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MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
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QUALIFICATION WORK

on the topic: «**DESIGN OF MOLECULES WITH ANTIMICROBIAL PROPERTIES IN THE SERIES OF DERIVATIVES OF 1-BENZYL-3-PHENYL-THIENO[3,2-D]PYRIMIDINE-2,4-DIONE DERIVATIVES»**

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ANNOTATION

Design, synthesis and molecular docking of novel thieno[3,2-d]pyrimidine derivatives were performed to evaluate their affinity toward bacterial TrmD. It was found that compound 4.1 is a promising antibacterial agent against *P. aeruginosa* and *M. tuberculosis*. The thesis consists of 3 chapters, general conclusions, and a reference list (55 sources), presented on 48 pages and containing 11 figures.

Keywords: Thienopyrimidine, TrmD, molecular docking, antibacterial activity, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*

АНОТАЦІЯ

Проведено дизайн, синтез та молекулярний докінг нових похідних тієно[3,2-d]піримідину для оцінки їх афінності до бактеріального ферменту TrmD. Виявлено, що сполука 4.1 є перспективною як антибактеріальний агент проти *P. aeruginosa* та *M. tuberculosis*. Робота складається з 3 розділів, загальних висновків та списку використаної літератури (55 джерел), викладена на 48 сторінках, містить 11 рисунків.

Ключові слова: тієнопіримідин, TrmD, молекулярний докінг, антибактеріальна активність, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*

CONTENTS

LIST OF ABBREVIATIONS

ACP	Acyl carrier protein
CNS	Central nervous system
COX-2	Cyclooxygenase-2
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
EtOAc	Ethyl acetate
Hex	Hexane
HIV	Human immunodeficiency virus type
MIC	Minimum inhibitory concentration
NSAID	Non-Steroidal Anti-Inflammatory Drug
RNA	Ribonucleic Acid
SAM	S-adenosylmethionine
SAR	Structure–activity relationship
TLC	Thin-Layer Chromatography
TrmD	tRNA-(N^1G37)-methyltransferase
tRNA	Transfer ribonucleic acid
QcrB	quinol-cytochrome c reductase subunit B

INTRODUCTION

Relevance of the topic. The rise of antibiotic resistance is one of the most pressing challenges in modern medicine, leading to reduced effectiveness of bacterial infection treatments and an increase in mortality rates. Gram-negative bacteria, in particular *Pseudomonas aeruginosa*, and resistant strains of *Mycobacterium tuberculosis* pose a particular threat, requiring the development of new antibacterial agents with unconventional mechanisms of action.

One enzyme that has gained particular attention is tRNA-(N^1G37)-methyltransferase (TrmD) – a bacterial enzyme absent in humans, which is responsible for methylation of tRNA and is critical for pathogen viability. TrmD is considered a promising target for next-generation antimicrobial therapy due to its high selectivity potential and low toxicity to eukaryotic cells.

In the search for TrmD inhibitors, thienopyrimidine derivatives have emerged as attractive candidates. These heterocyclic scaffolds combine well-known pharmacophoric fragments and exhibit a broad spectrum of biological activity. Many thienopyrimidines are known to inhibit key microbial enzymes involved in DNA replication, protein synthesis, and nucleotide metabolism.

In this study, thieno[3,2-d]pyrimidine derivatives were selected as research objects. These compounds were modified at key positions of the heterocyclic core to evaluate the effect of substitution on TrmD inhibition. This choice is supported by the synthetic accessibility, structural versatility, and established antimicrobial potential of the thienopyrimidine scaffold, making it a promising foundation for the development of novel selective antibacterial agents capable of targeting resistant pathogens.

The purpose of the study. The aim of this study is to synthesize and computationally model thieno[3,2-d]pyrimidine derivatives to evaluate their potential as TrmD enzyme inhibitors in multidrug-resistant pathogens *P. aeruginosa* and *M. tuberculosis*, with a focus on the impact of structural substitutions on target affinity.

In order to achieve the objective, the following *tasks* had to be accomplished:

- To analyze and summarize literature data on the pharmacological activity of thieno[2,3-d]pyrimidine derivatives, particularly their antibacterial effects, and to evaluate pharmacophore features influencing biological activity.
- To design a series of target molecules based on SAR analysis and pharmacophore modeling principles.
- To synthesize thieno[3,2-d]pyrimidine derivatives modified at key positions of the heterocyclic core (N^1, C3, and C7).
- To characterize the obtained compounds using ^{1}H NMR spectroscopy and elemental analysis.
- To perform molecular docking studies of the target compounds with TrmD isolated from *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*.
- To analyze the ligand–protein interaction profiles and identify structure–activity relationships (SAR) relevant to TrmD binding affinity.
- To evaluate the potential of the synthesized compounds as multitarget antibacterial agents.

The object of the study. Design, synthesis and molecular docking of potential antibacterial agents.

The subject of the study. Thieno[2,3-d]pyrimidine derivatives, drug-design, affinity, TrmD enzymes

The methods of the study. Thieno[3,2-d]pyrimidine derivatives were synthesized using classical methods of organic chemistry. The structures of the compounds were confirmed by ^{1}H NMR spectroscopy and elemental analysis. Molecule design was performed in BIOVIA Draw 2017R2, and geometry optimization was carried out using Chem3D and HyperChem 7.5. Molecular docking was conducted using AutoDock Vina and AutoDockTools 1.5.6 with TrmD proteins (*P. aeruginosa*, *M. tuberculosis*). Interaction visualization was performed in Discovery Studio Visualizer 2017R2.

The practical value of the results. The results obtained are important for the early-stage development of new antibacterial agents aimed at overcoming antibiotic resistance. The designed thieno[3,2-d]pyrimidine derivatives demonstrated potential as inhibitors of TrmD – a promising bacterial target that is absent in the human organism. The use of computational methods in compound selection reduces time and resource costs in the search for active molecules.

The identified TrmD-affine compounds (*P. aeruginosa*, *M. tuberculosis*) can serve as scaffolds for further optimization, analogue development, and in vitro evaluation. Thus, this study opens a perspective for the creation of novel multitarget antimicrobial agents with enhanced selectivity and safety.

Elements of scientific research. A comprehensive study was conducted to address the urgent issue of antibiotic resistance by developing new antibacterial agents among thieno[3,2-d]pyrimidine derivatives. For the first time, novel compounds were synthesized, their physicochemical and spectral characteristics described, and their affinity to TrmD from *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* evaluated. A promising molecule – 1-benzyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione – was identified as a potential lead for the further development of multitarget antibacterial agents.

Structure and scope of the qualification work.

The paper consists of an introduction, three sections, conclusions, and a list of references. The work is presented on 48 pages and contains 11 figures. The list of the used literary sources contains 55 titles.

CHAPTER 1

ANTIBIOTIC RESISTANCE. PHARMACOLOGICAL ACTIVITY OF THIENO[2,3-D]PYRIMIDINE DERIVATIVES

(Literature review)

1.1. General description of the problem of microbial resistance to therapy

The problem of microbial resistance to therapy lies in the ability of pathogenic microorganisms – including bacteria, fungi, viruses, and parasites – to withstand the effects of therapeutic agents, leading to reduced or complete loss of treatment efficacy. This phenomenon arises from both natural processes, such as mutations and horizontal gene transfer, and anthropogenic factors, notably the excessive and irrational use of antimicrobial agents in medicine and veterinary practice [1].

The principal mechanisms underlying the development of therapeutic resistance include:

- structural modification of drug targets;
- activation of efflux systems that expel therapeutic agents from the cell;
- production of enzymes that inactivate drugs;
- reduction of cell wall permeability to therapeutic agents
- formation of biofilms that create a physical barrier to treatment [2].

The spread of resistant strains results in prolonged infections, more severe disease courses, increased mortality rates, the need to use more toxic or expensive drugs, and a significant economic burden on healthcare systems.

Overcoming this challenge requires a comprehensive, interdisciplinary approach, involving the development of novel therapeutic strategies, the implementation of antimicrobial stewardship programs, the strengthening of preventive measures, and robust surveillance of resistance trends [3].

1.2. Approaches to overcoming microbial resistance to therapy

Approaches to overcoming microbial resistance to therapy include a comprehensive set of measures at various levels – from molecular biology to healthcare systems (Fig. 1).

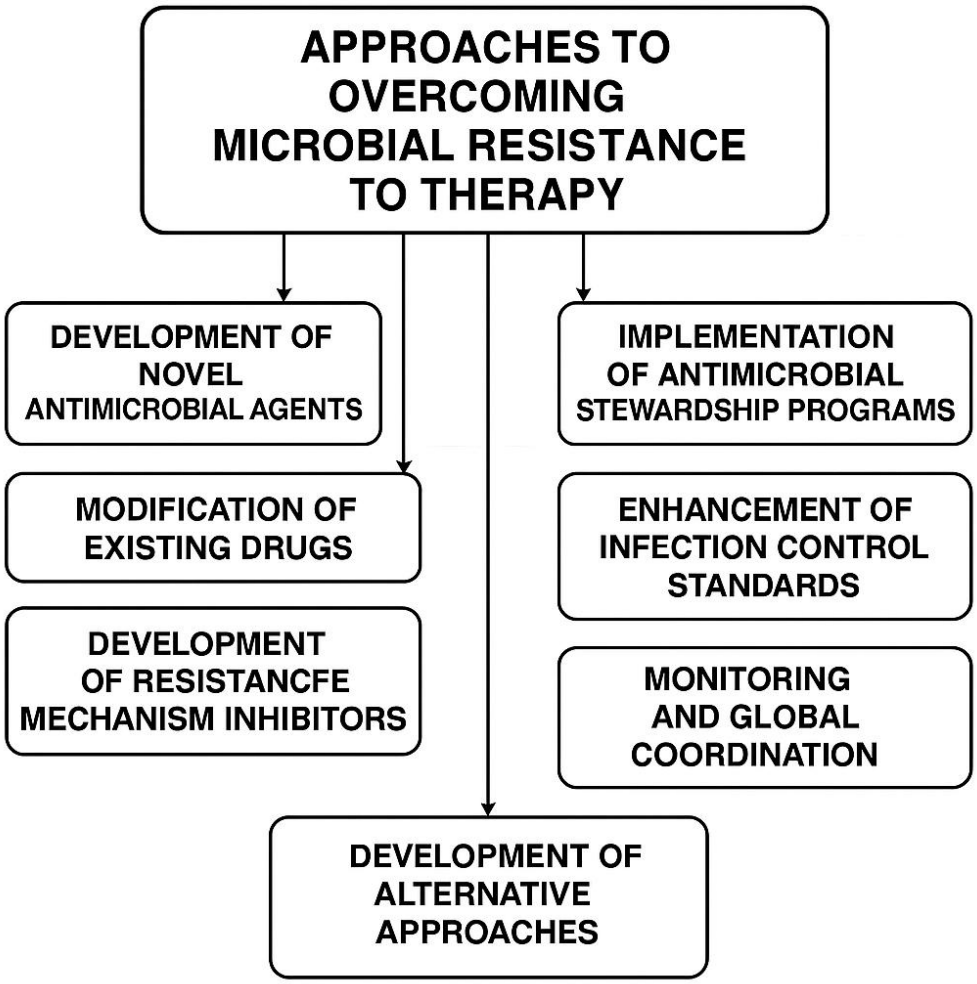


Fig.1.1. Scheme of ways to overcome microbial resistance to therapy

Development of new antimicrobial agents. The creation of new classes of antibiotics, antiviral, antifungal, and antiparasitic agents with fundamentally new mechanisms of action that can bypass existing resistance mechanisms.

Modification of existing therapeutic agents. Chemical optimization of known drugs to enhance their stability, permeability, or resistance to inactivation mechanisms.

Development of resistance mechanism inhibitors. The use of compounds that block efflux pumps or enzymes that degrade antimicrobial agents (e.g., beta-lactamase inhibitors) [4].

Combination therapy. The simultaneous use of multiple antimicrobial agents to reduce the likelihood of resistant mutant emergence and to achieve synergistic therapeutic effects.

Immunotherapy and vaccination. Enhancing the body's immune response using monoclonal antibodies, immunomodulators, or prophylactic vaccines to reduce infection rates and the need for antimicrobial therapy [5].

Development of alternative approaches. Application of phage therapy, antimicrobial peptides, CRISPR-based technologies, probiotics, and postbiotics as alternatives or complements to conventional therapies [6].

Implementation of antimicrobial stewardship programs. Rational use of antimicrobial agents in clinical practice, optimization of therapy duration and dosing, and prevention of unnecessary prescriptions.

Enhancement of infection control standards. Strengthening hygiene measures in hospitals and other healthcare facilities to prevent the spread of resistant strains.

