of available ketones, derivatives of cyanoacetic acid and elemental sulfur. These thiophenes, through standard pyrimidine ring formation reactions typical of quinazolines, yield a variety of thieno[2,3-d]pyrimidins. There is a lot of data on the biological activity of thieno[2,3-d]pyrimidins. For example, the basis of the innovative drug orgovyx (relugolix) for cancer therapy with a unique mechanism, which was approved as the first oral GnRH-R antagonist for the treatment of advanced prostate cancer, lies a fragment of thieno[2,3-d]pyrimidin-2,4-dione. Also, thieno[2,3-d]pyrimidins are preveleged heterocyclic scaffolds for the design of bacterial TrmD inhibitors, which are potentially considered as a new class of antibacterial agents with an innovative mechanism of action.

Purpose of the study. Synthesis of new thieno[2,3-d]pyrimidine derivatives and their study by molecular docking as TrmD inhibitors isolated from *M. tuberculosis* and *P. aeruginosa* in order to assess the potential of antimicrobial activity.

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

1. To systematize data from the literature on the treatment of tuberculosis, as a dangerous disease that is difficult to treat and requires the implementation of innovative approaches for the development of new antimicrobial agents.

2. Develop chemical methods to carry out the synthesis of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives; to confirm the structure of the obtained compounds by the complex application of physical and instrumental methods.

3. Computer modeling of binding to the active site of TrmD inhibitors isolated from *M. tuberculosis* and *P. aeruginosa* for the obtained derivatives of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d] pyrimidin-4(3H)-one using *in silico* methods,.

4. To evaluate the potential of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one as an innovative antibacterial agent and draw a conclusion about the leading compounds in accordance to the research results.

Object of the study. Derivatives of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one as antibacterial agents with an innovative mechanism of action.

Subject of the study. Methods of synthesis of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one and parameters of binding to TrmD isolated from *M. tuberculosis* and *P. aeruginosa*.

Methods of the study. Methods of organic synthesis, physical and instrumental methods of analysis of organic substances (measurement of the melting points, elemental analysis, ^{1}H NMR spectroscopy), standard methods of *in silico* biological activity research.

The practical value of the results. The methods developed and compounds tested during experimental studies can be used to create large libraries of 2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives suitable for screening studies using both high-throughput and classical methods pharmacological screening. The obtained computer screening data provide insight for the further development of antibacterial drugs with the potential to overcome antibiotic resistance of *M. tuberculosis* and *P. aeruginosa* strains.

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:

El-Mouddene H., Vlasov S.V. The efficient approach to 5,6-dimethyl-2-(alkylthio)-3- phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives. “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. 46.

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 73 sources. The content of the work is placed on 46 pages and contains 2 tables, 3 figure, 2 schemes.

CHAPTER 1. MODERN APPROACHES TO ANTITUBERCULOSIS PHARMACOTHERAPY

(Literature review)

1.1. Tuberculosis antibiotic resistance

Among the population of our planet, a significant number of people are carriers of the causative agent of tuberculosis. The causative agent of this disease, *Mycobacterium tuberculosis,* can cause disease in any part of the body (extrapulmonary tuberculosis), but the main and most problematic form of the disease is pulmonary tuberculosis, which is the most common. Latent tuberculosis (LT) is asymptomatic, non-transmissible, and remains under control in most individuals. Some people with compromised immunity may develop active tuberculosis due to worsening living conditions. Exacerbation can also be caused by other infectious diseases during this period of time, which reduce the body's resistance [1].

Poverty complicates the fight against HIV infection, which becomes an additional factor in the spread of the disease. HIV attacks the immune system responsible for controlling latent tuberculosis infection (LTI) and promotes the activation of this infection, which in turn leads to accelerated disease progression. At the same time, tuberculosis strengthens the influence and accelerates the course of HIV infection [2].

According to 2017 statistics, approximately 10 million people had tuberculosis, of which 1.3 million people died from the disease during the year, which is a very high mortality rate [3].

In many cases, TB is curable, and the regimen for drug-susceptible TB includes a combination of four drugs: isoniazid, rifampicin, pyrazinamide, and ethambutol, given for at least 6 months as part of directly observed therapy (DOT), followed by

supportive care for the patient with in order to prevent relapse of the disease. At the same time, a big problem in the treatment of tuberculosis is the wide spread of drug resistance, which is caused by the mutation of strains under the influence of therapy. [4]. The worst cases of resistance are considered complete resistance to drugs, which was registered in Italy, and later India and South Africa also recorded cases of such forms of the pathogen [5]

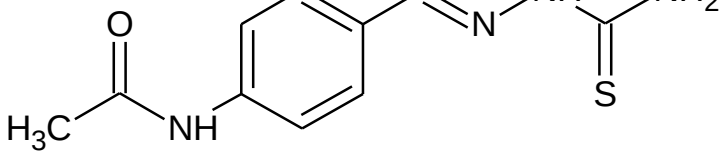
The most severe form of infection is caused by strains of *M. tuberculosis* that are resistant to all first- and second-line drugs. First-line anti-tuberculosis drugs are included in the standard four-drug regimen for drug-susceptible TB, while second-line drugs are used to treat drug-resistant TB. In 2016, multidrug-resistant tuberculosis was recorded in 490,000 people, with 6% of them being cases of broad-spectrum drug resistance [6].

In order to overcome resistance, a number of antibiotics, which have been repurposed for tuberculosis, have been added to the treatment protocols. Antibiotics of this group include drugs, the effectiveness of which has not yet been fully clarified or is ambiguous in the context of treatment of resistant tuberculosis. This group includes drugs such as thiacetazone, high-dose isoniazid, clofazimine, linezolid, amoxicillin plus clavulanate, macrolides, carbapenems, and thioridazine [7].

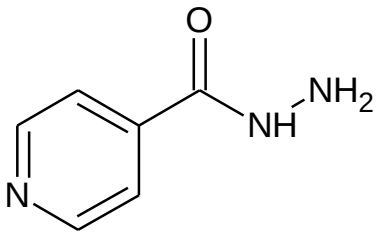
A new antituberculosis agent must meet the following criteria:

- must have a proven security profile;
- should contribute to the development of shorter, safer, cost-effective and effective alternatives for the treatment of multidrug-resistant tuberculosis;
- must be effective against new targets to overcome multidrug-resistant and broad-spectrum resistance of tuberculosis strains;
- should be compatible with antiretroviral therapy for the treatment of a significant number of patients suffering from co-infection with HIV and tuberculosis;

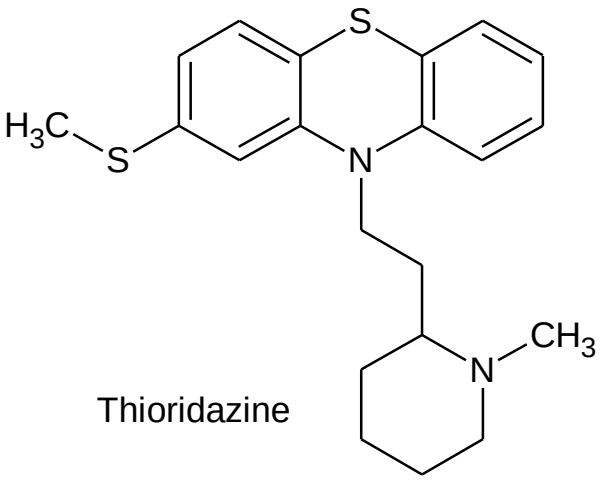
- should not cause interactions with other antituberculosis drugs or candidate drugs [8].



Thioacetazone



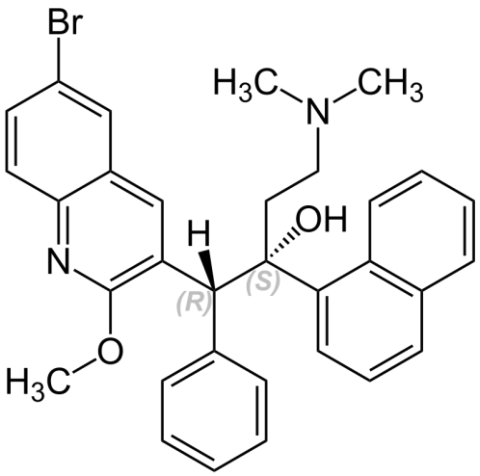
Isoniazid



Thioridazine

1.2. New antituberculosis drugs

The drug available on the market under the name Sirturo (Bedaquiline) belongs to the class of diarylquinoline compounds, which represents the modern class of antituberculosis drugs [9].



Sirturo (Bedaquiline)

The addition of to the standard treatment regimen for tuberculosis has been shown to be cost-effective and practical [10].

Unlike other drugs, bedaquiline directs its effect on the energy metabolism of mycobacteria. Even under stressful conditions such as hypoxia, energy production by ATP synthase is crucial for the survival of various mycobacterial species. This applies to both active and dormant mycobacteria, replicating or non-replicating, extracellular or intracellular, fermenting and non-fermenting species [11].

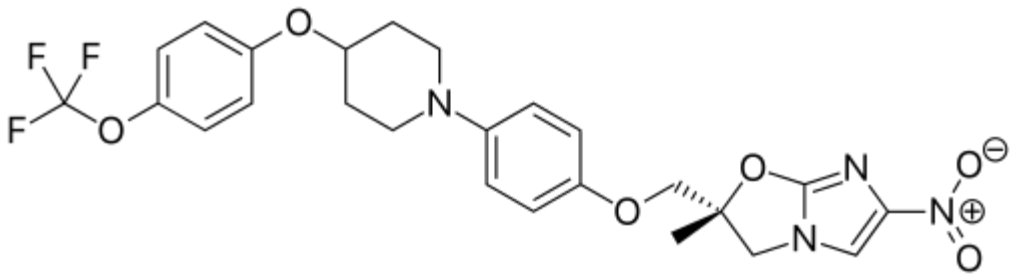
Bedaquiline inhibits the ATP synthase enzyme of *M. tuberculosis*, which is located in the membrane. ATP synthase converts ADP to ATP using an electrochemical gradient of ions (H+ or Na+) that crosses the transmembrane region. The C subunit of ATP synthase contains ion-binding sites that transport ions across the membrane, providing energy for ATP synthesis. The action of bedaquiline is to block these sites, interfering with the normal functioning of the proton pump and therefore reducing the level of intracellular ATP [12].

Bedaquiline has two chiral carbon atoms. Studies of the relationship between its structure and activity have shown that the (RS,SR) configuration of stereocenters exhibits higher antimicrobial activity compared to the (RR,SS) configuration. This is explained by the formation of an additional hydrogen bond between the hydroxyl part

of the (R,S) stereoisomer and the Glu-61 residue of subunit c. In addition, the dimethyl tertiary amine group in bedaquiline plays a key role in its activity by mimicking the functions of arginine and interfering with the proton pump of ATP synthase [13].

Bedaquiline exhibits high lipophilicity (logP 7.25) and has cationic amphiphilic properties. These characteristics allow it to interact with phospholipids of the intracellular space, which ultimately leads to its accumulation in tissues. This process causes the slow release of bedaquiline from peripheral tissues and determines the long terminal half-life. The specified high lipophilicity can also cause phospholipidosis as a side effect [14].

Delamanid is a medicine from the class of nitroimidazoles that has been approved by the European Medicines Agency for the treatment of tuberculosis infection caused by *Mycobacterium tuberculosis*. Compared to other antituberculosis drugs, delamanid has the lowest minimum inhibitory concentration and shows activity against both sensitive and drug-resistant strains of *M. tuberculosis*.



Delamanid

This drug also inhibits replication, including latent forms, and affects extracellular and intracellular growth of tuberculosis isolates [15].

Delamanid is known for its ability to inhibit the formation of two important components of mycolic acid, namely ketomycolic acid and methoxymycolic acid. Since mycolic acids are inherent only in the cell wall of mycobacteria and are absent in other

bacteria, unhealthy gram-positive or gram-negative, the ability of delamanid to inhibit this process is specific to mycobacteria [16].

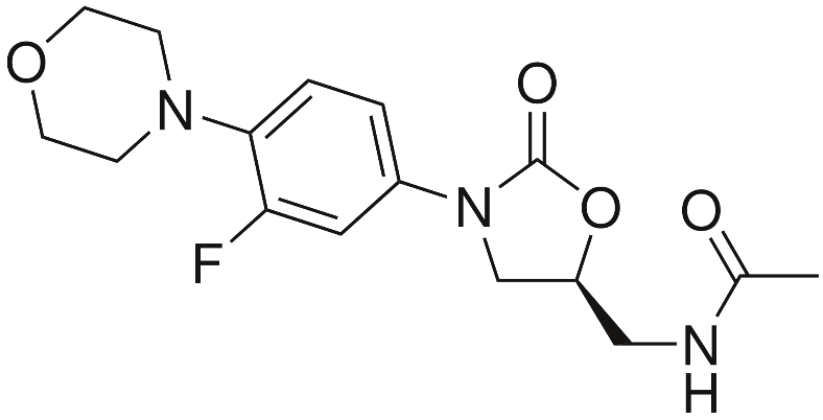
Delamanid is a prodrug that requires activation through reduction of the nitro group by the deazaflavin (F420)-dependent nitroreductase (Ddn) enzyme. Inhibition by delamanid likely involves the release of reactive radicals such as NO, which play a key role in the mammalian defense mechanism against mycobacterial infections [17].

The high tendency of delamanid to bind to plasma proteins, in particular to albumin (≥99.5%), leads to an increase in its volume of distribution. Most of delamanid is processed by plasma albumin, and only a small part is degraded by cytochrome P450 enzymes. The electron-withdrawing nitro group of delamanid results in an electron deficiency at the C-5 carbon, making it vulnerable. The amino acid residues of albumin function as nucleophiles, attacking this carbon and forming an albumin-delamanide adduct, which is further hydrolyzed and cleaved [18]. When absorbed through the mouth, the bioavailability of delamanid ranges from 35-60% in animals, and this figure increases when the drug is taken with food, especially fatty food [19].

So, pretomanid, like delamanid, belongs to the class of nitroimidazole drugs. Like delamanid, it is effective against both replicating and hypoxic non-replicating strains of *Mycobacterium tuberculosis* [20]. Pretomanid exhibits a dual mechanism of action, including inhibition of cell wall biosynthesis and respiratory toxicity. Although these mechanisms explain the effect of pretomanid on bacterial growth, they do not reveal the exact mechanism of its action on latent cells. The pharmacokinetic profile of pretomanid is improved compared to delamanid. It is easily absorbed, well tolerated and has high bioavailability. A long half-life (16-20 hours) allows the use of pretomanid in single daily doses [21].

Linezolid belongs to the class of oxazolidinones and was originally approved for the treatment of infections caused by gram-positive bacteria such as methicillin-

resistant staphylococcus and vancomycin-resistant enterococcus in 2000. It is sold under the brand name Zyvox [22].



Linezolid

The first candidate for the class of oxazolidinones was identified by E. I. du Pont de Nemours & Company in 1978. However, further development of this class of antibacterial drugs was halted because clinical trials revealed safety concerns, particularly hepatotoxicity. Later, in the 1990s, the development of resistance in Gram-positive bacteria led to a review of the development of oxazolidinones with favorable safety profiles. Almost two decades after the discovery of the oxazolidinones, the FDA approved the first oxazolidinon drug linezolid for clinical use in 2000 [23].

Some special properties of linezolid, lack of cross-resistance with other clinically approved anti-tuberculosis agents and excellent oral bioavailability make it the drug of choice for the treatment of tuberculosis [24]. Two new candidates in the oxazolidinone class, delpazolide and sutezolide, are in early clinical trials. Both delpazolid and sutezolid are less toxic and almost as effective as linezolid [25].

It is known that linezolid inhibits the process of protein synthesis that occurs in ribosomes. The bacterial ribosome is a large nucleoprotein complex consisting of a small (30S) and a large (50S) subunit. Each subunit consists of ribosomal RNA (rRNA) and many proteins (p-proteins) that work together to synthesize proteins for the cell.

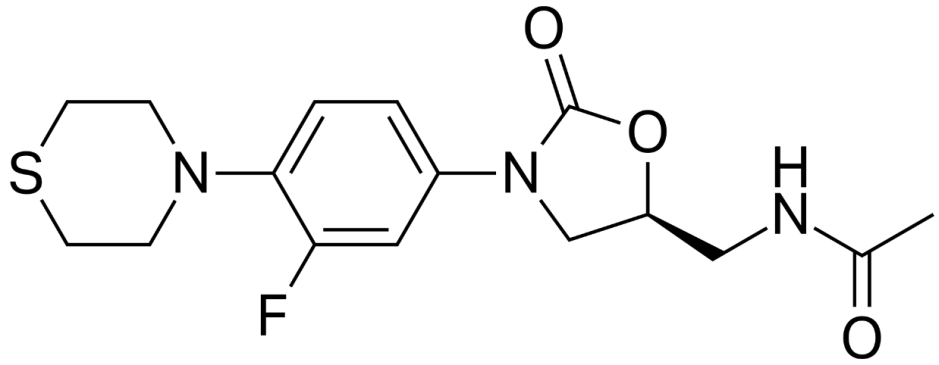
The process of protein synthesis consists of four main stages: initiation, elongation, termination and recycling. There are three characteristic sites of ribosomes involved in protein synthesis, the A, P, and E sites. During initiation, the small (30S) and large (50S) subunits of the ribosome come together to form the 70S ribosome, and template RNA (mRNA) aligns with transfer RNA (tRNA) at the P site of the ribosome to form a peptide bond [26]. In the elongation phase, aminoacylated tRNA (aa-tRNA) is transferred to the A-site of the ribosome. This leads to the formation of a peptide bond between the amino acids attached to the A- and P-sites of tRNA. Amino acids are continuously transported from the P-site to the A-site, resulting in the elongation of the peptide chain, which later exits through the E-site to the cytoplasm. The components are then recycled and the same cycle is repeated again. Linezolid binds to the A-site of the 50S peptidyltransferase center (PTC), occupying the space of the aminoacyl residue of aa-tRNA. This prevents the formation of a peptide bond between the A- and P-site tRNAs. This prevents the formation of a large 70S ribosomal complex, as a result, protein synthesis becomes difficult [24].

Since linezolid has already been successfully introduced into the clinic for the treatment of gram-positive infections and has been repurposed for the treatment of tuberculosis, its pharmacokinetics have already been well studied. Linezolid exhibits a very high oral bioavailability of approximately 100%, so its oral and injectable dosages are the same. Thus, a patient receiving intravenous therapy can be instantly switched to oral therapy when the condition becomes stable. This gives linezolid an advantage over other drugs administered only parenterally. Once administered, linezolid maintains significant serum levels, which means less frequent dosing at longer intervals. Another key characteristic of linezolid is its excellent penetration into the cerebrospinal fluid (CSF), which makes it suitable for the treatment of meningitis [27].

The presence of food does not have a significant effect on the absorption of linezolid, so this antibiotic can be taken with or without food. Metabolism of linezolid

occurs by non-enzymatic oxidation, and metabolites do not show antibacterial activity. Linezolid is not metabolized by cytochrome P450 and does not inhibit any of the important P450 isoforms. Linezolid is eliminated mainly through urine and intestines. However, the toxicity profile of linezolid limits the wider use of the drug. The drug has demonstrated a number of clinically significant side effects, some of which include peripheral neuropathy, myelosuppression, gastrointestinal disturbances, thrombocytopenia, and optic neuritis. Myelosuppression has been observed in patients receiving long-term linezolid therapy. Other common side effects include diarrhea, nausea, and headache.

Sutezolid was developed together with linezolid in 1996. After being out of development for several years, sutezolid has emerged as the second most promising candidate in the oxazolidinone class after linezolid, which is active against M. tuberculosis. This drug was active against resistant strains of M. tuberculosis and also demonstrated favorable pharmacokinetics and low toxicity in rat models. After showing promising results in mouse models, it was studied in humans and found to be safe and well tolerated [28].



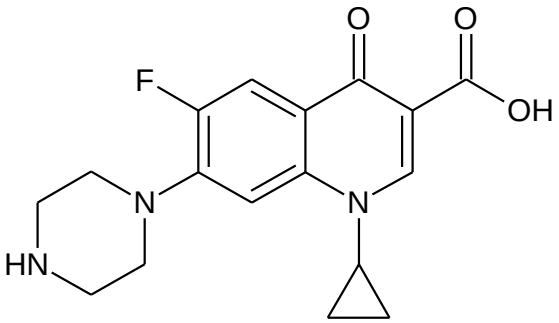
Sutezolid

Sutezolid shows superior efficacy compared to linezolid against *M. tuberculosis*. The use of linezolid is limited to the treatment of drug-resistant tuberculosis due to its low toxicity profile. Sutezolid, on the other hand, has a better safety profile and is 1-2 orders of magnitude more effective than linezolid in terms of antimycobacterial activity.