Surveillance and global coordination. Establishment of effective surveillance systems for monitoring resistance trends, sharing data across countries, and promoting international collaboration to rapidly identify and respond to emerging threats [7].

1.3. Thienopyrimidine derivatives as pharmacologically active substances

1.3.1. General characteristics of pharmacological activity

The search for new biologically active substances that would exhibit selectivity of action toward a biotarget and have low toxicity remains a pressing issue today. The joining of a pyrimidine ring with a thiophene ring can result in three different thienopyrimidine arrangements: thieno[2,3-d]pyrimidines, thieno[3,2-d]pyrimidines, and thieno[3,4-d]pyrimidines (Fig. 1.2). All three structural forms of thienopyrimidine have been the subject of scientific investigation, and numerous derived compounds have exhibited a wide array of biological effects, including anticancer, antioxidant, and central nervous system (CNS) protective properties. Certain compounds are currently undergoing clinical evaluation while others have

achieved market approval (e.g., Relugolix, a gonadotropin-releasing hormone (GnRH) receptor antagonist) [8].

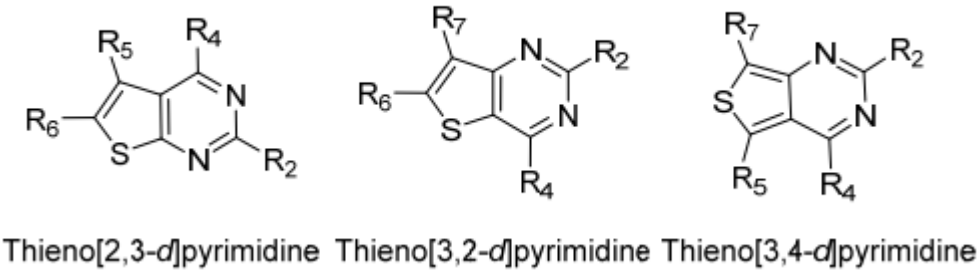


Fig. 1.2. Structure of thienopyrimidines [9]

Compounds with a thienopyrimidine skeleton, in particular thieno[2,3-d]pyrimidines, occupy a special place [9], attracting the attention of scientists around the world due to the possibility of diverse functionalization and various directions of biological activity, the main ones of which are shown in Fig. 1.3.

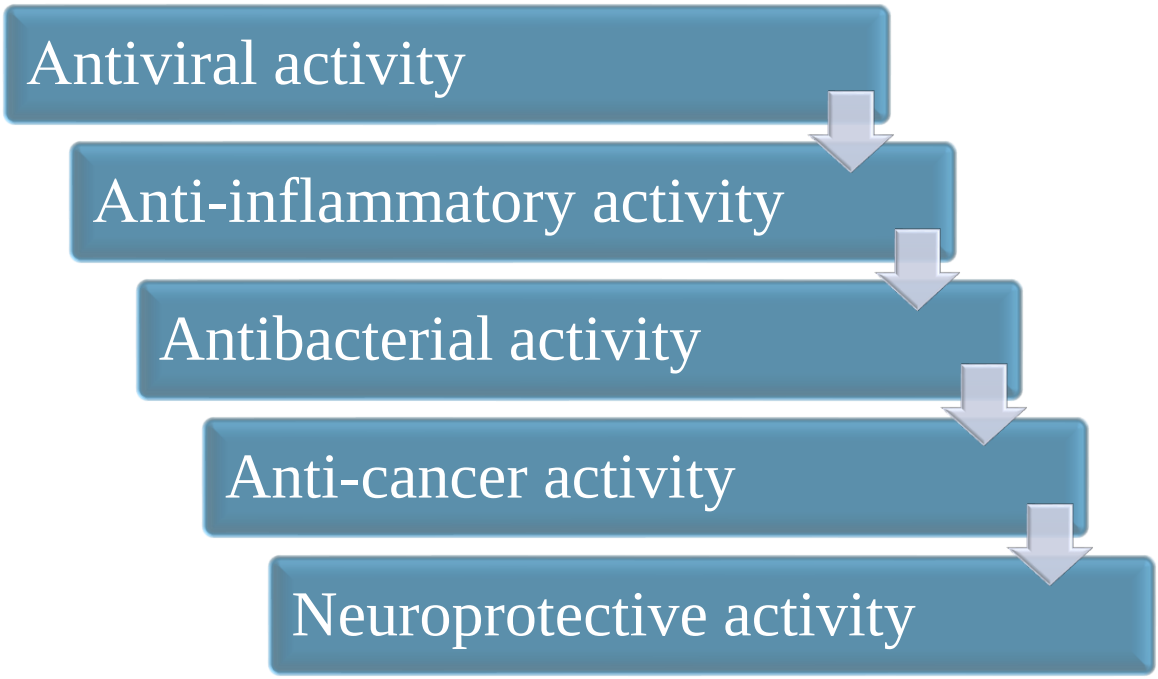


Fig.1.3. Pharmacological activity of thienopyrimidine derivatives

Thienopyrimidine derivatives are of considerable interest in the development of new antimicrobial agents due to their wide range of biological activities, including

antibacterial, antifungal, antiviral and antiparasitic effects. Their structural diversity allows them to interact with different targets in microorganisms, making them promising candidates to combat the growing problem of antimicrobial resistance.

The mechanisms of antimicrobial action of thienopyrimidine derivatives can include:

1. Inhibition of microbial cell enzymes. Many thienopyrimidine derivatives exhibit antimicrobial activity by inhibiting key enzymes essential for the viability of microorganisms. These can include enzymes involved in nucleic acid synthesis (e.g., DNA gyrase, topoisomerase), protein synthesis (e.g., ribosomal enzymes), metabolic pathways (e.g., enzymes of folic acid synthesis), or cell wall synthesis [10].
2. Interaction with nucleic acids. Some thienopyrimidine derivatives are capable of intercalating between DNA or RNA strands, disrupting their structure and function, leading to the inhibition of replication and transcription [11].
3. Disruption of cell membrane structure and function. Certain compounds in this series can interact with the lipid bilayer of microbial cell membranes, altering their permeability and disrupting ion balance, leading to cell death [12].
4. Inhibition of biofilm biosynthesis. Biofilm formation is a crucial virulence factor for many bacteria and fungi, as they provide protection against antimicrobial agents and the immune system. Some thienopyrimidine derivatives have shown the ability to inhibit biofilm formation or disrupt pre-formed biofilms [13].

Advantages of thienopyrimidine derivatives as antimicrobial agents:

- Broad spectrum of activity. Many compounds exhibit activity against Gram-positive and Gram-negative bacteria, fungi, viruses, and parasites [9].
- Structural diversity. The thienopyrimidine core provides a flexible platform for the introduction of various substituents, allowing for fine-tuning of the pharmacological properties and spectrum of activity of the compounds [9].

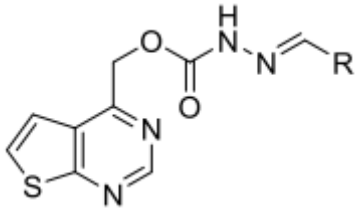
- Potential to overcome resistance. Research indicates that some thienopyrimidine derivatives can be active against microorganisms resistant to existing antibiotics [9].
- Potential for developing multitarget agents. Due to their structural flexibility, thienopyrimidine derivatives can be designed to simultaneously target multiple pathways in microorganisms, a promising approach for combating resistance [9].

1.3.2. Thienopyrimidines with antituberculosis activity

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a global health challenge, with 10 million cases and 1.5 million deaths reported in 2020. Vulnerable populations include those with weakened immune systems (e.g., HIV-infected individuals). While treatable, multi-drug-resistant TB (MDR-TB) remains a serious threat, infecting around 206,000 people in 2019 [14].

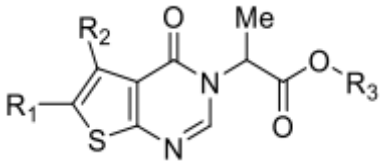
Rashmi and colleagues synthesized a series of thieno[2,3-d]pyrimidine derivatives and evaluated their antitubercular activity against the *Mycobacterium tuberculosis* H37Rv strain by determining the minimum inhibitory concentration (MIC). The structure–activity relationship (SAR) analysis revealed that the introduction of electron-donating substituents, particularly at the para- or ortho-positions of the phenyl ring, significantly enhanced antitubercular efficacy. Specifically, compounds **1.1** exhibited markedly improved activity, with MIC values ranging from 32 to 71 μM, compared to the unsubstituted compound 1.1a, which showed a much higher MIC of 320 μM. The antitubercular activity of these derivatives was comparable to that of the reference drug pyrazinamide (MIC ≈ 60.97 μM), suggesting that the thieno[2,3-d]pyrimidine scaffold is a promising platform for further optimization. Moreover, the incorporation of a bulkier 3,4,5-trimethoxyphenyl group, as in compound **1.1g**, was well tolerated without significant loss of activity. Cytotoxicity assessments indicated that the most active compounds exhibited low toxicity toward the THP-1 human monocytic cell line,

suggesting a favorable therapeutic index and supporting their potential as candidates for further preclinical development as novel antituberculosis agents [15].



1.1 a-g

Ananthan et al. conducted a high-throughput screening campaign involving 100,997 compounds to identify novel agents with activity against *Mycobacterium tuberculosis* H37Rv. Through this large-scale screening, several thienopyrimidinone derivatives, specifically compounds 1.2 a–e, were identified as exhibiting moderate to high levels of antimycobacterial activity. Despite these promising initial findings, the ability to draw definitive conclusions regarding the SAR of these compounds were limited. This limitation was primarily attributed to the relatively small number of active thienopyrimidine derivatives identified within the screened library, as well as the absence of reported IC_{90} values (90% inhibitory concentration) for standard reference drugs, which impeded direct comparisons of potency and efficacy. Nevertheless, the results highlight the potential of thienopyrimidinone scaffolds as starting points for the development of new antituberculosis therapeutics [16].

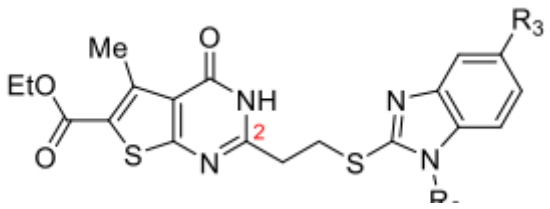


1.2 a-e

1.3.3. Thienopyrimidines with antihelminthic activity

Recent studies have increasingly focused on the discovery of novel antihelminthic agents, particularly those targeting infections caused by helminths such as *Trichinella spiralis*. Trichinellosis is a parasitic disease that arises when the larvae of *T. spiralis* invade and encyst within the muscular tissues of the infected

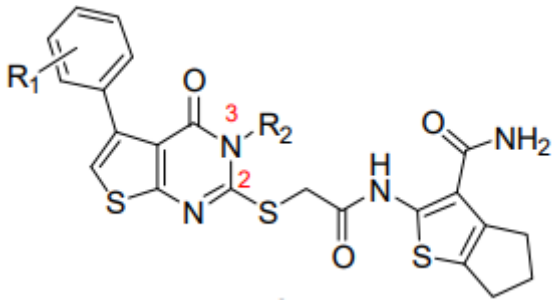
host, leading to significant morbidity. In an effort to address the need for new therapeutic options, Mavrova et al. reported the synthesis and biological evaluation of a series of thieno[2,3-d]pyrimidine derivatives as potential antihelminthic agents [17]. Their investigation highlighted the critical importance of specific structural features for bioactivity, notably the substitution at the 2-position of the thienopyrimidine scaffold. The replacement of the native alkyl chain with a benzimidazole moiety at this position was found to be essential for significant antihelminthic activity. Among the compounds tested, the derivative **1.3a** exhibited the highest efficacy, demonstrating a fivefold improvement in activity against *T. spiralis* larvae after 48 hours of incubation when compared to albendazole, a clinically used standard antihelminthic agent selected by the authors as the reference. Furthermore, the introduction of a sulfide linker between the ethyl chain and the benzimidazole ring was explored; this modification was generally well tolerated, with compounds 1.3 a and 1.3c achieving 59.75% and 80.05% efficacy after 48 hours, respectively. However, it was noted that compound 1.3 c showed a lack of activity in vitro, suggesting that additional factors may influence biological performance. Beyond their antihelminthic properties, further in vivo studies revealed that these thieno[2,3-d]pyrimidine derivatives also exhibited antiprotozoal activity against *Lamblia muris*, indicating their potential as promising lead structures for the development of broad-spectrum antiparasitic therapies. The findings of this study not only underscore the structural requirements necessary for antihelminthic efficacy but also open avenues for the rational design of multifunctional antiparasitic agents based on the thieno[2,3-d]pyrimidine core [17].