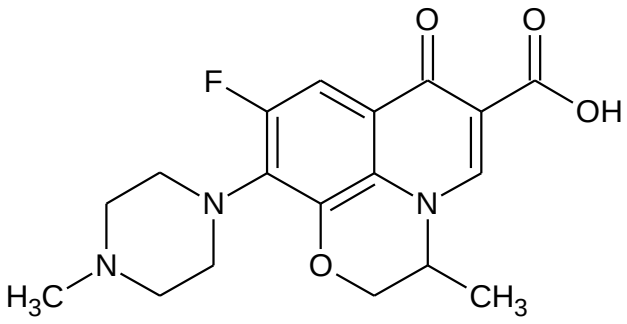
Sutezolid is a thiomorpholine analogue of linezolid, the mechanism of action of which is similar to that of linezolid. It inhibits protein biosynthesis by binding to the 23S rRNA of the large 50S subunit of the ribosome. Sutezolid is converted to an active sulfoxide metabolite that is more potent than sutezolid against extracellular tuberculosis. However, for the treatment of intracellular tuberculosis in pulmonary tuberculosis infection, it was found that the parent molecule sutezolid is 17 times more effective than its metabolite [29]. In addition, sutezolid is effective against both drug-susceptible and drug-resistant tuberculosis. The drug and its metabolite exhibit a relatively short plasma half-life (approximately 4 hours), favoring a divided dose rather than a single dose [30].

Fluoroquinolones are fluoroderivatives of quinolones that include fluorine atoms in their structure. Quinolones are compounds with bicyclic rings and are divided into two categories: 2-quinolones and 4-quinolones. The most widely used clinically quinolones are representatives of 4-quinolones. The first clinically approved quinolone for the treatment of urinary tract infections in humans was nalidixic acid, approved in 1962 [31].

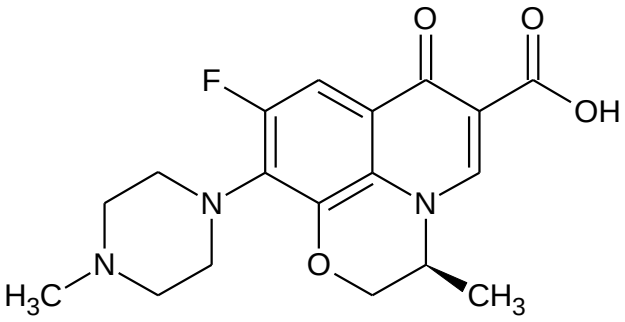
The addition of fluorine atoms to quinolones led to the creation of a new class of drugs - fluoroquinolones. These drugs are distinguished by a wider spectrum of antimicrobial action and an improved pharmacokinetic profile. Ciprofloxacin and ofloxacin (drugs of the second generation), levofloxacin (third generation), as well as moxifloxacin and gatifloxacin (fourth generation) can be distinguished among the key representatives of the class of fluoroquinolones [32].



Ciprofloxacin



Ofloxacin



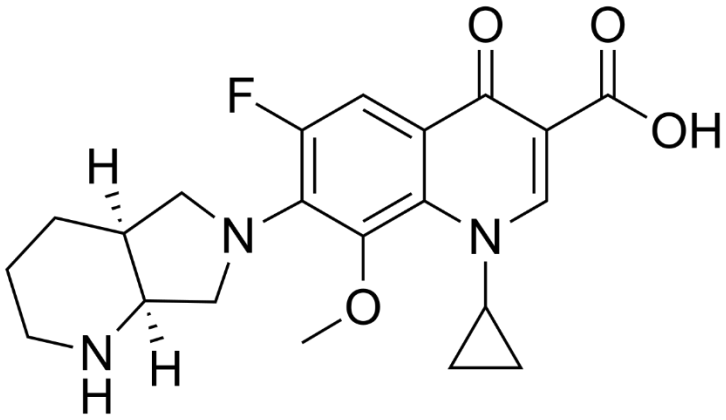
Levofloxacin

Currently, fluoroquinolones such as ciprofloxacin, ofloxacin, and levofloxacin are recommended as second-line drugs for the treatment of tuberculosis.

While these drugs are being used, two other members of the fluoroquinolone class, namely moxifloxacin and gatifloxacin, are currently being evaluated for their efficacy and potential anti-tuberculosis activity [33].

Moxifloxacin may become a potential first-line anti-tuberculosis drug in phase III clinical trials. However, it is important to consider that the wide spectrum of action of this class of drugs and their high bioavailability when taken orally can lead to excessive use of this agent. [34]

Moxifloxacin is a fluoroquinolone antibiotic that is used to treat tuberculosis, particularly tuberculosis that is resistant to treatment with standard drugs. In the future, studies of the drug in combination with bedaquiline, pretomanid and pyrazinamide or rifapentine are being conducted. Current evidence suggests the use of moxifloxacin in patients who are intolerant of any first-line antituberculosis drug or are resistant to isoniazid. However, the drug does not demonstrate effectiveness in reducing the duration of treatment regimens.



Moxifloxacin

In mycobacteria, the bactericidal effect of moxifloxacin is achieved by inhibiting DNA gyrase, which in turn disrupts the replication of bacterial DNA. [35] DNA gyrase plays a role in breaking the DNA strand by forming an enzyme-DNA complex. Moxifloxacin, like other fluoroquinolones, interacts with this enzyme-DNA complex and stabilizes it, forming a drug-enzyme-DNA complex. This leads to the blocking of the replication fork and causes chromosome fragmentation [36].

DNA gyrase, which is a member of a class of enzymes known as topoisomerases, causes the formation of supercoils in the DNA molecule. [37].

Topoisomerase enzymes play a role in maintaining DNA topology during replication, transcription, translation, and recombination processes in both prokaryotic

and eukaryotic cells. Thus, inhibition of DNA gyrase leads to cell death [38]. DNA gyrase is represented by a heterotetramer, which includes two subunits of type A and two subunits of type B. In particular, the enzyme topoisomerase II, which is present in *M. tuberculosis*, is a heterotetramer and consists mainly of subunits GyrA and GyrB [39].

The GyrA subunit is responsible for the cleavage and rejoining of DNA molecules. The active center of the enzyme contains a tyrosine fragment, and the phenolic group of this tyrosine acts as a nucleophilic center that participates in the cleavage of phosphodiester bonds in the DNA molecule. [40] The GyrB subunit includes an ATP-binding pocket that facilitates ATP hydrolysis. [41]

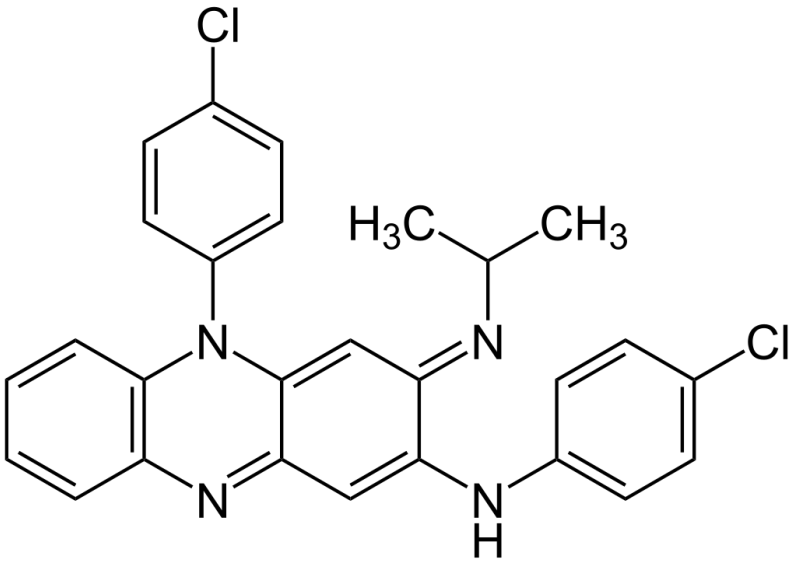
The absence of this enzyme in eukaryotes creates an opportunity for the development of new anti-tuberculosis drugs, as it makes it an attractive area to target in the treatment of this disease. All fluoroquinolone antibiotics, including moxifloxacin, target the GyrA subunit. On the other hand, the natural product novobiocin, which belongs to the aminocoumarin class of antibiotics, targets the GyrB subunit. Ofloxacin, a fluoroquinolone antibiotic used clinically to treat tuberculosis, also interacts with the GyrA subunit. Resistance to fluoroquinolones and toxicity of novobiocin have prompted interest in targeting the GyrB subunit. Studies have shown that antibiotics from the aminobenzimidazole class target the ATP binding site of GyrB [42].

When taken orally, moxifloxacin has a bioavailability of more than 90%. Moxifloxacin is widely distributed and penetrates well into the cerebrospinal fluid, making it an important component of combination therapy with rifampicin for the treatment of tuberculous meningitis [43]. Moxifloxacin and its metabolites have no effect on enzymes of the cytochrome P450 system. [44]

Instead, the drug is metabolized in the liver and excreted in the urine. Moxifloxacin also serves as a substrate for glycoprotein, while this protein interacts with the absorption, distribution and excretion of this drug. Taking this drug simultaneously with food leads to only minor effects, so it can be taken regardless of

food intake. The interaction of moxifloxacin with other antituberculosis drugs, such as bedaquiline, delamanid and clofazimine, leads to prolongation of the QT interval [45].

Fluoroquinolones tend to interact with multivalent cations, which can lead to a decrease in their absorption. Therefore, it is not recommended to take moxifloxacin at the same time as multivitamin supplements that contain iron or zinc [46]. This can pose a problem for patients with HIV-TB co-infection, as they are often prescribed multivitamin supplements. It is important to consider this interaction when developing treatment regimens for patients with similar conditions [47].



Clofazimine

The drug Clofazimine, which belongs to the riminophenazine group of antibiotics, is a recognized leprosy drug that is often prescribed for the repeated treatment of tuberculosis caused by mycobacteria [48].

The drug, which is available under the brand name Lamprene, was originally developed to treat tuberculosis. Clofazimine has shown significant antimycobacterial

activity *in vitro*, but its further development was halted due to its therapeutic inefficiency in humans and side effects such as skin discoloration and psychiatric disorders. Due to the discovery of more effective remedies for the treatment of tuberculosis, interest in the antimycobacterial action of clofazimine has decreased. Nevertheless, in 1981, WHO recommended the use of clofazimine in the treatment of multidrug-resistant leprosy [49].

With the growing epidemic of hospital-resistant tuberculosis, there has been renewed interest in clofazimine, which is now a key element of new TB treatment regimens [50].

Clofazimine is a prodrug, and the exact mechanism of its action has not yet been fully elucidated. Studies suggest the existence of a redox cycle mechanism in which clofazimine is first reduced by NADH-quinone oxidoreductase type 2 (NDH-2) and then re-oxidized to form reactive oxygen species (ROS) [51].

Reactive oxygen species (ROS) play an essential role in the regulation of *M. tuberculosis*. During normal respiration, they are formed as a byproduct and are neutralized by antioxidants. However, increased generation of ROS disrupts the balance between ROS and antioxidants, causing a condition known as oxidative stress. Increased accumulation of these reactive oxygen species (ROS) can cause cell death through damage to nucleic acids, proteins, lipids, and other biomolecules [52].

While this redox mechanism contributes to the antimycobacterial activity of clofazimine, it does not explain why clofazimine does not lose significant antimycobacterial activity under anaerobic or hypoxic conditions [53].

This led the researchers to conclude that clofazimine exhibits different mechanisms of action in different environmental conditions. In addition, menaquinone can stabilize the secondary membrane, which can reduce the destructive effect of the drug on the bacterial membrane [54].

It is also unknown why Gram-negative bacteria, which are normally susceptible to the antimicrobial action of reactive oxygen species (ROS), are not sensitive to clofazimine [55].

These results suggest that the activity of clofazimine may depend on alternative or multifaceted mechanisms. In addition, clofazimine exhibits cross-resistance with bedaquiline due to overexpression of the efflux pump MmpL5. However, recent studies also suggest that mutations in the pepQ gene may influence cross-resistance between bedaquiline and clofazimine [56, 57] .

However, the exact target of action of clofazimine is not yet fully understood, NDH-2 enzyme is considered as a potential target of clofazimine. NDH-2 is a membrane-bound protein that contains an FAD fragment [58, 59]. It is a key enzyme in the respiratory chain of mycobacteria. NDH-2 catalyzes the transfer of electrons from NADH to menaquinone, converting it to menaquinol. The resulting menaquinol further supplies electrons to oxidoreductase enzymes in the respiratory chain. The FAD fragment in the menaquinone molecule carries electrons that compete with clofazimine and menaquinone [51, 60].

Clofazimine shows low solubility, but shows bioavailability when taken orally [61]. The drug contains three ionized amine groups, which are protonated in an acidic environment and receive a positive charge. This leads to an increase in the solubility of the drug, which is otherwise highly lipophilic, in an acidic environment [62].

The volume of distribution of clofazimine is extremely large, and the half-life is extremely long, reaching 70 days. As stated earlier, the main difficulty with clofazimine is its lipophilic nature. To improve the absorption of clofazimine, it is recommended to use it in the form of a microcrystalline suspension on an oil-wax basis [63].

The high lipophilicity of clofazimine leads to its accumulation in the adipose tissue of organs such as the lungs, liver, brain, spleen, and bone marrow. The gradual

increase in tissue accumulation of clofazimine causes skin discoloration, which is the most common side effect of this drug [64].

Clofazimine also forms crystal-like drug inclusions in macrophages, which may be associated with various side effects. It is important to note that clofazimine is well tolerated, and side effects associated with it disappear after discontinuation of the drug [65].

Conclusions to the Chapter 1

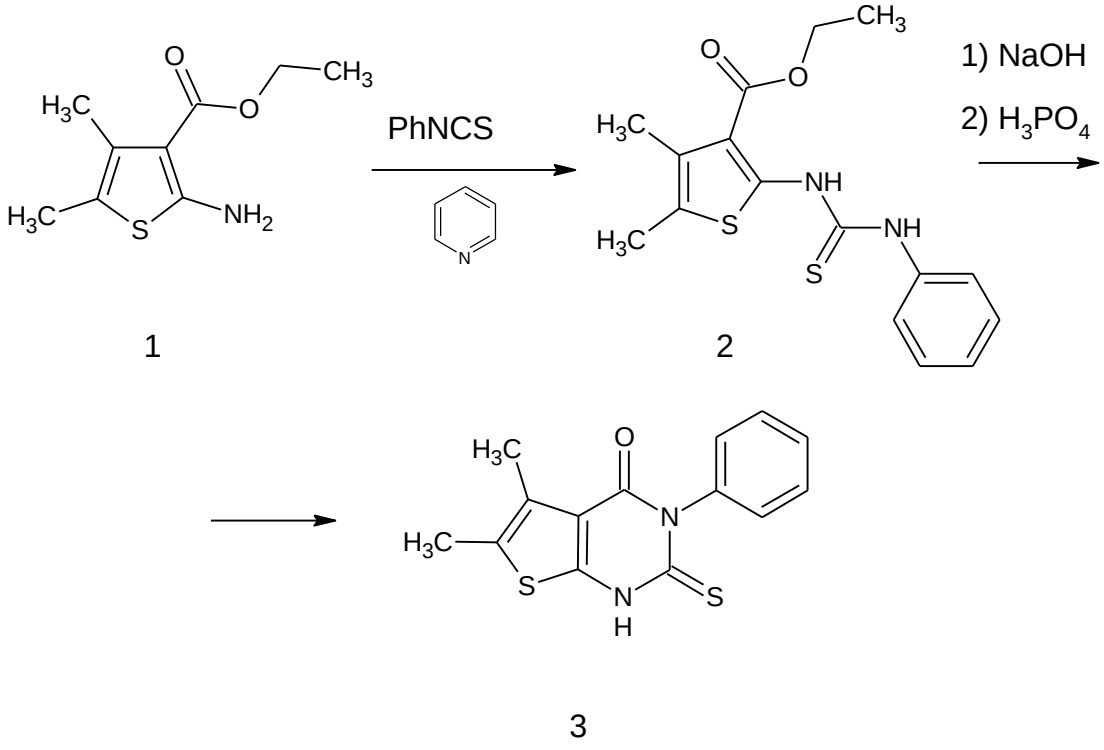
1. Tuberculosis is a dangerous disease that mainly affects the lungs and rarely other organs and is caused by *M. tuberculosis*; this disease currently has approved treatment regimens, however, it is dangerous if mycobacteria develop resistance to these drugs.
2. New drugs such as Bedaquiline, Delamanid, oxazolidinones and fluoroquinolones are proposed to be used in the market to overcome resistance; in some cases, Clofazimine is used, which is poorly studied and has been shown to inhibit the growth of mycobacteria in difficult cases.
3. The problem of overcoming bacterial resistance to existing drugs is urgent and may require new approaches to its solution.

CHAPTER 2. CONSTRUCTION OF 5,6-DIMETHYL-2-(ALKYLTHIO)-3-PHENYLTHIENO[2,3-d]PYRIMIDIN-4(3H)-ONE DERIVATIVES

2.1. Synthesis of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives

The synthesis of target compounds 2-(alkylthio)-5,6-dimethyl-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one was carried out according to schemes 2.1 and 2.2.

Scheme 2.1

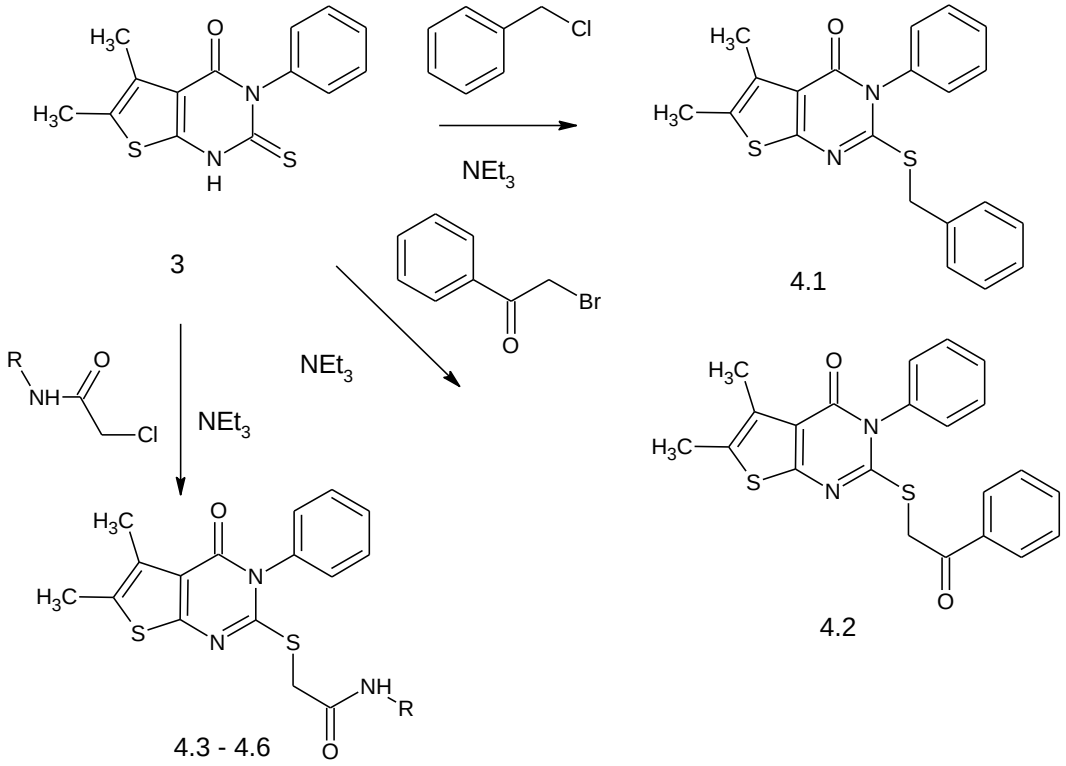


At the first stage, intermediate thiourea **2** was obtained from ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate **1** available by the Gewald reaction at heating in pyridine. Further cyclization of compound **2** into the key building block - 5,6-dimethyl-3-phenyl -2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one **3** was carried out by heating with three equivalents of sodium hydroxide in an ethanol medium (96%). The

precipitate of the target product **3** was isolated by neutralizing of the alkali with orthophosphoric acid. The precipitate **3** that formed was filtered and thoroughly washed with distilled water.

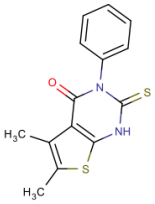
Further modification of **3** was carried out according to scheme 2.2 by alkylation with benzyl chloride, α-bromoacetophenone and a series of 2-chloroacetamides. As a result, a number of derivatives of **4** were obtained. The structure of the obtained compounds 4 was confirmed by the data of chromato-mass spectra and elemental analysis

Scheme 2.2



2.2. Structure assignmet of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives

Quality control of intermediate compound 3 was carried out with the help of chromatography-mass spectroscopy; spectrum, which indicates purity in the sample of at least 95% (Fig. 2.1.)