1.3 a-c

1.3.4. Thienopyrimidines with antimalarial activity

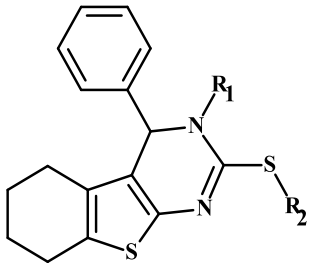
The growing resistance of malaria parasites to current medications, such as artemisinin-based drugs, poses a significant threat to public health. Consequently, the creation of new antimalarial treatments is a critical and immediate need [18]. Zhu and colleagues synthesized a small collection of thieno[2,3-d]pyrimidine compounds designed to inhibit falcipain-2 [19]. Falcipain-2 (FP-2), a cysteine protease found in *Plasmodium falciparum*, is a key enzyme responsible for breaking down hemoglobin in the parasite's erythrocytic trophozoite stage [19]. Blocking FP-2 prevents this hemoglobin digestion, halting the parasite's development. Therefore, the FP-2 enzyme is considered a promising target for the development of antimalarial drugs. Enzyme activity tests demonstrated that the entire series of synthesized compounds had the potential to inhibit FP-2. The degree of inhibition by these compounds ranged from 53.0 to 94.3% at a concentration of 10 μM (Table 1). Existing FP-2 inhibitors described in scientific literature are typically peptide-like molecules with very high potency, showing IC50 values in the nanomolar range. In contrast, the compounds synthesized by Zhu et al. showed moderate activity, with IC50 values in the micromolar range. The IC50 values of these compounds against FP-2 indicated that having an allyl, cyclohexyl, or a phenyl group with a substituent at the para- or meta-position at the third position of the molecule was acceptable for maintaining activity (compounds 1.4). The presence of a para-chloro-phenyl or a benzyl group at this position resulted in a slight decrease in potency.



1.4

1.3.5 Thienopyrimidines with anti-inflammatory activity

In 1991, Vega and co-workers reported the design and synthesis of 4-phenyl-thioxobenzo[4,5]thieno[2,3-d]pyrimidine compounds of general formula 1.5, which act as thiophene bioisosters of the non-steroidal anti-inflammatory drugs (NSAIDs) procurasone and ciproquasone [20].

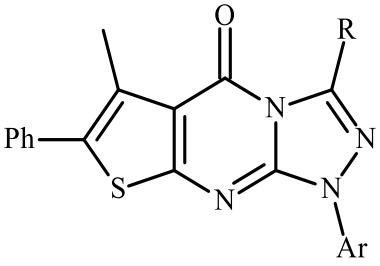


1.5

In 1994, the same research group conducted more extensive investigations into their anti-inflammatory properties. This subsequent study aimed to quantify the anti-inflammatory efficacy of the most promising compounds identified in the preliminary screening. Furthermore, the researchers extended their evaluation to explore potential adverse effects of these active analogs, specifically focusing on their impact on the CNS, the gastric mucosa (lining of the stomach), and primary hemostasis (the initial process of blood clot formation). The ability of these compounds to inhibit carrageenan-induced edema, granuloma formation, arthritis, and phorbol ester-induced ear edema was assessed across all synthesized derivatives. For comparative analysis of anti-inflammatory potency, piroxicam, aspirin, and ibuprofen were employed as standard NSAIDs. The tested compounds demonstrated the most significant activity in derivatives that exhibited anti-inflammatory effects comparable to aspirin, but were less potent than both piroxicam and ibuprofen in these in vivo models of inflammation [20].

In 2007, El-Gazzar and co-workers reported the synthesis of a series of triazolothieno[2,3-d]pyrimidine derivatives, represented by general formulas 1.6 and 1.7. Their study involved a comprehensive pharmacological evaluation,

specifically assessing their analgesic (pain-relieving), anti-inflammatory (inflammation-reducing), and ulcerogenic (ulcer-causing potential) activities. This evaluation was conducted in direct comparison to the well-established nonsteroidal anti-inflammatory drugs indomethacin and aspirin, which served as reference standards for both efficacy and safety profiles. The aim of this research was to identify novel compounds within the triazolothienopyrimidine class that could offer comparable or superior analgesic and anti-inflammatory effects while potentially exhibiting a reduced risk of gastrointestinal side effects, a common concern with traditional NSAIDs like indomethacin and aspirin. The study employed various in vivo models to assess these different pharmacological activities, providing a detailed understanding of the potential therapeutic value and safety liabilities of the synthesized compounds [21].



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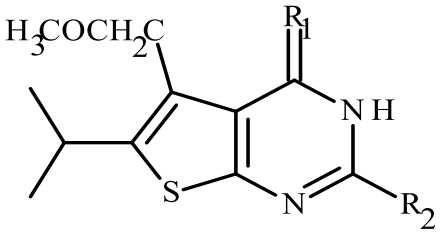


1.7

In 2016, El-Sayed et al. synthesized a new series of thieno[2,3-d]pyrimidine steroid heterocyclic compounds. The design and SAR studies of the synthesized compounds are illustrated in Fig. 1.4 [22].

Steroid-based chemotherapeutic agents possess several significant advantages, including high bioavailability, low toxicity, and reduced susceptibility to multidrug resistance (MDR). These benefits can be attributed to their ability to effectively penetrate cell membranes and undergo predictable and controlled one- or two-step *in vivo* transformation into inactive metabolites via a known enzymatic deactivation process [23].

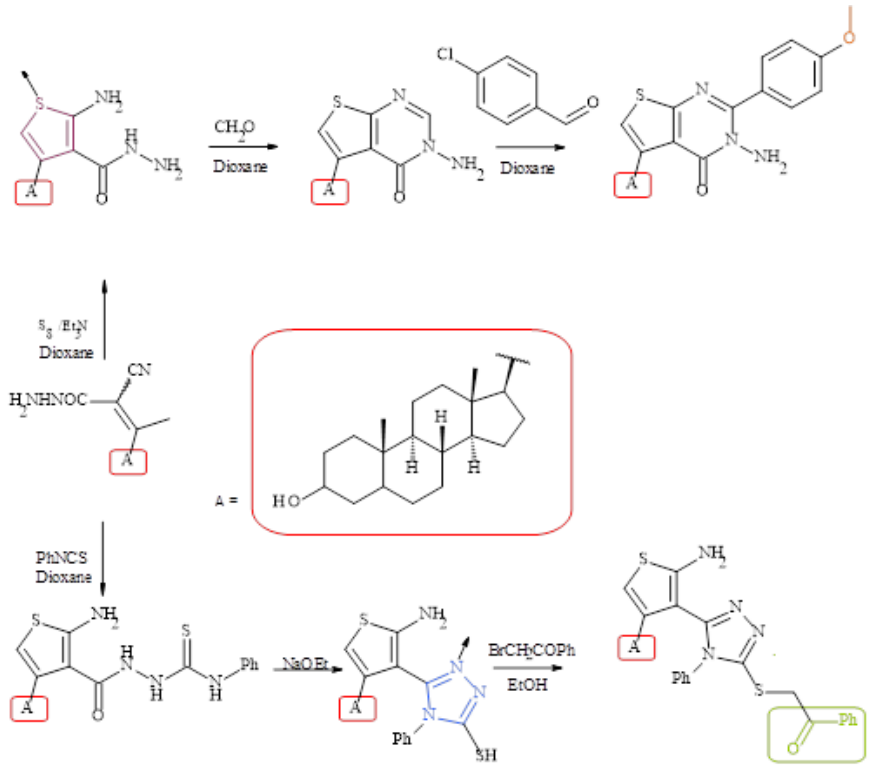
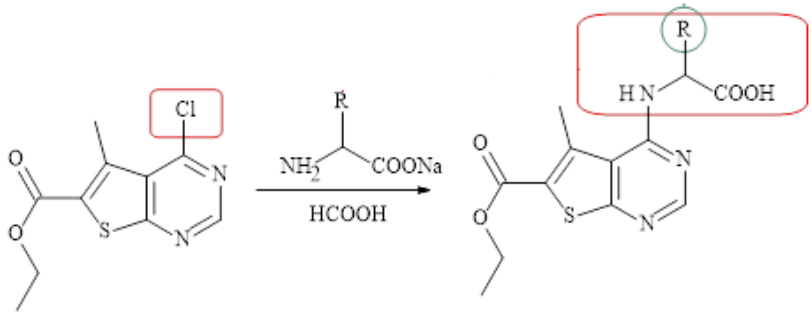


Fig. 1.4. Design and SAR study of steroidal thieno[2,3-d]pyrimidine derivatives

In 2016, Zaher et al. presented a synthetic approach for thieno[2,3-d]pyrimidine scaffolds linked to various terminal amino acid moieties. The resulting products were tested for their post-irradiation protective effect on young rats. The researchers synthesized a series of these novel compounds and tested their ability to mitigate the harmful effects of gamma radiation in young rats. The key finding was that many of these synthesized derivatives demonstrated a significant protective effect, suggesting their potential as radioprotective agents [24].The study also delved into the possible mechanism of action. The researchers proposed that the protective effects might be mediated through the regulation of a key inflammatory pathway involving nuclear factor kappa B (NFκB). NFκB is a transcription factor that plays a crucial role in inflammatory responses. Following irradiation, its activity can lead to the upregulation of pro-inflammatory cytokines like TNF-α and IL-6, as well as enzymes like COX-2 (involved in inflammation and pain) and the CYP2E1 gene (involved in the metabolism of certain compounds, and its upregulation can contribute to oxidative stress) [25]. The experimental data supported this proposed

mechanism by showing that γ-irradiation led to a significant increase in the pro-inflammatory cytokines TNF-α and IL-6 in the control group. However, the amino acid-linked thienopyrimidine derivatives appeared to counteract this effect, suggesting a role in modulating the inflammatory response triggered by radiation. Furthermore, the study highlighted the importance of the amino acid component, as derivatives containing amino acid moieties (general formula 1.8) showed a more pronounced protective effect compared to a derivative without an amino acid (compound 1.9). This structure-activity relationship suggests that the incorporation of amino acids into the thienopyrimidine scaffold enhances its radioprotective properties. The overall conclusion is that these novel amino acid-containing heterocyclic derivatives hold promise as potential therapeutic agents for protecting against organ damage caused by γ-irradiation [25].

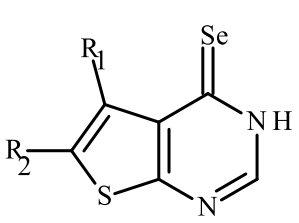


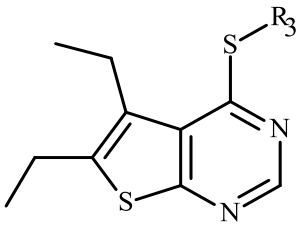
1.8 **1.9**

1.3.6. Thienopyrimidines with antimicrobial activity

Over the past three years, the thieno[2,3-d]pyrimidine scaffold has attracted significant attention from medicinal chemists due to its promising potential in the development of new effective antimicrobial agents. This heterocyclic system serves as a structural basis for the design of various series of biologically active compounds exhibiting high activity against a broad spectrum of pathogenic microorganisms [26].

In 2015, Kolomieitsev et al. reported the synthesis of new derivatives of thieno[2,3-d]pyrimidine, namely R¹, R²-substituted thieno[2,3-d]pyrimidin-4(3H)-ones, thiones, and selenones. Structural modifications of these compounds allowed the generation of series of derivatives with different sets of functional groups, which significantly influenced their biological activity. All synthesized compounds with general structures 1.1 and 1.2 were subjected to biological screening to evaluate their antifungal and antimicrobial activities [27]. Compounds 1.1 and 1.2 demonstrated notable antimicrobial activity against *E. coli*, *S. aureus*, and *M. luteum*. Among them, compound 1.1 exhibited the most pronounced fungistatic effect against *A. niger*, producing inhibition zones of 22.0 mm and 12.0 mm at concentrations of 0.5% and 0.1%, respectively. The minimum inhibitory concentration (MIC) for both compounds was determined to be 31.2 μg/mL [27].





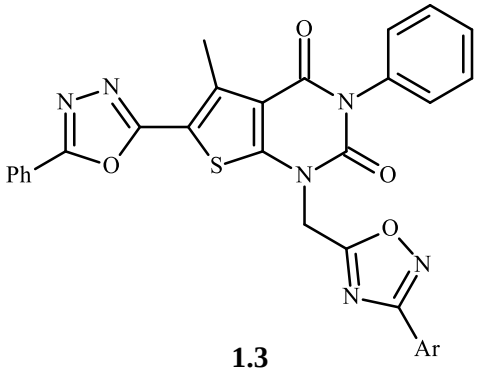
1.1 **1.2**

The assessment of antimicrobial properties was carried out using the agar well diffusion method, one of the classical and widely used techniques for the primary determination of the biological activity of compounds. The compounds were tested against several strains of Gram-positive and Gram-negative bacteria, as well as certain pathogenic fungi. The study results demonstrated that certain derivatives exhibited strong inhibitory activity against microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, confirming the potential of the thieno[2,3-d]pyrimidine scaffold as a promising foundation for the development of new antimicrobial agents [27]. Thus, the modification of the thieno[2,3-d]pyrimidine core enables targeted influence on the spectrum and degree of

antimicrobial activity, opening opportunities for further optimization of such compounds towards enhanced bioavailability, reduced toxicity, and the development of agents with novel mechanisms of action [27].

Additionally, in 2015, Vlasov et al. introduced a new group of derivatives based on the 5-methyl-3-phenyl-6-(5-phenyl-1,3,4-oxadiazol-2-yl)thieno[2,3-d]pyrimidine-2,4-dione scaffold. These synthesized compounds, corresponding to the general structure 1.23, were subjected to biological screening to assess their potential as antimicrobial agents [28]. The antimicrobial activity of the obtained derivatives was evaluated against several bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*.

The results demonstrated that these compounds exhibited significant inhibitory effects against the tested microorganisms. In particular, the zones of microbial growth inhibition observed for the tested compounds were comparable to those produced by well-known standard antibiotics such as streptomycin and metronidazole. Such findings suggest that the introduction of the 1,3,4-oxadiazole moiety into the thieno[2,3-d]pyrimidine core may significantly enhance antimicrobial properties. This highlights the potential of these structures as promising candidates for the development of new antimicrobial therapeutics targeting resistant bacterial strains [28].

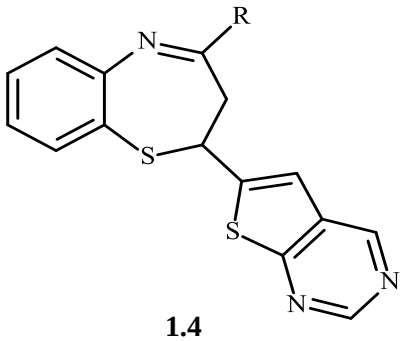


1.3

In 2017, Prabhakar et al. synthesized a new series of benzo[b][1,5]thiazepine derivatives incorporating a thieno[2,3-d]pyrimidine core, with the general structure 1.4. The antimicrobial activity of these compounds was evaluated using the agar diffusion method. The screening results demonstrated that the synthesized

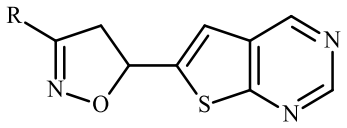
compounds exhibited notable activity against two Gram-positive bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis*, as well as two Gram-negative bacterial strains, *Pseudomonas aeruginosa* and *Escherichia coli* [29].

Furthermore, significant antifungal activity was observed against *Candida albicans*. In several cases, the antimicrobial and antifungal activities of the tested compounds were found to be comparable to, or even higher than, those of standard reference drugs such as ampicillin and nystatin. These findings suggest that the introduction of the benzo[b][1,5]thiazepine moiety into the thieno[2,3-d]pyrimidine scaffold can considerably enhance the biological profile of the resulting compounds, making them promising candidates for the development of new broad-spectrum antimicrobial and antifungal agents [29].



1.4

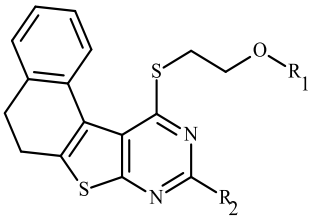
Throughout 2017, the same research group reported the synthesis of various series of isoxazoline derivatives incorporating the thieno[2,3-d]pyrimidine core. All synthesized compounds, corresponding to the general structure 1.38, were evaluated for their antimicrobial activity [30]. The biological screening revealed that the derivatives exhibited good antimicrobial efficacy against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. The antimicrobial activity was assessed using amoxicillin as the reference standard. Sensitivity was determined based on the measurement of growth inhibition zones against both Gram-positive and Gram-negative bacterial strains. The results confirmed that the introduction of the isoxazoline moiety into the thieno[2,3-d]pyrimidine framework can significantly enhance the antibacterial properties of the molecules, suggesting their potential for further development as effective antimicrobial agents [30].



1.38

1.3.7. Thienopyrimidines with antiviral activity

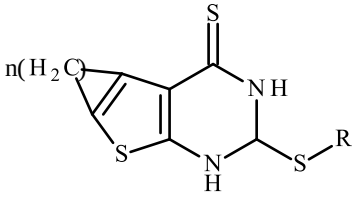
In 2006, Rashad et al. synthesized several cyclic thieno[2,3-d]pyrimidine nucleoside derivatives. The synthesized compounds, represented by the general structure 1.43, were evaluated for their inhibitory effects against herpes simplex virus type 1 (HSV-1) and hepatitis A virus (HAV) [31]. The screening assay was designed to determine the virokidal activity, adsorption and effect on virus replication. Compound 1.43 demonstrated the highest activity against HSV-1. On the other hand, the HAV virus was resistant to all tested compoundsThe biological screening was conducted to assess the potential of these derivatives as antiviral agents. The evaluation focused on measuring the ability of the compounds to inhibit viral replication in infected cell cultures. Although detailed quantitative data on antiviral potency were not disclosed, the study indicated that some of the synthesized nucleoside analogues exhibited promising inhibitory activity, suggesting that the incorporation of the thieno[2,3-d]pyrimidine scaffold into nucleoside structures could be a valuable strategy for the development of new antiviral therapies [31].



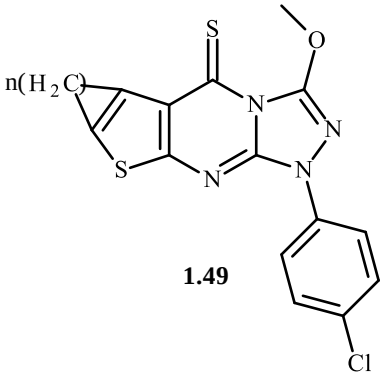
1.43

In 2010, Hafez et al. synthesized a new series of thienopyrimidine derivatives, specifically thieno[2,3-d]pyrimidine-2,4-dithione derivatives. The newly synthesized compounds, corresponding to general structures 1.48 and 1.49, were evaluated for their antiviral activity *in vitro* against herpes simplex virus type 1

(HSV-1) and human immunodeficiency virus type 1 (HIV-1) [32]. The biological screening involved testing the ability of the compounds to inhibit viral replication in infected cell lines. Preliminary results indicated that several of the synthesized derivatives exhibited promising antiviral activity, suggesting that modifications of the thieno[2,3-d]pyrimidine core, particularly the incorporation of dithione functionalities, may contribute positively to antiviral efficacy. These findings highlight the potential of thieno[2,3-d]pyrimidine-based structures as valuable scaffolds for the development of new antiviral agents targeting a broad range of viral infections [32].



1.48



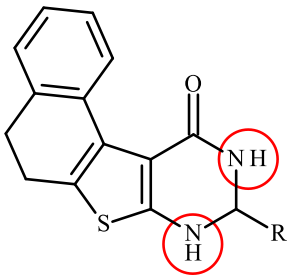
1.49

To determine the cytotoxic activity of the studied compounds, Vero cells were cultured. Cytotoxicity was assessed as the concentration causing approximately 50% loss of the monolayer surrounding plaques induced by HSV-1. Acyclovir, an antiviral, antimitotic, and antibiotic drug, was used as a control. Among the tested compounds, those containing dithione (2C=S groups) exhibited the highest cytotoxicity, with IC50 values < 0.1 μM/L. In contrast, S-glycosides, particularly S-b-D-arabinofuranosylthienopyrimidine derivatives, showed the lowest cytotoxic effect (IC50 > 1.5 mM/L).

On the other hand, the anti-HIV-1 activity of the investigated compounds was determined by their ability to protect T4 lymphocytes from death induced by HIV-1. The median effective concentration (EC50) of the tested compounds in infected cells was compared to their cytotoxic effect (IC50) in uninfected cultures, where these compounds served as controls. Zidovudine was used as a positive control. The obtained results showed that only 2,4-dithione derivatives exhibited slight activity

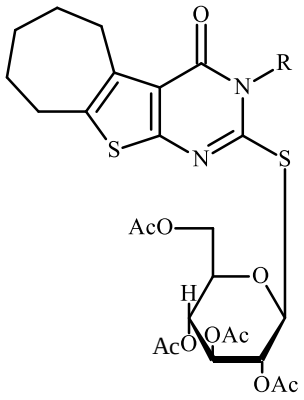
against HIV-1 with EC50 values of 6.5 and 5.25 mM/L, respectively. The remaining tested compounds did not show significant activity. Their weak activity is attributed to the absence of substitution at the 2-position of the thieno[2,3-d]-pyrimidine core, which negatively affects antiviral activity against HIV [33].

In 2010, Rashad et al. successfully synthesized a series of novel derivatives containing the thieno[2,3-d]pyrimidine ring system [34]. These newly obtained compounds, characterized by general formula 1.56, were subsequently evaluated for their inhibitory activity against the H5N1 strain of the avian influenza virus, commonly known as bird flu. The LD50 and EC50 were determined and confirmed using a plaque reduction assay on MDCK cells. The obtained data indicated that the compounds were the safest and most effective derivatives with high therapeutic indices. Zanamivir was used as a standard drug [34].

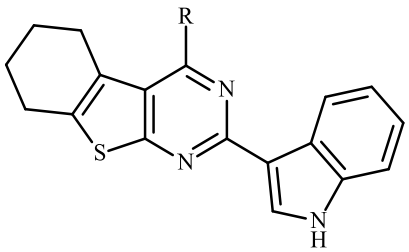


1.56

In 2013, Hassan et al. [35] reported the synthesis of a novel series of thioglycoside derivatives of the thieno[2,3-d]pyrimidine scaffold, characterized by general formula 1.61. To evaluate the antiviral activity of these compounds against Hepatitis A virus (HAV), a plaque reduction assay was conducted. Among the investigated derivatives demonstrated the most significant effect against Hepatitis A virus at a concentration of 40 µg/mL [35].



In 2016, AbdEl-All et al. synthesized and characterized a novel series of thieno[2,3-d]pyrimidines incorporating pyrazole, pyrrolidine, and/or indole rings. These new compounds, represented by general formula 1.66, were subsequently evaluated for their antiviral activity against the neuraminidase enzyme of influenza A virus [36]. The obtained data demonstrated that all tested compounds exhibited significant inhibitory effects against the neuraminidase (NA) enzyme of influenza A (H3N2) virus. Notably, compounds featuring a pyrrolidine-2,5-dione core showed the highest efficacy against neuraminidase (NA), with IC50 values of 0.021 μM and 0.067 μM.



1.66

Conclusions for Chapter 1:

This chapter summarizes the pharmacological potential of thienopyrimidine-based compounds. A comprehensive review of the literature was performed to organize and evaluate current knowledge regarding their anti-inflammatory, antimicrobial, and antiviral activities. Additionally, data on their influence on the central nervous system, gastric mucosa, and primary hemostasis were examined—factors essential for assessing their potential toxicity and safety. Thienopyrimidine

derivatives emerge as strong candidates in the search for new antimicrobial drugs, owing to their structural variability and wide-ranging biological activity. Continued research in this field could contribute to the development of effective and safe therapeutics in response to the growing challenge of antimicrobial resistance.

CHAPTER 2

SELECTION OF RESEARCH OBJECTS AND SYNTHESIS OF TARGETED THIENO[2,3-D]PYRIMIDINE DERIVATIVES

2.1. Justification for the selection of research objects

Thieno[2,3-d]pyrimidine derivatives have established themselves as a promising class of heterocyclic compounds with a broad spectrum of antimicrobial activity, as evidenced by numerous literature sources. These structures have attracted considerable scientific interest due to their ability to act against both Gram-positive and Gram-negative microorganisms, including certain strains resistant to conventional antibacterial agents.

A significant contribution to the development of this research area has been made by scientists at the National University of Pharmacy under the supervision of Doctor of Pharmaceutical Sciences, Professor Sergii Vlasov and Hanna Severina. Within the scope of their work, a range of novel thieno[2,3-d]pyrimidine derivatives were synthesized and evaluated for antimicrobial properties. Notably, high biological activity was observed among 4-carboxamide derivatives of general structure I (Figure 1) [37], as well as triazole-fused analogs of type II [38].

Particular scientific interest is also associated with 6-heteryl-substituted thieno[2,3-d]pyrimidines, including derivatives bearing 1,3,4-oxadiazole (III) [39], 2-aminothiazole (IV) [40], and 1,3-benzoxazole rings – both in the form of thieno[2,3-d]pyrimidin-4-ones and thieno[2,3-d]pyrimidin-4-thiones (V) [41].