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L753140F
LCMS-33
SUPOR_30.M
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MaxPeak: 95.8%

#	RT	DAD1A	DAD1B	MSD1	MSD2	ELSD	MSD1 ions	MSD1 rt	MSD2 ions	MSD2 rt	Info
1	0.338	3.5%	---	---	---	---	---	---	---	---	
2	0.982	0.7%	1.1%	2.8%	2.2%	---	273.0(100)	0.991	271.2(100)	0.991	
3	1.056	---	---	10.5%	1.9%	---	305.0(100)	1.066	303.0(100)	1.066	
4	1.086	95.8%	98.9%	86.7%	95.9%	100.0%	289.0(96),311.0(4)	1.098	287.0(82),597.0(10),289.2(8)	1.098	P +H+,P NEG

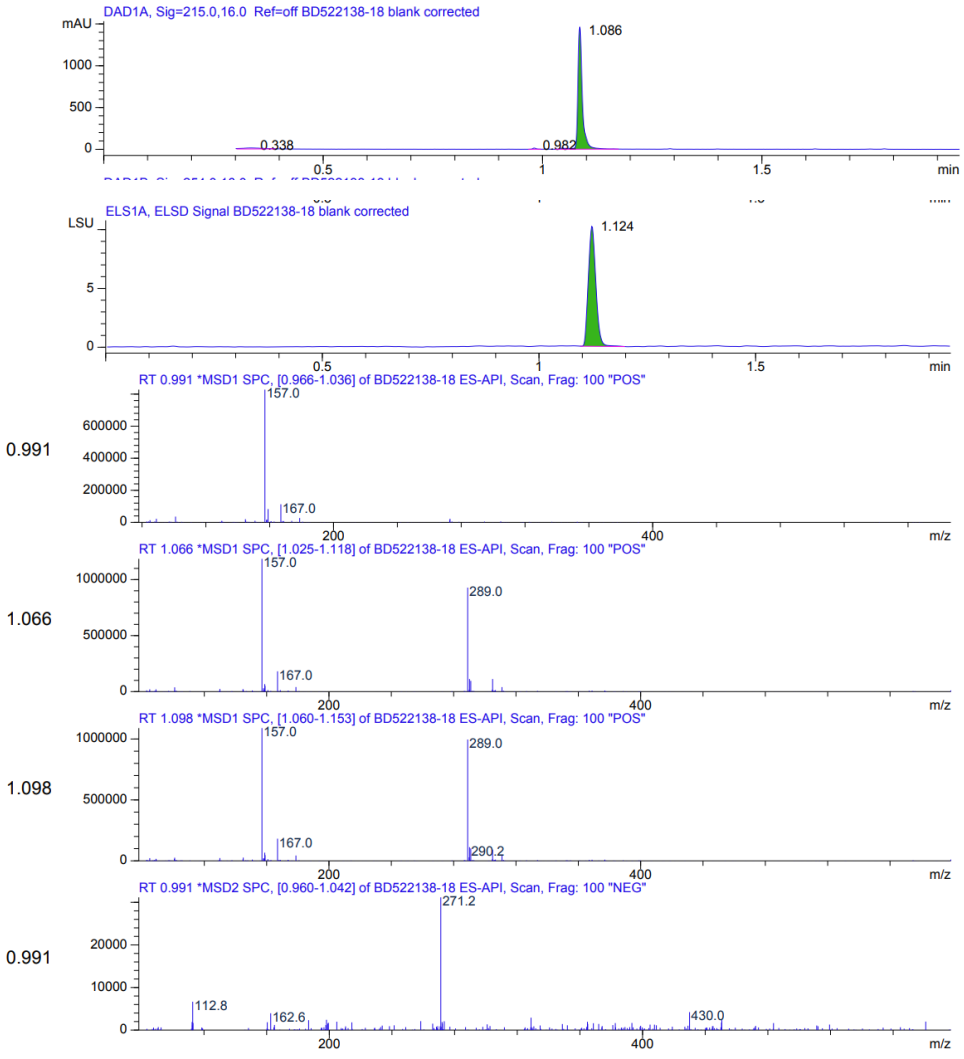


Fig. 2.1. Chromato-mass spectrum of 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one **3**

In order to determine the structure, the ^{1}H NMR spectroscopy method was also used. According to the data of ^{1}H NMR spectra, all synthesized compounds **4** have proton signals of two CH_3 groups of the thiophene nucleus in the form of two singlet signals in the range from 2.29 ppm. to 2.37 ppm The appearance of an alkyl radical in the molecule is evidenced by the characteristic signals of the protons of the methylene group, which for the compound with the benzyl radical - 2-(benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-d]pyrimidin-4(3H)- one **4.1** is at 4.31 ppm. For compound **4.2**, the signal of the methylene group is at 4.69 ppm, which clearly correlates with the appearance of an electron-withdarwing carbonyl group nearby.

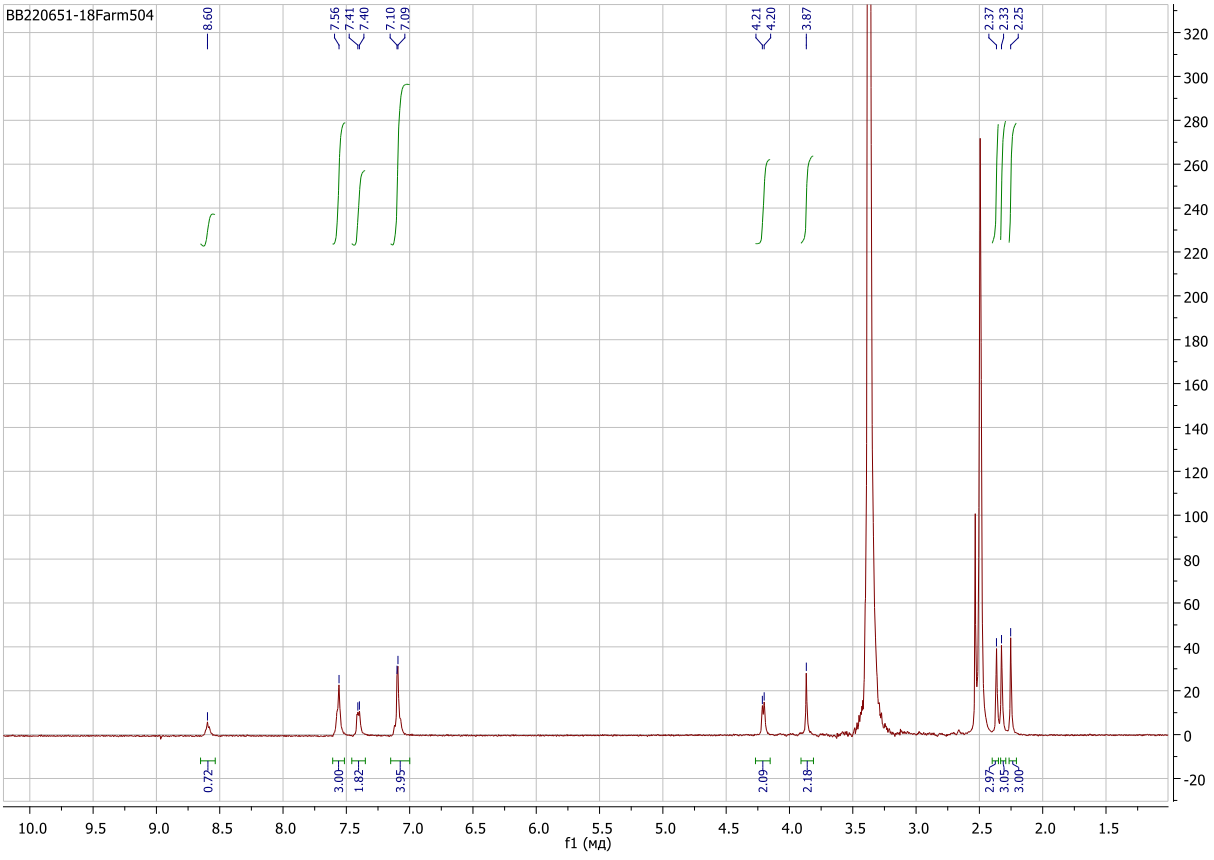


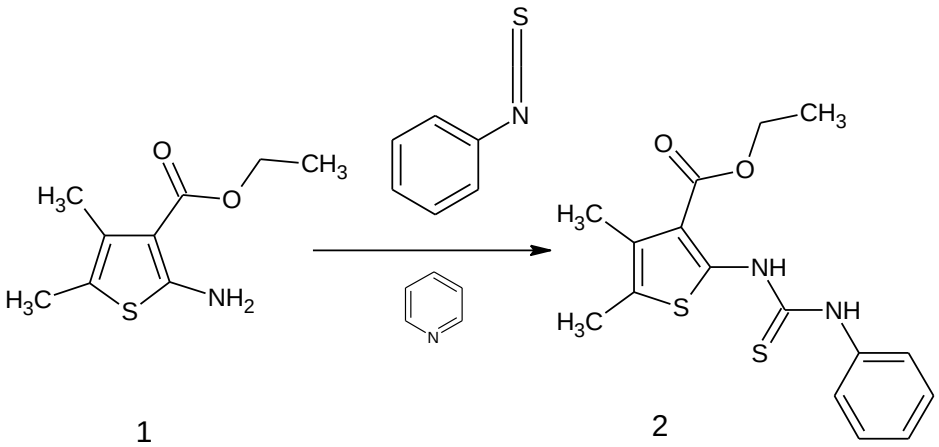
Fig. 2.2. ^{1}H NMR spectrum of 2-[(5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio]-3-phenyl-*N*-(4-methylbenzyl)acetamide **4.5**

For amides **4.3**–**4.6**, the signals of the methylene protons of the acetic acid residue are located in the range of 3.86–4.04 ppm, at the same time, for benzyl amides, the signals of the methylene group of benzyl residues will appear as doublets in the range of 4.18–4.26 ppm. For compound **4.5** 2-[(5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl)thio]-3-phenyl-N-(4-methylbenzyl)acetamide the spectrum is characterized by the presence of a methyl group signal at 2.25. ppm, and for the compound with a methoxy group **4.6** 2-[(5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-2-yl)thio]-3-phenyl-N-(4-methoxybenzyl)acetamide signal of this group appears at 3.71 ppm in the form of a singlet signal.

2.3. 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. 1H NMR spectra for the compounds were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz and on a Varian-400 at 400 MHz respectively, solvents - DMSO-d6, internal standard TMS. 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.

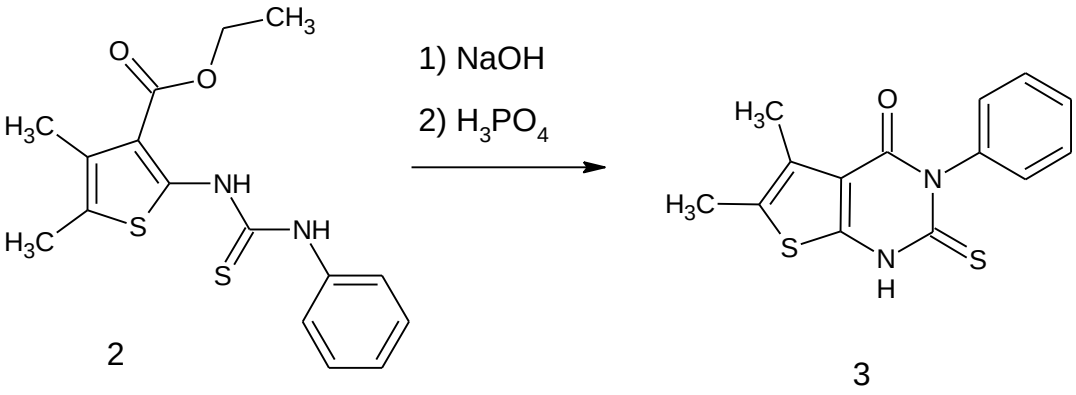
Preparation method of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-ones 4



1 2

Synthesis of ethyl 4,5-dimethyl-2-[(phenylcarbamothioyl)amino]thiophene-3-carboxylate 2

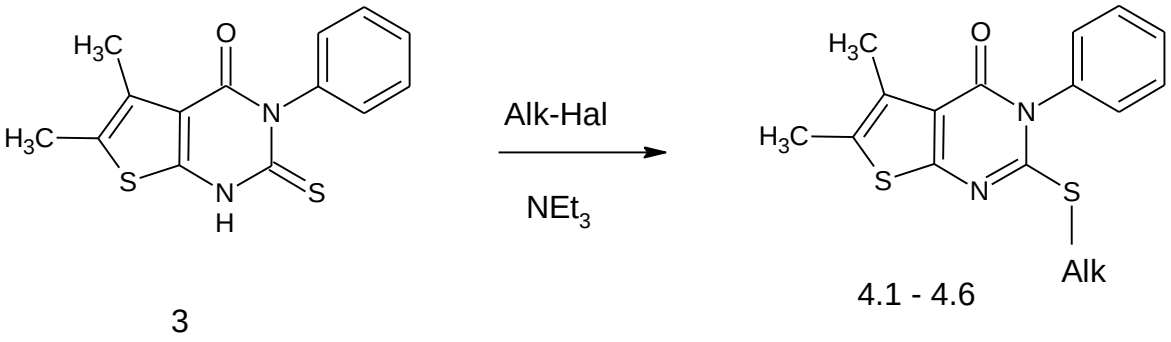
Phenylisothiocyanate 4.1 ml (0.03 mol) was added to 6 g (0.03 mol) of ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate **1** in 10 ml of pyridine, and the mixture was heated with stirring at 60º C. After cooling, the reaction mixture was diluted with water, and the precipitate ethyl 4,5-dimethyl-2-[(phenylcarbamothioyl)amino]thiophene-3-carboxylate **2**, which was formed, was filtered and dried and used in further transformations without additional purification.



2 3

Synthesis of 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one 3

Sodium hydroxide 3.0 g (0.075 mol) was added to 8.35 g (0.025 mol) of ethyl 4,5-dimethyl-2-[(phenylcarbamothioyl)amino]thiophene-3-carboxylate **2** and the mixture was boiled in 60 ml of ethanol. After 3 hours, the reaction mixture was cooled and 8.5 ml (0.075 mol) of orthophosphoric acid was added to it. The reaction of the medium was checked, and the precipitate 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one **3**, which formed in an acidic medium, was filtered and washed with a large amount of water.

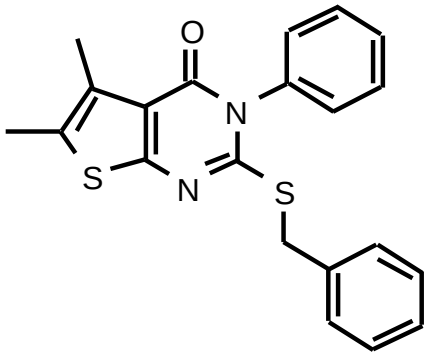


3 4.1 - 4.6

General method of alkylation of 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one 3 for preparation of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-ones 4

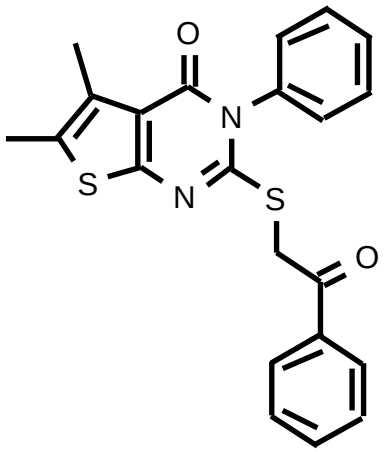
Dimethylformamide (3 ml) and 0.14 ml of triethylamine were added to 0.29 g (0.001 mol) of 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one 3 (0.001 mol), and then 0.001 mol of an alkylating agent was added and the reaction mixture was heated at 70ºC for 8 hours. Then the reaction mixture was diluted with water and the precipitate that fell out was filtered and washed with water and dried. Additionally, compounds 4 were purified by boiling in ethyl alcohol.

4.1. 2-(Benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one



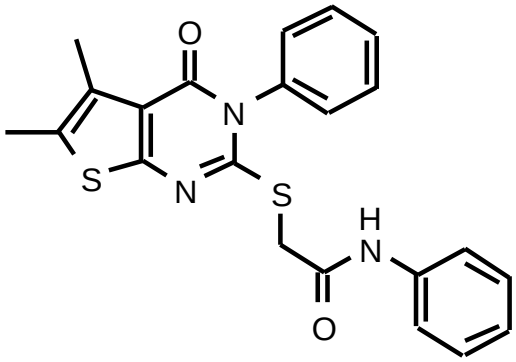
Yield – 67 %, white crystals. M. p. 213-214 °C. Anal. Calcd. for $C_{21}H_{18}N_2OS_2$, % (378,52): C, 66.64; H, 4.79; N, 7.40. Found, C, 66.72; H, 4.80; % N, 7.55. ^{1}H NMR (500 MHz, DMSO) δ 7.52 (d, *J* = 6.9 Hz, 3H), 7.36 (dd, *J* = 12.8, 6.7 Hz, 5H), 7.27 (t, *J* = 7.3 Hz, 2H), 7.22 (t, *J* = 7.1 Hz, 1H), 4.31 (s, 2H), 2.36 (s, 3H), 2.32 (s, 3H). LC-MS m/z (ES+) 379.0 (MH^+).

4.2. 5,6-Dimethyl-2-[(2-oxo-2-phenylethyl)thio]-3-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one



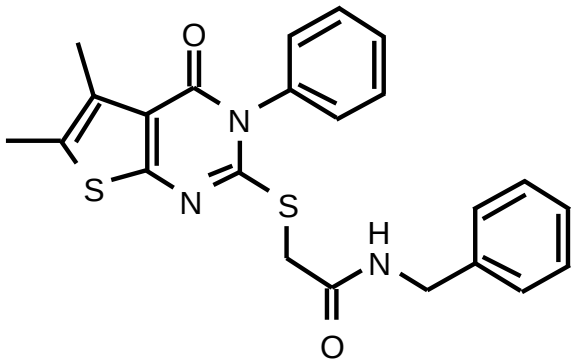
Yield – 58 %, white crystals. M. p. > 250 °C. Anal. Calcd. for $C_{22}H_{18}N_2O_2S_2$, % (406.53): C, 65.00; H, 4.46; N, 6.89. Found, C, 65.12; H, 4.52; % N, 6.95. ^{1}H NMR (400 MHz, DMSO) δ 8.01 (d, *J* = 7.6 Hz, 2H), 7.68 (t, *J* = 7.2 Hz, 1H), 7.57 (dd, *J* = 15.9, 7.4 Hz, 5H), 7.42 (d, *J* = 5.8 Hz, 2H), 4.69 (s, 2H), 2.29 (s, 6H). LC-MS m/z (ES+) 407.0 (MH^+).

4.3. 2-[(5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio]-3-phenyl-*N*-phenylacetamide



Yield – 82 %, white crystals. M. p. 218-220 °C. Anal. Calcd. for $C_{22}H_{19}N_3O_2S_2$, % (421.54): C, 62.69; H, 4.54; N, 9.97. Found, C, 62.73; H, 4.56; % N, 9.98. ^{1}H NMR (500 MHz, DMSO) δ 10.28 (s, 1H), 7.61 – 7.48 (m, 5H), 7.42 (d, *J* = 6.7 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 7.04 (t, *J* = 6.9 Hz, 1H), 4.04 (s, 2H), 2.34 (s, 3H), 2.32 (s, 3H). LC-MS m/z (ES+) 422.0 (MH^+).

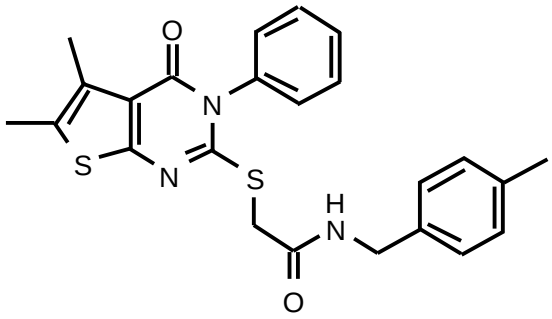
4.4. *N*-Benzyl-2-[(5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio]-3-phenyl-acetamide



Yield – 73 %, white crystals. M. p. 238-240 °C. Anal. Calcd. for $C_{23}H_{21}N_3O_2S_2$, % (435,57): C, 63.42; H, 4.86; N, 9.65. Found, C, 63.52; H, 4.88; % N, 9.67. ^{1}H NMR (500 MHz, DMSO) δ 8.64 (s, 1H), 7.56 (m, 3H), 7.41 (d, *J* = 6.2 Hz, 2H), 7.28 (d, *J* =

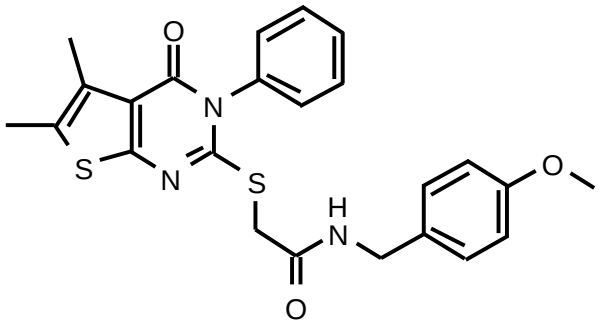
6.9 Hz, 2H), 7.23 (d, *J* = 7.2 Hz, 3H), 4.26 (d, *J* = 5.6 Hz, 2H), 3.89 (s, 2H), 2.37 (s, 2H), 2.33 (s, 3H). LC-MS m/z (ES+) 436.0 (MH^+).

4.5. 2-[(5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio]-3-phenyl-*N*-(4-methylbenzyl)acetamide



Yield – 83 %, white crystals. M. p. >250 °C. Anal. Calcd. for $C_{24}H_{23}N_3O_2S_2$, % (449,60): C, 64.12; H, 5.16; N, 9.35. Found, C, 64.12; H, 5.18; % N, 9.37. ^{1}H NMR (400 MHz, DMSO) δ 8.60 (s, 1H), 7.56 (s, 3H), 7.40 (m, 2H), 7.10 (m, 4H), 4.21 (d, *J* = 5.8 Hz, 2H), 3.87 (s, 2H), 2.37 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H). LC-MS m/z (ES+) 450.0 (MH^+).