The choice of thieno[2,3-d]pyrimidine derivatives as target compounds for this study is grounded in their proven potential as scaffolds with diverse biological activities, particularly antimicrobial, antiviral, anticancer, and anti-inflammatory effects. Their structural versatility, which allows for various modifications at different positions of the heterocyclic core, makes them an attractive platform for medicinal chemistry research aimed at developing new pharmacologically active agents.

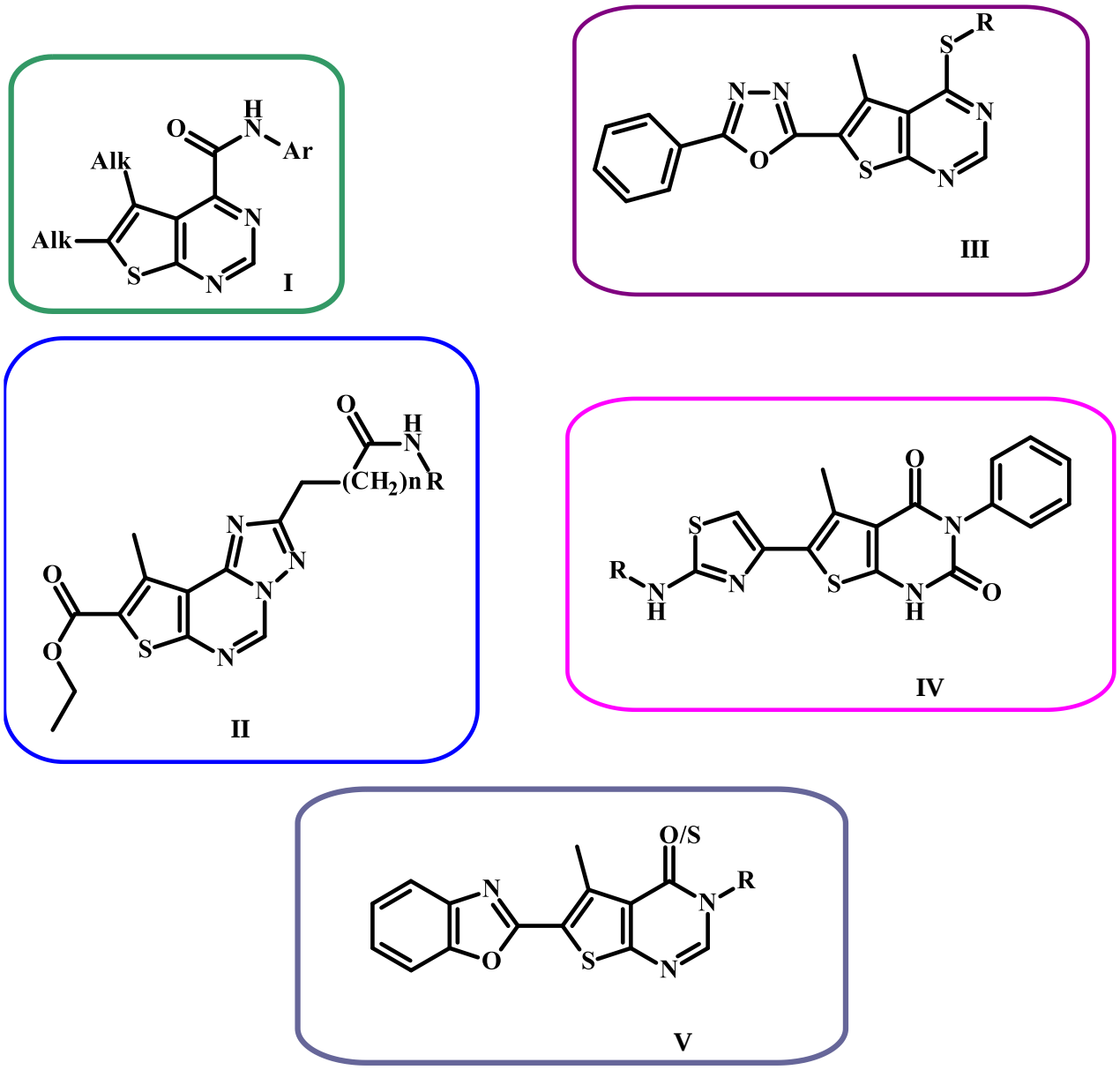


Fig. 2.1. Thieno[2,3-d]pyrimidine derivatives with antimicrobial properties synthesized in NUPh

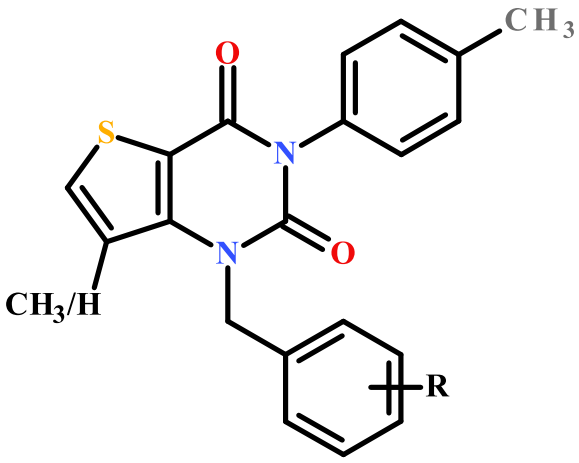
A thorough review of the scientific literature indicates that even slight alterations in the substituents of thieno[2,3-d]pyrimidine molecules can significantly influence their pharmacological profile and target specificity. Such derivatives can interact with multiple biological targets, including enzymes, receptors, and nucleic acids, which supports the concept of their potential multi-target mechanism of action – a highly desirable feature for the treatment of resistant infections and complex pathologies.

In light of these considerations, the synthesis and pharmacological evaluation of novel thieno[2,3-d]pyrimidine derivatives were identified as a rational and promising direction for the present research.

Continuing a series of experiments aimed at discovering novel antimicrobial agents, a set of virtual compounds based on the thienopyrimidine scaffold was designed for further targeted synthesis of potential antibacterial candidates. This design was guided by SAR analysis of previously obtained data.

It is well known that the outer envelope of Gram-negative microorganisms consists of an outer membrane covered with lipopolysaccharides, a thin layer of peptidoglycan, and an inner cytoplasmic membrane. This complex structure serves as an effective barrier against the penetration of exogenous substances and is a major contributor to the intrinsic nonspecific resistance of such bacteria to antibiotics [42].

Diffusion across the membrane is feasible primarily for highly lipophilic molecules. Therefore, in designing the target thienopyrimidines, a 4-methylphenyl group was introduced at position 3 to enhance lipophilicity, along with a benzyl substituent at position 1. Additionally, the potential influence of a methyl group at position 7 of the thieno[3,2-d]pyrimidine core was considered. The structures of the designed target compounds are presented are presented below :



Target compounds

2.2. Synthesis of targeted derivatives of 1-benzyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione

The synthesis of two key intermediates, 3-(p-tolyl)-thieno[3,2-d]pyrimidine-2,4-dione (3.1 and 3.2), was carried out by reacting methyl 3-aminothiophene-2-carboxylate (1.1) or methyl 3-amino-4-methylthiophene-2-carboxylate (1.2) with 1-isocyanato-4-methylbenzene (2) in DMF at 80 °C for 2 hours. (Fig. 2.2)

Regarding the reaction mechanism, the interaction between methyl 3-amino-thiophene-2-carboxylate (1.1, 1.2) and 1-isocyanato-4-methylbenzene (2) leads to the formation of a carbamoyl intermediate, which subsequently undergoes cyclization to yield a thieno[3,2-d]pyrimidine derivative.

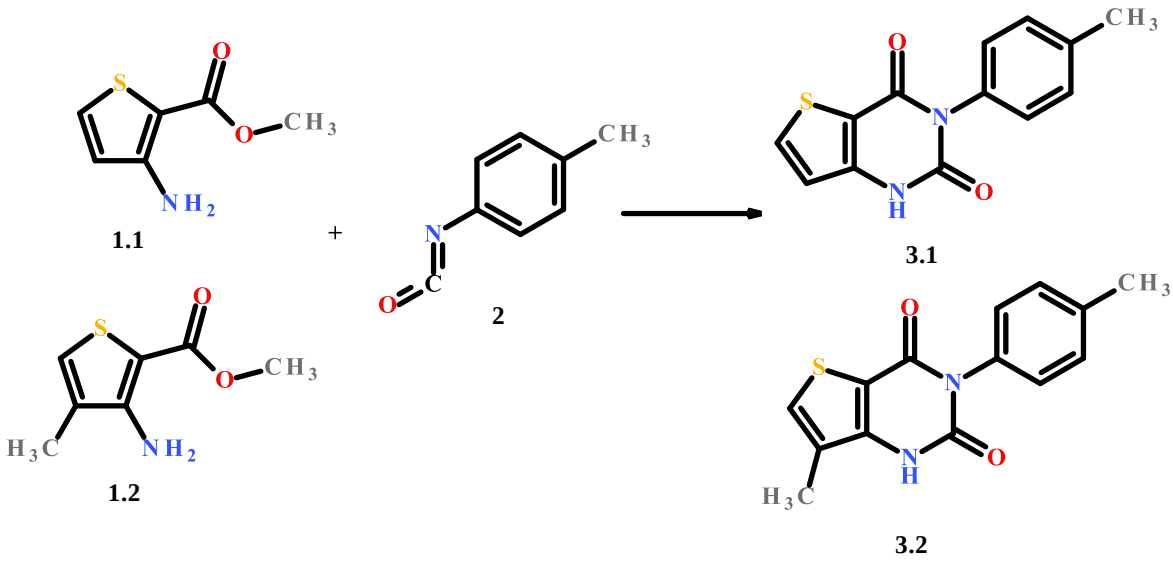


Fig. 2.2. Synthesis of 3-(p-tolyl)-thieno[3,2-d]pyrimidine-2,4-dione 3.1, 3.2

Next alkylation of 3-(p-tolyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione (1.1, 1.2) was performed via reaction with 1-(chloromethyl)benzene derivatives in the presence of potassium carbonate (K_2CO_3) in dimethylformamide (DMF) (Fig/ 2.3).

Potassium carbonate acts as a mild base, deprotonating the NH group at position 1 of the thienopyrimidine ring, thus generating a nucleophilic nitrogen center. This nucleophile subsequently undergoes an SN2 substitution with the benzylic halide (e.g., 1-(chloromethyl)benzene), leading to the formation of an N-

benzylated thienopyrimidine derivative. The chloride ion released as a by-product is neutralized by potassium carbonate, minimizing potential side reactions.

This reaction is typically carried out under gentle heating (50–80 °C), and DMF serves as a polar aprotic solvent that stabilizes ionic intermediates and enhances nucleophilic substitution efficiency.

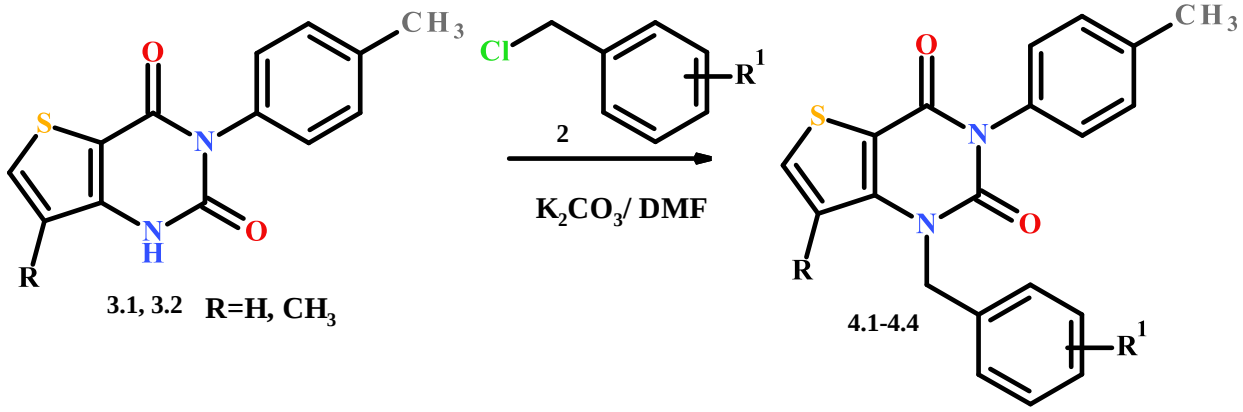


Fig. 2.3. Scheme of alkylation of 3-(p-tolyl)-thieno[3,2-d]pyrimidine-2,4-dione 3.1, 3.2

This alkylation approach offers a practical method for structural diversification of the thieno[3,2-d]pyrimidine scaffold by introducing benzyl substituents, which can modulate biological activity, improve membrane permeability, and influence binding interactions with target biomolecules.

2.3. Description of physicochemical and spectral characteristics of synthesized derivatives

Structural characterization of the synthesized compounds 4.1-4.4 (1-aryl(benzyl)-substituted thieno[3,2-d]pyrimidine-2,4-diones) was performed using ^{1}H NMR spectroscopy and elemental analysis, which collectively confirmed the identity and purity of the target molecules.

The ^{1}H NMR spectra of all compounds exhibited well-resolved singlet signals corresponding to the benzylic methylene protons (–CH_2–Ar), appearing consistently

in the range of δ 5.11–5.24 ppm, indicative of substitution at the N1-position of the thienopyrimidine scaffold. These signals are diagnostic for N-alkylation and are absent in the spectra of the starting unsubstituted thienopyrimidinones.

In addition to the methylene region, the spectra displayed multiplets in the aromatic region (δ ~8.10–7.10 ppm), corresponding to protons of both the p-tolyl and benzyl (or aryl) fragments, as well as the thieno[3,2-d]pyrimidine core. The methyl group of the p-tolyl substituent consistently appeared as a singlet around δ 2.50–2.35 ppm, confirming the retention of the para-substituted tolyl moiety.

As an example, the ^{1}H NMR spectrum of 1-benzyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione (4.1) is presented in Figure 2.4, illustrating the characteristic chemical shifts and signal patterns that confirm the proposed structure.

Fig. 2.4. ^{1}H NMR spectrum of 1-benzyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione (4.1)

The presence of a singlet at δ 5.24 ppm for 2 protons confirms N-benzylation at the pyrimidine nitrogen. The aromatic region shows the expected complexity for

a disubstituted aromatic system (tolyl and phenyl rings), with 10 protons in the range of δ 7.14–7.39 ppm. The singlet at δ 2.33 ppm clearly corresponds to the methyl group of the p-tolyl fragment. The downfield doublet at δ 8.11 ppm is consistent with a deshielded pyrimidine ring proton, coupled to a neighbouring proton on the heterocycle.

The overall signal patterns, chemical shift ranges, and integration values fully support the proposed substitution patterns and molecular frameworks. Furthermore, elemental analysis (C, H, N) data for each compound were within ±0.4% of the theoretical values, further corroborating the molecular compositions.

These findings collectively affirm the successful formation of the desired N-substituted thieno[3,2-d]pyrimidine-2,4-diones and the structural consistency of the series 4.1–4.4 with the intended molecular designs.

2.4. Experimental part

All chemicals and solvents were purchased from commercial suppliers and used without additional purification. Melting points were measured in capillary tubes using a digital melting point apparatus (Electrothermal IA9100X1, Bibby Scientific Ltd., Staffordshire, UK). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Unity Plus 400 MHz spectrometer. Elemental analyses were carried out on a Euro Vector EA-3000 microanalyzer (Eurovector SPA, Redavalle, Italy), with results deviating by no more than 0.4% from the calculated values.

Synthesis of 3-(p-tolyl)-thieno[3,2-d]pyrimidine-2,4-dione derivatives (3.1 and 3.2)

To a stirred solution of methyl 3-aminothiophene-2-carboxylate (1.1) (0.84 g, 5.0 mmol) or methyl 3-amino-4-methylthiophene-2-carboxylate (1.2) (0.91 g, 5.0 mmol) in 10 mL of N, N-dimethylformamide (DMF), 1-isocyanato-4-methylbenzene (2) (0.68 g, 5.0 mmol) was added dropwise at room temperature. The reaction mixture was then heated to 80 °C and stirred for 2 hours. After completion

(monitored by TLC), the reaction mixture was cooled to room temperature and poured into 50 mL of cold water with vigorous stirring. The resulting precipitate was filtered, washed with water, and dried. The crude product was recrystallized from ethanol to afford pure compound 3.1 or 3.2 as a pale-yellow solid. Yield (3.1): 1.23 g (85%), Yield (3.2): 1.30 g (88%). Rf (TLC, EtOAc:Hex 3:1): 0,57.

General Procedure for the Synthesis of 1-Aryl(benzyl)-substituted thieno[3,2-d]pyrimidine-2,4-diones (4.1-4.4)

A mixture of 3-(p-tolyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione 1.1., 1.2 (1.0 mmol), potassium carbonate (K_2CO_3, 2.0 mmol), and the appropriate aryl- or benzyl halide (1.1 mmol) was suspended in 10 mL of dry dimethylformamide (DMF). The reaction mixture was stirred at room temperature for 15 minutes to allow activation of the nucleophile. Subsequently, the reaction was heated to 60–80 °C and stirred for 4–8 hours under a nitrogen atmosphere.

Reaction progress was monitored by thin-layer chromatography (TLC) using a solvent system of ethyl acetate:hexane (3:1). Upon completion, the reaction mixture was cooled to room temperature and poured into 50–60 mL of cold distilled water with vigorous stirring.

The precipitate was collected by filtration, washed thoroughly with water to remove inorganic salts and DMF residues, and dried under reduced pressure. The crude product was recrystallized from ethanol or ethyl acetate/hexane, depending on solubility, to afford the pure 1-aryl(benzyl)-substituted thienopyrimidine derivative. Yields: 75–90%. Appearance: Pale yellow to off-white crystalline solids. Purity: Confirmed by TLC and ^{1}H NMR.

1-Benzyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione (4.1):

Molecular formula: $C_{20}H_{16}N_2O_2S$, Molecular weight: 348.43 g/mol, Exact mass: 348.09. Elemental composition: C68.95%, H 4.63%, N 8.04%, O 9.18%, S 9.20%, ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 8.11 (doublet, J = 5.3 Hz, 1H) – proton of the thieno[3,2-d]pyrimidine ring, likely at position C-6; 7.39–7.14 (multiplet, 10H) – aromatic protons from both p-tolyl and benzyl phenyl rings; 5.24 (singlet, 2H) – benzylic -CH_2 - protons attached to the nitrogen at position 1 of the

pyrimidine ring; 2.33 (singlet, 3H) – methyl group (–CH_3) on the p-tolyl substituent at position 3.

1-Benzyl-7-methyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione (4.2) :

Molecular formula: $C_{21}H_{18}N_2O_2S$, Molecular weight: 362.45 g/mol, Exact mass: 362.10, Elemental composition: C 69.59%, H 5.01%, N 7.73%, O 8.83%, S 8.85%, ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 7.75 (singlet, 1H) – proton of the thienopyrimidine ring; 7.33 (triplet, J = 7.6 Hz, 2H) – aromatic protons of the benzyl phenyl ring; 7.26–7.08 (multiplet, 7H) – remaining aromatic protons of the p-tolyl and benzyl rings; 5.40 (singlet, 2H) – benzylic –CH_2– protons at N1; 2.32 (singlet, 3H) – methyl group on the p-tolyl ring; 2.19 (singlet, 3H) – methyl group at position 7 of the thienopyrimidine core.

1-[(3,5-dimethylphenyl)methyl]-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione (4.3):

Molecular formula: $C_{22}H_{20}N_2O_2S$, Molecular weight: 376.47 g/mol, Exact mass: 376.12, Elemental composition: C 70.19%, H 5.36%, N 7.44%, O 8.50%, S 8.51%; 1H NMR (400 MHz, DMSO-d_6) δ, ppm: 7.73 (s, 1H) – singlet proton of the thieno[3,2-d]pyrimidine ring; 7.22–7.03 (m, 6H) – multiplet, aromatic protons from the p-tolyl and 3,5-dimethylphenyl rings; 5.18 (s, 2H) – singlet, benzylic CH_2 group attached to N1; 2.31 (s, 3H) – singlet, methyl group of the p-tolyl ring (para-substituted); 2.26 (s, 6H) – singlet, two equivalent methyl groups at the 3- and 5-positions of the dimethylphenyl moiety.

1-[(3,5-dimethylphenyl)methyl]-7-methyl-3-(p-tolyl)thieno[3,2-d]pyrimidine-2,4-dione (4.4) :

Molecular formula: $C_{23}H_{22}N_2O_2S$, Molecular weight: 390.50 g/mol, Exact mass: 390.14, Elemental composition: C 70.75%, H 5.68%, N 7.17%, O 8.20%, S 8.21%; ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 7.70 (s, 1H) – singlet proton of the thieno[3,2-d]pyrimidine core; 7.20–7.02 (m, 6H) – multiplet, aromatic protons from the p-tolyl and 3,5-dimethylphenyl rings; 5.19 (s, 2H) – singlet, benzylic CH_2 group attached to N1; 2.31 (s, 3H) – singlet, methyl group on the p-tolyl ring (para-

position); 2.25 (s, 6H) – singlet, two methyl groups at the 3- and 5-positions of the dimethylphenyl ring; 2.17 (s, 3H) – singlet, methyl group at position 7 of the thienopyrimidine ring.

Conclusions for Chapter 2

1. Based on preliminary structure–activity relationship (SAR) analysis and prior knowledge of the biological potential of thieno[3,2-d]pyrimidines, a rational design approach was implemented to develop novel derivatives as potential antimicrobial agents. Structural modifications such as N1-benzylation and methyl substitution were introduced to improve lipophilicity and membrane permeability, particularly to overcome the intrinsic resistance of Gram-negative bacteria.

2. Key intermediates (3.1, 3.2) were synthesized by reacting methyl 3-aminothiophene-2-carboxylates with 1-isocyanato-4-methylbenzene (2) in DMF at 80 °C for 2 hours, providing thienopyrimidine-2,4-dione cores for further functionalization.

3. A series of novel 1-aryl(benzyl)-substituted thieno[3,2-d]pyrimidine-2,4-dione derivatives (4.1–4.4) was then synthesized by N-alkylation of compounds 3.1 and 3.2 with various substituted benzyl halides under mild basic conditions in DMF, resulting in good to excellent yields and high regioselectivity.

4. The structures of all synthesized compounds were thoroughly confirmed using ^{1}H NMR spectroscopy and elemental analysis. The NMR spectra exhibited characteristic singlet signals for benzylic methylene protons in the range of δ 5.11–5.40 ppm, unambiguously confirming N1-substitution. The aromatic regions of the spectra (δ ~7.0–7.8 ppm) as well as the signals of methyl groups (δ ~2.1–2.3 ppm) were consistent with the proposed substitution patterns, supporting the integrity of the synthesized molecular structures.

5. Elemental analysis data matched theoretical values within ±0.4% for all compounds, confirming the high analytical purity and correctness of molecular composition.

CHAPTER 3

DETERMINATION OF AFFINITY OF SYNTHESIZED DERIVATIVES TO ANTIBACTERIAL BIOTARGETS

Selective inhibition of key enzymatic systems within bacterial cells represents a high-priority strategy in the search for novel mechanisms of antibacterial action. One of the most notable advantages of thieno[2,3-d]pyrimidine derivatives is their demonstrated ability to effectively inhibit a variety of microbial enzymes, particularly those of *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*.

These compounds have shown significant activity against *M. tuberculosis* strains that are resistant to isoniazid, with a mechanism of action attributed to the inhibition of enoyl-acyl carrier protein (ACP) reductase, a critical enzyme in mycolic acid biosynthesis. Importantly, this mechanism does not require activation by catalase-peroxidase, which is essential for isoniazid activity and commonly mutated in resistant strains [43, 44].

In addition, recent studies have identified 4-amino-thieno[2,3-d]pyrimidines as a promising and innovative scaffold for the development of inhibitors targeting QcrB(quinol-cytochrome c reductase subunit B), a subunit of the cytochrome bc_1 complex involved in the electron transport chain. This target is gaining recognition as a novel and attractive biochemical site for anti-tuberculosis drug development [45]. QcrB is a component of the cytochrome bc_1 enzyme complex, which participates in the transfer of electrons from ubiquinol (QH_2) to cytochrome *c*, accompanied by the generation of a proton gradient across the membrane. This process is critically important for oxidative phosphorylation, the primary mechanism of ATP production in the cell [46].

Inhibition of QcrB leads to disruption of oxidative phosphorylation, resulting in energy depletion and cell death in *M. tuberculosis*. Due to its essentiality and distinct mechanism, QcrB inhibition holds potential for overcoming resistance associated with conventional drugs and is currently being explored as a next-generation strategy in the treatment of tuberculosis [47].