4.6. 2-[(5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio]-3-phenyl-*N*-(4-methoxybenzyl)acetamide



Yield – 90 %, white crystals. M. p. 223-224 °C. Anal. Calcd. for $C_{24}H_{23}N_3O_3S_2$, % (465.60): C, 61.91; H, 4.98; N, 9.02. Found, C, 62.02; H, 4.99; % N, 9.05. ^{1}H NMR (400 MHz, DMSO) δ 8.57 (s, 1H), 7.56 (s, 3H), 7.39 (s, 2H), 7.14 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 2H), 4.18 (d, *J* = 5.6 Hz, 2H), 3.86 (s, 2H), 3.71 (s, 3H), 2.36 (s, 3H), 2.32 (s, 3H). LC-MS m/z (ES+) 466.0 (MH^+).

Conclusions to the Chapter 2

1. An effective method for obtaining 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-ones by alkylating 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one was developed.
2. The structures of the obtained target 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-ones and intermediate compounds were confirmed using a series of instrumental methods of analysis.

CHAPTER 3. STUDY OF MOLECULAR MECHANISMS OF ANTIBACTERIAL ACTIVITY OF 5,6-DIMETHYL-2-(ALKYLTHIO)-3-PHENYLTHIENO[2,3-d]PYRIMIDINE-4(3H)-ONE DERIVATIVES

3.1. TrmD importance for bacterial survival

Among the population of our planet, a significant number of people are carriers of the causative agent of tuberculosis. The causative agent of this disease, *M. tuberculosis*, can cause disease in any part of the body (extrapulmonary tuberculosis), but the main and most problematic form of the disease is pulmonary tuberculosis, which is the most common of all. The treatment regimen for tuberculosis includes a combination of four drugs: isoniazid, rifampicin, pyrazinamide, and ethambutol, administered for at least 6 months. But in cases of persistent tuberculosis, this scheme may not be effective. Therefore, innovative approaches to the creation of anti-tuberculosis drugs are relevant.

tRNA methyltransferase-(N(1)G37) methylates guanosine at position 37 (G37) in prokaryotic tRNA. The resulting modified nucleotide, N1-methylguanosine at position 37 (m1G37), is present in tRNA where the sequence G36G37 is located in the anticodon region. This is seen in tRNAs from all three domains of life, and G37 is the base adjacent to the anticodon at the 3' end [66]. Mutations in the TrmD gene lead to growth disorders associated with increased translational frameshifting, which in turn leads to incorrect protein synthesis [67]. TrmD belongs to a unique class of S-adenosyl-L-methionine (SAM)-dependent methyltransferases known as the SpoU-TrmD (SPOUT) superfamily of RNA methyltransferases or class IV methyltransferases. Proteins belonging to this family differ in their structure because they do not contain a consensus methyltransferase fold. TrmD and other members of the SPOUT family have a characteristic deep trefoil architecture in the catalytic region that forms an L-shaped pocket for SAM binding. In comparison, methylation of G37 in eukaryotes is carried out by the enzyme Trm5, which belongs to the class I methyltransferase family [68,

69]. Previous research suggests differences in substrate requirements for TrmD and Trm5. Trm5 recognizes the general tertiary structure of tRNA with base G37, whereas TrmD interacts mainly with the stem and anticodon loop of tRNA, including bases G36G37. Trm5 functions as a monomer, binding to S-adenosyl-L-methionine (SAM) in the Rossmann fold region of the active site, unlike dimeric TrmD, which has a methyl donor binding site in a trefoil shape. In addition, the SAM adopts a unique bent conformation in TrmD compared to the extended conformation in Trm5 and many other conventional methyltransferases. These structural differences, substrate requirements, and ligand binding conformations between TrmD and its human ortholog provide an opportunity to develop novel and selective inhibitors for bacterial TrmD. [68, 69]. Additional studies in Gram-negative bacteria indicate a key role for m1G37 tRNA methylation by TrmD in regulating the synthesis of membrane proteins such as drug efflux pumps. This is achieved by controlling protein translation at proline codons near the start of the open reading frames. Therefore, tRNA modification by TrmD is an important factor in the regulation of membrane protein synthesis, at least in Gram-negative bacteria. As a result, inhibition of TrmD significantly impairs the development of drug resistance, contributing to the sensitization of these organisms to antibiotics [70].

Previous drug development efforts targeting TrmD in *Haemophilus influenzae* and *Pseudomonas aeruginosa* have resulted in specific inhibitors with high biochemical activity against TrmD isozymes in vitro. However, these compounds generally showed only limited antibacterial efficacy when tested against a variety of bacterial species. The unfavorable result encourages scientists to consider targeting TrmD using a structure-guided fragment-based strategy. They systematically tested the biochemical and antimicrobial activity at each step of the iteration to determine its success against microbes [71, 72].

3.2. Prediction of mycobacterial TrmD interaction

Search among a number of synthetically available 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives for potential mycobacterial TrmD inhibitors that may be effective against resistant strains tuberculosis alone or in combination with other anti-tuberculosis agents. We used the model of mycobacterial TrmD for the studies.

Table 3.1

The results of the docking studies to micobacterial TrmD for 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidine-4(3H)-one drivatives **4**

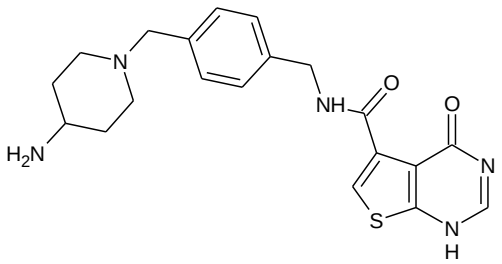
Code/Stucructure	**Interaction with the active site***	**Interacting amino acids**
1	2	3
Native inhibitor 	+	PRO83 THR84 PRO85 GLU112 SER132 ILE133 GLY134 TYR136 VAL137 LEU138 GLY140 GLY141 ALA144

Table 3.1 (continued)

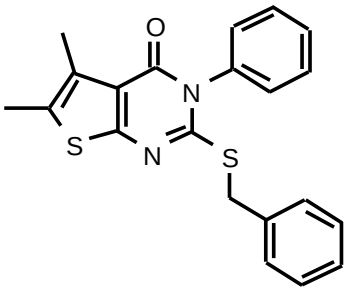
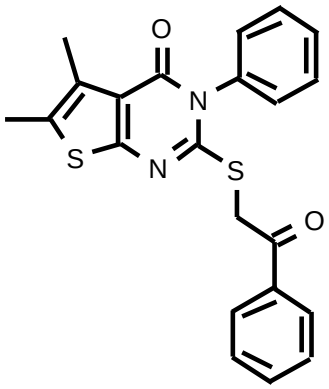
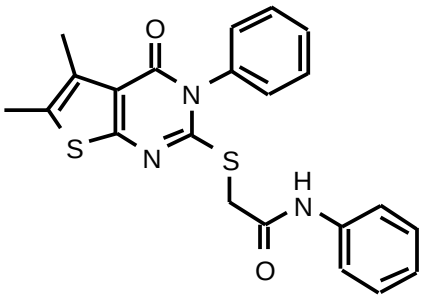
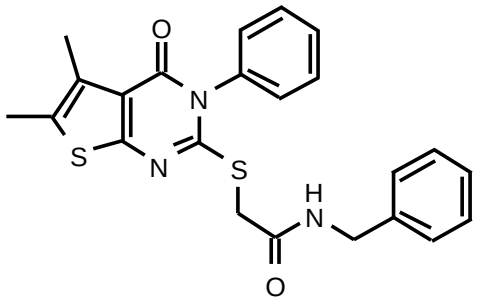
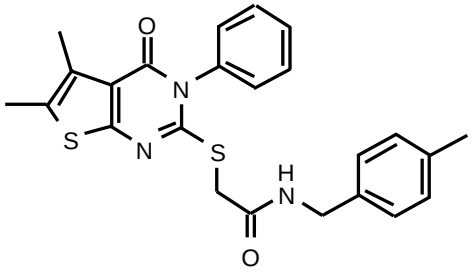
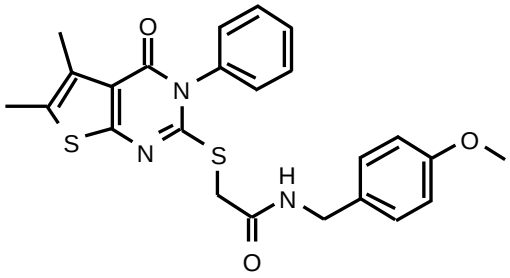
1	2	3
4.1. 	+	PRO83 THR84 PRO85 GLY109 TYR111 GLU112 GLY113 ILE114 ILE133 TYR136 LEU138
4.2. 	-	-
4.3. 	-	-

Table 3.1 (continued)

1	2	3
4.4. 	-	-
4.5. 4 	+ / - (partially matches native ligand)	THR84 PRO85 GLU112 ILE114 GLN116 GLU130 LEU138 GLY140
4.6. 	-	-

* 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 P. aeruginosa TrmD interaction

According to the results of docking studies of a number of synthetically available derivatives of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one, it was established that the best binding parameters with the active site of inhibitors mycobacterial TrmD had an S-benzyl derivative. According to the obtained results the benzyl or smaller substituent at the exocyclic sulfur atom is preferable, while substitution of the sulfur with 1-phenyl-2-ethanone or the derivatives of acetic acid form too bulky molecules to fit the active site.

The visualization of the interaction of the compound with the best binding parameters - 2-(benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one 4.1 is presented in the figure 2.1.

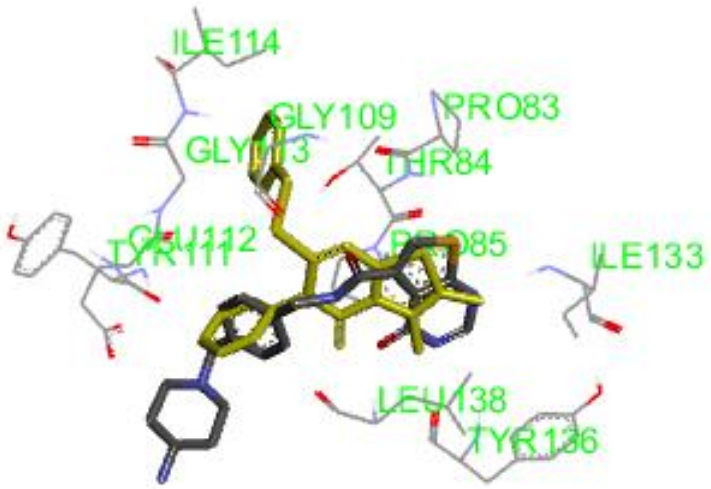


Fig. 2.1. The visual presentation of the interaction of 2-(benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **4.1** (yellow) with the active site of mycobacterial TrmD in comparison with the native ligand

TrmD is the key enzyme in the cell of *P. aeruginosa* which is a threteinig hospital infection with the opportunity to develop resistance againt many antibiotics. In view of this we tried the obtained 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidine-4(3H)-one drivatives **4** as possible ligand for the enzyme isolated from *P. aeruginosa* [73]. The results presented in the table 3.2 showed that most of the compounds **4** cannot effectively interct with the active site of the enzyme. Only inhibitors with patial binding were found.