3.1. *In silico* study of affinity to the TrmD inhibitor site of an inhibitor isolated from *Pseudomonas aeruginosa*

TrmD, or tRNA-(N^1G37)-methyltransferase, is a member of the SpoU-TrmD family of RNA methyltransferases that catalyzes the transfer of a methyl group from S-adenosylmethionine (SAM) to the N^1 position of guanosine at position 37 in bacterial tRNA [9, 48]. This enzyme has been shown to be essential for the growth of several pathogenic bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Mycobacterium abscessus*, and *Haemophilus influenzae* [48]. In 2013, Hill et al. [49] reported the discovery of thienopyrimidinone-based inhibitors of TrmD that bind within the SAM-binding pocket, thereby competing with the natural cofactor and blocking tRNA methylation. A subsequent study presented a structurally guided design and synthesis of a focused library of novel thienopyrimidine derivatives as selective inhibitors of bacterial TrmD enzymes [50].

Co-crystal structures of TrmD from *S. aureus*, *P. aeruginosa*, and *M. tuberculosis* in complex with these inhibitors revealed specific binding modes within the active site. Notably, they highlighted the involvement of a tyrosine-flipping mechanism, where a critical tyrosine residue undergoes conformational change upon ligand binding. This mechanism, which appears to be unique to *P. aeruginosa* TrmD, renders the enzyme inaccessible to the SAM cofactor and likely also to its natural tRNA substrate.

To support the design of novel analogues and predict their potential antimicrobial mechanisms, molecular docking studies were performed on compounds 4.1-4.4 using the crystal structure of TrmD from *Pseudomonas aeruginosa* (PDB ID: 5ZHN). As a reference ligand, N-(4-((octylamino)methyl)benzyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-5-carboxamide was used (Figure 3.1), a competitive TrmD inhibitor with potent antibacterial activity against both Gram-positive and Gram-negative bacteria,

including *Mycobacterium tuberculosis*. The binding energy of the reference compound was calculated to be -8.2 kcal/mol.

Docking methodology was previously validated and is described in detail in reference [51-55].

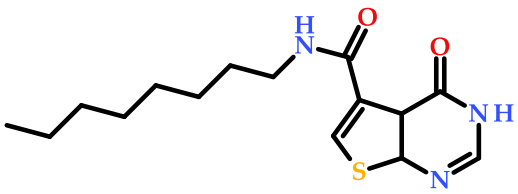


Fig. 3.1. Reference ligands and inhibitors of TrmD

The highest affinity toward TrmD was demonstrated by ligands 4.1 and 4.2, which lack a methyl group at position 7 (Table 3.1). Ligand 4. forms a complex network of strong and selective non-covalent interactions within the active site of the *P. aeruginosa* TrmD protein, including1 (Fig. 3.2a): one conventional hydrogen bond, one weak C–H···O hydrogen bond, one electrostatic π-anion interaction, one π–sulfur interaction, two amide–π stacking contacts, and five π-alkyl and alkyl hydrophobic interactions (Table 3.1). This interaction profile is highly favorable for selective and stable binding within the enzyme's active site and highlights compound 4.1 as a promising TrmD inhibitor.

In contrast, ligand 4.3 (Fig. 3.2b), which bears a bulkier 3,5-dimethylbenzyl group at the N^1-position, showed a slightly different interaction profile. While it lacks some of the specific polar interactions seen in 4.1 (such as π-anion and π–sulfur interactions), it compensates through a greater number of hydrophobic contacts, including additional interactions with ILE138 and TYR141, and retains the key hydrogen bonds with TYR91 and LEU143. However, the increased hydrophobicity and steric bulk of the N^1-substituent in 4.3 may influence binding orientation and reduce selectivity.

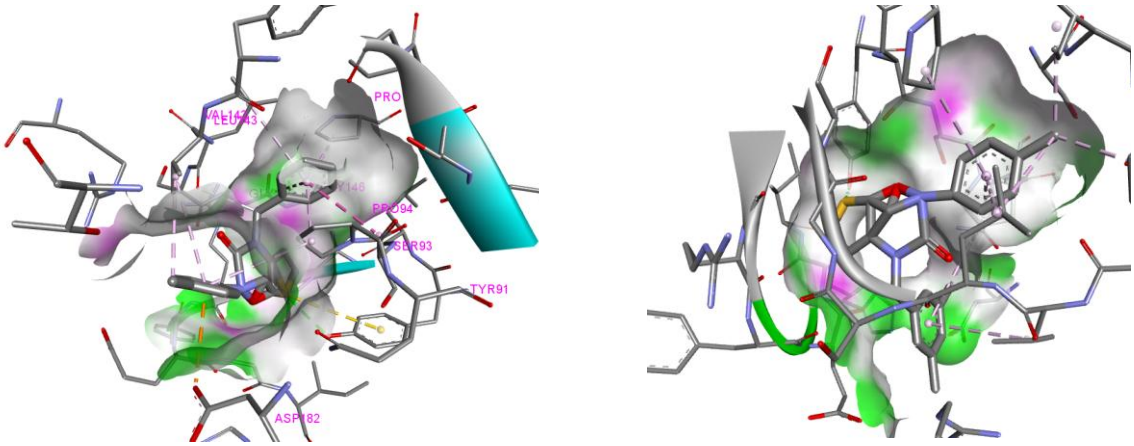
Overall, ligand 4.1 remains the most promising TrmD inhibitor in this series due to its strong polar interactions and selective binding pattern, whereas ligand 4.3,

though still active, may benefit from further optimization to restore critical polar contacts while retaining its expanded hydrophobic profile.

Table 3.1

Results of molecular docking of thienopyrimidine derivatives into the active sites of TrmD

Compound	Scoring function kcal/mol	Hydrophobic interactions	Hydrogen bonds	Other interactions
	TrmD, *P. Aeruginosa*			
Native ligand 15	-8.2	Tyr141, Ser93(2)#, Pro94 (4), Pro149(2), Ile138, Leu143, Gly 45, Gly146	Ser59(2), Tyr60, Lys296(2)	Cys397 Pi-Sulfur
4.1	-9.3	SER93, PRO94, GLY145, GLY146, VAL142, PRO94, LEU143, PRO149, PRO94, VAL142	TYR91, LEU143,	ASP182, TYR91 Electrostatic Pi-Anion Pi-Sulfur
4.2	-8.4	LEU228, PRO94, LEU181	–	ASP178, ASP182 Electrostatic Pi-Anion
4.3	-9.3	SER93, PRO94, LEU92, PRO149, VAL142, TYR141	TYR91, LEU143	ASP182 Electrostatic Pi-Anion
4.4	-8.7	PRO94, ILE138, TYR141, VAL142, PRO149	TYR91, LEU143	-
	TrmD, *M. Tuberculosis*			
Native ligand AZ54	-7,8	LEU138(2), THR84, PRO85(2), THR84, PRO85(3), ALA144(2), ILE133	Leu138(2), Glu112, Gly134O	–
4.1	-7.8	THR84, VAL137, PRO85, LEU138, PRO85	LEU138, GLY140	GLU112(2)



a b

Fig. 3.2. 3D visualization of the conformational arrangement of ligands 4.1 and 4.3 in the active site of TrmD

Compared to compound 4.4, ligand 4.2 exhibits a less complex interaction network, consisting mainly of weaker electrostatic contacts and a few hydrophobic interactions, particularly with residues LEU181, LEU228, and PRO94.

In contrast, ligand 4.4 demonstrates a richer and more balanced binding profile, including two classical hydrogen bonds (one of them with TYR91), an electrostatic π-anion interaction with ASP182, an amide–π stacking interaction, and an extended network of hydrophobic contacts involving LEU, VAL, PRO, and TYR residues.

When compared to ligand 4.1, ligand 4.4 retains similar hydrogen bonding and π-anion interactions, but shows fewer stacking interactions while compensating with a broader range of hydrophobic contacts, particularly with TYR141 and LEU92. Its binding appears to be more hydrophobically driven, which could offer advantages in terms of membrane permeability, although it may result in reduced selectivity toward the TrmD active site.

Overall, ligand 4.1 exhibits stronger affinity in terms of target selectivity, primarily due to its specific polar interactions, such as π-anion, π-sulfur, and π-stacking contacts. In contrast, ligand 4.4 may possess a better hydrophobic balance and pharmacokinetic potential, but requires structural optimization to enhance its specific interactions with the enzymatic target.

The most promising ligand is compound 4.1, which exhibits the highest selectivity and affinity toward the TrmD enzyme from *P. aeruginosa* due to a balanced network of specific polar interactions (hydrogen bonds, π-anion, and π-sulfur) and π-stacking contacts, ensuring stable binding within the active site.

Regarding the structure–activity relationship:

- Higher selectivity is observed with an unsubstituted position 7, an aromatic benzyl fragment, and the presence of polar π-interactions (compound 4.1).
- Hydrophobic enhancement without polar compensation leads to reduced selectivity, although it may improve pharmacokinetic properties (compounds 4.2 and 4.3).
- A balance of hydrophobic and polar interactions provides a structurally promising profile, but requires further optimization (compound 4.4).

3.2. *In silico* study of affinity to the TrmD inhibitor site isolated from *Mycobacterium tuberculosis*

The affinity of the investigated ligand 4.1 toward TrmD from *Mycobacterium tuberculosis* (PDB ID 6JOF) was comparable to that of the reference ligand AZ54, with a binding energy of –7.8 kcal/mol. Interaction analysis revealed the presence of one strong conventional hydrogen bond with GLU112, several additional C–H···O contacts, and hydrophobic interactions with PRO85, VAL137, and LEU138, as well as contacts with peptide amide groups, which may possess a π-character.

This interaction profile supports the formation of a stable complex within the active site of *M. tuberculosis* TrmD. However, π-anion and π–sulfur interactions, which were characteristic for binding to *P. aeruginosa* TrmD, were not observed in this case. Thus, the binding affinity is mainly attributed to a combination of conventional hydrogen bonding and hydrophobic contacts, rather than specific π-interactions.

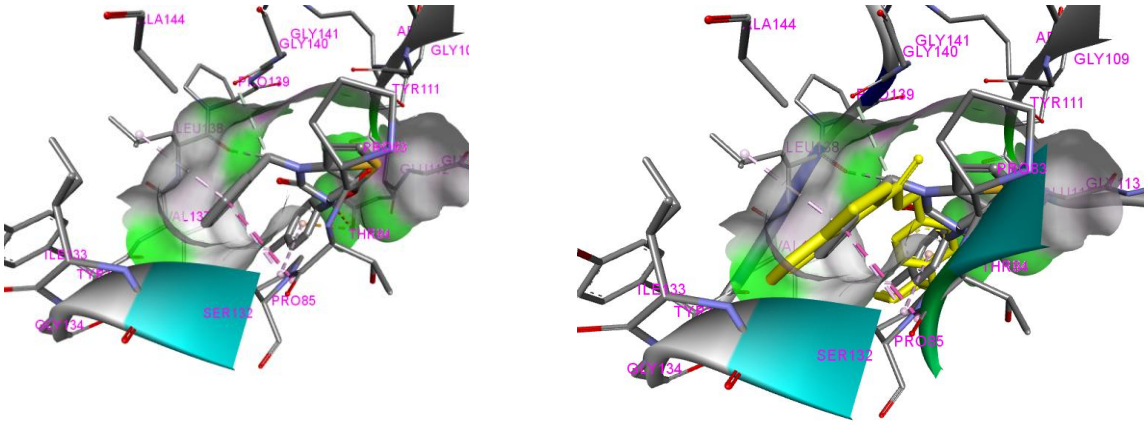


Fig. 3.3. 3D interaction of derivative 4.1 with amino acid residues of the active site of *M. tuberculosis* TrmD and compatible conformation with the native ligand (yellow)

Conclusions for Chapter3:

1. Based on molecular docking results, ligand 4.1, which contains an unsubstituted position 7 and a simple benzyl substituent at the N^1-position, demonstrated the highest affinity toward TrmD. This structure provides an optimal balance of polar interactions (hydrogen bonds, π-anion, π-sulfur) and π-stacking interactions, ensuring high selectivity for the enzyme's active site.

2. Ligand 4.3, featuring a 3,5-dimethylbenzyl substituent and lacking a methyl group at position 7, compensates for the absence of polar contacts through extended hydrophobic interactions, particularly with ILE138, TYR141, PRO94, and LEU143. However, the lack of π-anion and stacking interactions indicates reduced selectivity for the active site.

3. Ligands 4.2 and 4.4, both containing a methyl group at position 7, showed lower selectivity for TrmD compared to 4.1. Ligand 4.2 forms only weak electrostatic interactions (with ASP178 and ASP182) and a few hydrophobic contacts, lacking specific π-interactions. Ligand 4.4, while retaining key hydrogen bonds (TYR91, LEU143) and a π-anion interaction, displays fewer π-stacking contacts and a predominance of hydrophobic interactions (with LEU92, TYR141, PRO149, VAL142). Thus, substitution at position 7 reduces the polarity-driven binding, negatively affecting affinity toward TrmD.