Table 3.2

The results of the docking studies to *P. aeruginosa* TrmD for 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidine-4(3H)-one drivatives **4**

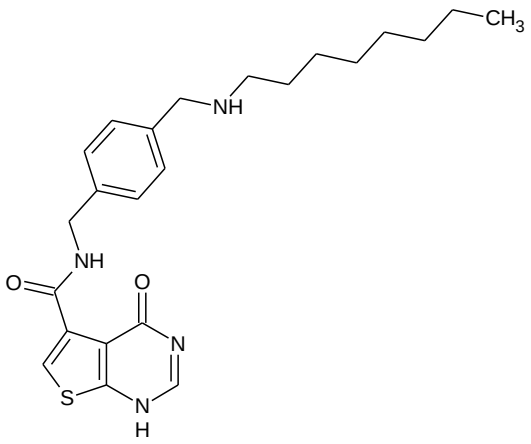
Code/Stucructure	**Interaction with the active site***	**Interacting amino acids**
1	2	3
Native inhibitor 	-	LEU92 TYR120 SER137 ILE138 GLY139 TYR141 LEU143 GLY145 ASP178 LEU180 LEU181 ASP182 CYS183

Table 3.2 (continued)

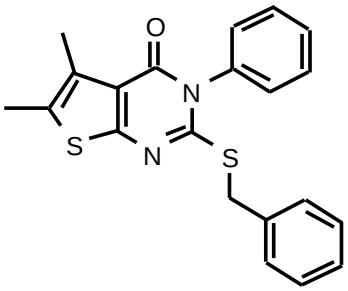
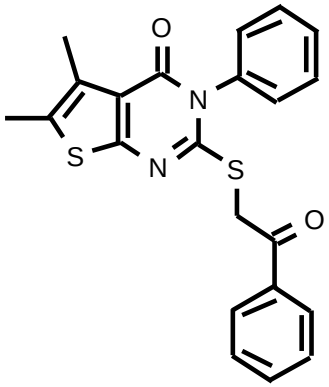
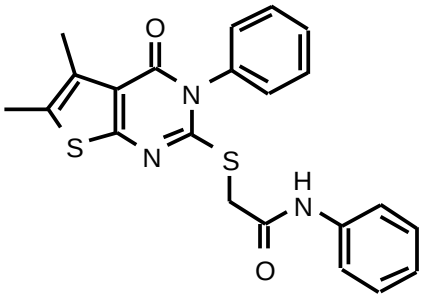
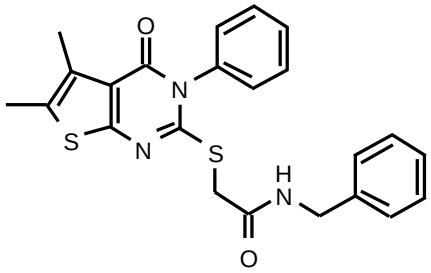
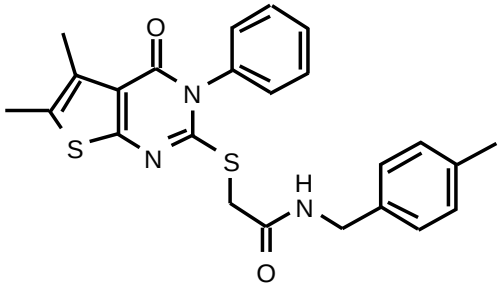
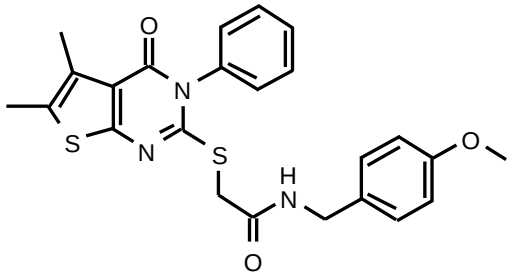
1	2	3
4.1. 	-	-
4.2. 	+/- (partial interaction with active site)	TYR120 SER137 GLY139 ASP140 TYR141 VAL142 LEU180 ASP182 ARG225
4.3. 	+/- (partial interaction with active site)	GLN95 TYR120 VAL142 LEU143 ASP178 LEU180 LEU181 ASP182

Table 3.2 (continued)

1	2	3
4.4. 	+/- (partial interaction with active site)	PRO94 TYR120 VAL142 LEU143 ASP178 LEU180 ASP182
4.5. 	-	-
4.6. 	+/- (partial interaction with active site)	TYR91 GLN95 ARG119 TYR120 VAL142 GLN101 ARG105 ARG159 ASP178 HIS185

* 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

3.4. 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. The potein model from pdb bank (https://www.rcsb.org/) with the code 6jof was used for the molecular modeling studies for TrmD of *M. tuberculosis* and for the studies of *P. aeruginosa* TrmD inhibitory activity the structure with code 5zhn was used.

Conclusions to the Chapter 3

1. The docking studies revealed that 2-(benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one is a potential inhibitor of mycobacterial TrmD and is a potential innovative drug for overcoming tuberculosis resistant to standard pharmacotherapy.
2. The docking studies showed that most of the synthesized 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidine-4(3H)-one derivatives cannot effectively interact with the active site of *P. aeruginosa* TrmD. Only inhibitors with patial binding were found.

GENERAL CONCLUSION

1. Tuberculosis is a dangerous disease that mainly affects the lungs and less often other organs and is caused by *M. tuberculosis* and is dangerous in case of the formation of mycobacterium resistance to these drugs. The problem of overcoming tuberculosis resistance to existing drugs is urgent and may require new approaches to its solution.

2. An effective method for obtaining 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-ones by alkylating 5,6-dimethyl-3-phenyl-2-thioxo- 2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one has been developed.

3. The structures of the obtained final 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-ones and intermediate compounds were confirmed using a number of instrumental methods of analysis.

4. The docking studies revealed that 2-(benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one is a potential inhibitor of mycobacterial TrmD, while the studies performed for showed that most of the synthesized compounds cannot effectively interact with the active site of TrmD isolated from *P. aeruginosa*.

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Appendices

Appendix A

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ГРАМОТА

за участь

отримав(ла)

El-Mouddene Hassnae

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

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

УДК 615.1

Редакційна колегія: проф. Котвіцька А. А., проф. Владимирова І. М.
Укладачі: Зуйкіна Є. В., Боднар Л. А., Сурікова І. О.,

Актуальні питання створення нових лікарських засобів: матеріали XXX міжнародної науково-практичної конференції молодих вчених та студентів (17-19 квітня 2024 р., м. Харків). – Харків: НФаУ, 2024. – 475 с.

Збірка містить матеріали міжнародної науково-практичної конференції молодих вчених та студентів «Актуальні питання створення нових лікарських засобів, які представлені за пріоритетними напрямами науково-дослідної роботи Національного фармацевтичного університету. Розглянуто теоретичні та практичні аспекти синтезу біологічно активних сполук і створення на їх основі лікарських субстанцій; стандартизації ліків, фармацевтичного та хіміко-технологічного аналізу; вивчення рослинної сировини та створення фітопрепаратів; сучасної технології ліків та екстемпоральної рецептури; біотехнології у фармації; досягнень сучасної фармацевтичної мікробіології та імунології; доклінічних досліджень нових лікарських засобів; фармацевтичної опіки рецептурних та безрецептурних лікарських препаратів; доказової медицини; сучасної фармакотерапії, соціально-економічних досліджень у фармації, маркетингового менеджменту та фармакоекономіки на етапах створення, реалізації та використання лікарських засобів; управління якістю у галузі створення, виробництва й обігу лікарських засобів; суспільствознавства; фундаментальних та мовних наук.

УДК 615.1

THE EFFICIENT APPROACH TO 5,6-DIMETHYL-2-(ALKYLTHIO)-3-PHENYLTHIENO[2,3-d]PYRIMIDIN-4(3H)-ONE DERIVATIVES

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Introduction. Thieno[2,3-d]pyrimidine derivatives have shown themselves in many screening experiments as effective antimicrobial agents, and even approximate data on the possible mechanisms of their influence on bacterial growth were not clear until now. The synthetic availability of thieno[2,3-d]pyrimidines is also a beneficial factor for these structures as possible drugs. Basic research on the development for new TrmD inhibitors confirmed the status of these structures as privileged to inhibit bacterial growth by blocking such an important enzyme for the synthesis of bacterial proteins as TrmD. The leading compounds are potential innovative antibiotics with possible antimycobacterial activity.

Aim. To develop effective methods that would make it possible to obtain new thieno[2,3-d]pyrimidine derivatives suitable for further rational search among them for means to fight against mycobacteria tuberculosis.

Materials and methods. Methods of organic synthesis and instrumental methods of chemical analysis (^{1}H NMR, etc.). Methods of virtual screening of organic molecules (molecular docking) were applied for prediction of biological activity.

Results and discussion. At the first stage, an intermediate thiourea was obtained from ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate available by the Gewald reaction when heated in pyridine. Further cyclization of thiourea into the key building block - 5,6-dimethyl-3-phenyl-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one was carried out by heating with three equivalents of sodium hydroxide in the media of ethanol (96%). Further modification was carried out by alkylation with benzyl chloride, α-bromoacetophenone and a number of 2-chloroacetamides. As a result, a number of 5,6-dimethyl-2-(alkylthio)-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one derivatives were obtained. Subsequently, docking studies of their binding to the active site of microbacterial TrmD inhibitors were conducted for the obtained compounds.

Conclusions. An effective procedure was developed that made it possible to obtain a number of potential antituberculosis agents, among which, according to docking studies into the active site of mycobacterial TrmD inhibitors, the leader is 2-(benzylthio)-5,6-dimethyl-3-phenylthieno[2,3-d]pyrimidine -4(3H)-one.

DETERMINATION OF AFFINITY OF TETRAZOLE DERIVATIVES TO MICROSOMAL PROSTAGLANDIN SYNTHASE

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Introduction. The problem of peptic ulcer disease occupies a central place in gastroenterology. Chronic recurrent course, polyetiological and pathogenicity, polymorphism of clinical manifestations, severe complications that characterize peptic ulcer disease, as well as determining the role of *Helicobacter pylori* in the development of the disease, determine the complexity of its successful