4. Ligand 4.1 exhibited high affinity toward TrmD from *Mycobacterium tuberculosis* (–7.8 kcal/mol), comparable to AZ54. Its inhibition is driven by a strong hydrogen bond, hydrophobic contacts, and interactions with peptide amide groups, indicating the potential of 4.1 as a TrmD inhibitor.

CONCLUSIONS

1. The targeted design of thieno[3,2-d]pyrimidine derivatives was carried out based on preliminary SAR analysis, leading to the creation of a series of compounds with potential antibacterial activity. A 4-methylphenyl substituent at position 3 and various benzyl groups at the N^1-position were selected as key fragments. Modifications at position 7 were introduced to optimize lipophilicity and predicted permeability.

2. The synthesis of target compounds was successfully achieved in two stages: condensation of 3-aminothiophene-2-carboxylates with 4-methylphenyl isocyanate, followed by alkylation of the resulting thieno[3,2-d]pyrimidine-2,4-diones with benzyl halides. The structures of the synthesized molecules were confirmed using ^{1}H NMR spectroscopy and elemental analysis.

3. Molecular docking studies focused on TrmD (tRNA-(N^1G37)-methyltransferase)—a key bacterial enzyme involved in tRNA methylation. The highest affinity was observed for ligand 4.1, which formed a network of hydrogen bonds, π-anion, π-sulfur, and π-stacking interactions with TrmD from *P. aeruginosa*, and also demonstrated stable binding to TrmD from *M. tuberculosis* via a classical hydrogen bond (GLU112) and hydrophobic stabilization.

4. SAR analysis revealed that methyl substitution at position 7 and bulky benzyl groups (as in 4.2–4.4) shift the binding mechanism toward hydrophobic interactions, reducing selectivity for TrmD. In contrast, the absence of substitution at position 7 and the presence of a simple benzyl group (as in 4.1) provide the most selective and favorable binding profile.

5. The results confirm the potential of thieno[3,2-d]pyrimidine derivatives as multitarget antibacterial agents, capable of inhibiting TrmD in different bacterial systems, including *P. aeruginosa* and *M. tuberculosis*. Ligand 4.1 stands out as a promising hit candidate for further optimization and experimental in vitro validation.

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APPLICATION

XXXI Міжнародна науково-практична конференція молодих вчених та студентів
«АКТУАЛЬНІ ПИТАННЯ СТВОРЕННЯ НОВИХ ЛІКАРСЬКИХ ЗАСОБІВ»

SYNTHESIS OF 1-BENZYL-3-(4-METHYLPHENYL)-THIENO[3,2-D]PYRIMIDINE-2,4-DIONE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

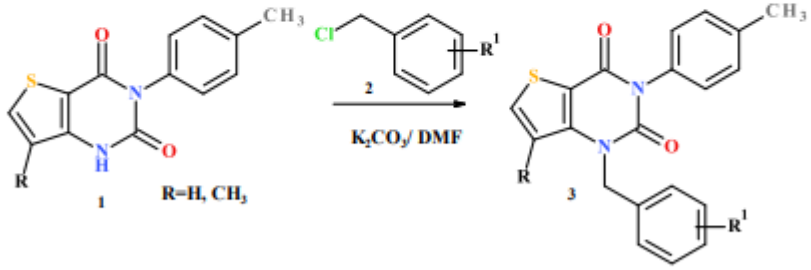
Dounia Miri, Vlasov S.V.
Scientific supervisor: Severina H.I.
National University of Pharmacy, Kharkiv, Ukraine
douniamiri@gmail.com

Introduction. The rapid growth of microbial resistance to existing antibiotics is one of the most serious threats to modern medicine. Inadequate and uncontrolled use of antimicrobial agents has led to the formation of multiple resistance among pathogenic bacterial strains, which greatly complicates the treatment of infectious diseases. Today, there is an urgent need to develop new antimicrobial agents with new mechanisms of action that can overcome resistant forms of microorganisms. Research in this area is aimed at both modifying existing molecules and discovering fundamentally new chemical structures. It is important to study the use of modern methods of molecular modelling and bioinformatics for the rational design of new drugs. One of the most promising areas is the study of thienopyrimidine derivatives, heterocyclic compounds that combine biologically active fragments of thiophene and pyrimidine in their structure. These compounds exhibit a wide range of pharmacological activities, including antimicrobial, antiviral, anti-inflammatory and antitumor effects. Due to their structural variability and ability to interact with biotargets, thienopyrimidine derivatives are considered a promising platform for the development of new antimicrobial drugs.

Aim. The aim of the present work was to synthesize new 1-benzyl-3-(4-methylphenyl)-thieno[3,2-d]pyrimidine-2,4-dione derivatives with potential antimicrobial activity.

Materials and methods. 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 ^{1}H NMR spectra were recorded using Varian Unity Plus 400 (400 MHz) spectrometers. 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. All reagents were purchased from commercial suppliers and used as received without further purification.

Results and discussion. The starting 3-(4-methylphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione and 7-methyl-3-(4-methylphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione were synthesized earlier at the Department of Pharmaceutical Chemistry. Alkylation of 3-(4-methylphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione 1 was carried out by reaction with 1-(chloromethyl)-benzenes in the medium of dimethylformamide and in the presence of potassium carbonate, which was added to bind chloride ions, which are by-products of the reaction (scheme 1).



The structure of the obtained compounds 3 was confirmed using ^{1}H spectroscopy, and the spectral data for these compounds indicate their correspondence to the suggested structures. In the spectra, the signals of methylene protons of the benzylic fragment are reflected in the range of 5.11–5.16 ppm. The set and nature of the signals and the resonance range of the aromatic photons correspond to the proposed structures for both compounds 3.

Conclusions. An effective procedure of obtaining 1-benzyl-3-(4-methylphenyl)-thieno[3,2-d]pyrimidine-2,4-dione derivatives by alkylation of synthetically available 3-(4-methylphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione and 7-methyl-3-(4-methylphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione with 1-(chloromethyl)-benzenes. Since the significant antimicrobial activity of various thieno[3,2-d]pyrimidine derivatives were previously revealed, the synthesized new derivatives should be investigated for *in vivo* activity.

THEORETICAL STUDIES ON THE ANTI-INFLAMMATORY ACTIVITY OF VITEXIN WITH THE PHOSPHOLIPASE A2 ENZYME

Maslov O. Yu., Akhmedov E.Yu., Karpova S.P.
Scientific supervisor: Kolisnyk S.V.
National University of Pharmacy, Kharkiv, Ukraine
alexmaslov392@gmail.com

Introduction. The use of anti-inflammatory drugs is often associated with numerous side effects. For example, steroidal medications can lead to osteoporosis, adrenal atrophy, and immune system suppression, while non-steroidal drugs may cause bronchospasms. Given these concerns, the search for new anti-inflammatory compounds derived from herbal sources remains highly relevant. One promising target for such research is the phospholipase A2 enzyme, which plays a crucial role at the beginning of inflammation.

Aim. To perform molecular docking of vitexin with the phospholipase A2 enzyme.

Materials and methods. A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6. Genetic algorithm parameters were applied for ligand interaction, with 10 runs of this criterion. Phospholipase A2 (PDB ID: 3hsw) structure was obtained from PDB database. The resolution of 3hsw was 3.00 Å. The ligand structures of vitexin (CID_5280441) was obtained from PubChem database. The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins. As a standard was taken diclofenac sodium. We applied the following classification of selectivity: IC50<0.001 mM (high selective); 0.05>IC50>0.01 (medium selective); IC50>0.05 mM (low selective).

Results and discussion. The vitexin had a high value of free energy value (-13.89 kcal/mol), whereas IC50 was 0,00000006539 mmol, so vitexin belong to high selective inhibitor. Comparing result with diclofenac sodium standard, the affinity of vitexin was 45% more than of diclofenac sodium (-7.65 kcal/mol, IC50 – 0.00248 mmol).

Conclusions. It was established that vitexin is a potentially high selective inhibitor of phospholipase A2 enzyme. So, the extract with vitexin can be applied for developing a new anti-inflammatory drug.

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

СЕРТИФІКАТ УЧАСНИКА

Цим засвідчується, що

Dounia Miri, Vlasov S.V.
Scientific supervisor: Severina H.I.

брав(ла) участь у роботі
XXXI Міжнародної науково-практичної конференції молодих вчених та студентів
«АКТУАЛЬНІ ПИТАННЯ СТВОРЕННЯ НОВИХ ЛІКАРСЬКИХ ЗАСОБІВ»

В.о. ректора
Національного фармацевтичного
університету

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ Національний фармацевтичний університет №02010936

Алла КОТВІЦЬКА

23-25 квітня 2025 р, м. Харків

Ф А 2.2.1–32-366

National University of Pharmacy

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

APPROVED
The Head of
Department of
pharmaceutical
chemistry

Victoriya GEORGIYANTS
" 3 " September 2024 year

ASSIGNMENT
FOR QUALIFICATION WORK OF
AN APPLICANT FOR HIGHER EDUCATION

Dounia MIRI

1. Topic of qualification work: «Design of molecules with antimicrobial properties in the series of derivatives of 1-benzyl-3-phenyl-thieno[3,2-d]pyrimidine-2,4-dione derivatives», supervisor of qualification work: Hanna Severina, DSc, professor

approved by order of NUPh from "27th" of September 2024 № 237

2. Deadline for submission of qualification work by the applicant for higher education: May 2025

3. Outgoing data for qualification work: Study describes the synthesize and computationally model thieno[3,2-d]pyrimidine derivatives to evaluate their potential as TrmD enzyme inhibitors in multidrug-resistant pathogens *P. aeruginosa* and *M. tuberculosis*, with a focus on the impact of structural substitutions on target affinity.

4. Contents of the settlement and explanatory note (list of questions that need to be developed): to analyze and summarize literature data on the pharmacological activity of thieno[2,3-d]pyrimidine derivatives, particularly their antibacterial effects, and to evaluate pharmacophore features influencing biological activity; to design a series of target molecules based on SAR analysis and pharmacophore modeling principles; to synthesize thieno[3,2-d]pyrimidine derivatives modified at key positions of the heterocyclic core (N^1, C3, and C7); to characterize the obtained compounds using ^{1}H NMR spectroscopy and elemental analysis; to perform molecular docking studies of the target compounds with TrmD isolated from Pseudomonas aeruginosa and Mycobacterium tuberculosis; to analyze the ligand–protein interaction profiles and identify structure–activity relationships (SAR) relevant to TrmD binding affinity; to evaluate the potential of the synthesized compounds as multitarget antibacterial agents..

5. List of graphic material (with exact indication of the required drawings): figures 11

6. Consultants of chapters of qualification work

Chapter	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1	Hanna SEVERINA, professor of higher education institution of the Department of pharmaceutical chemistry	04.09.2024	04.09.2024
2	Hanna SEVERINA, professor of higher education institution of the Department of pharmaceutical chemistry	25.10.2024	25.10.2024
3	Hanna SEVERINA, professor of higher education institution of the Department of pharmaceutical chemistry	25.12.2024	25.12.2024
4	Hanna SEVERINA, professor of higher education institution of the Department of pharmaceutical chemistry	29.01.2025	29.02.2025

7. Date of issue of the assignment: "_3st_" of September 2024

CALENDAR PLAN

№	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1.	Antibiotic resistance. pharmacological activity of thieno[2,3-d]pyrimidine derivatives (Literature review)	**September-November 2024**	**done**
2.	Selection of research objects and synthesis of targeted thieno[2,3-d]pyrimidine derivatives	**October- December 2024**	**done**
3.	Determination of affinity of synthesized derivatives to antibacterial biotargets	**January 2025– February 2025**	**done**
4.	Preparation of qualification work and submission to the Examination Commission	**February-April 2025**	**done**

An applicant of higher education _____ Dounia MIRI

Supervisor of qualification work _____ Hanna SEVERINA

ВИТЯГ З НАКАЗУ № 237

По Національному фармацевтичному університету

від 27 вересня 2024 року

Затвердити теми кваліфікаційних робіт здобувачам вищої освіти 5-го курсу Фм20(4,10д) 2024-2025 навчального року, освітньо-професійної програми – Фармація, другого (магістерського) рівня вищої освіти, спеціальності 226 – Фармація, промислова фармація, галузь знань 22 Охорона здоров'я, денна форма здобуття освіти (термін навчання 4 роки 10 місяців), які навчаються за контрактом (мова навчання англійська та українська) згідно з додатком № 1.

Прізвище, ім'я здобувача вищої освіти	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• **по кафедрі фармацевтичної хімії**				
Мірі Дуніа	Дизайн молекул з антимікробними властивостями в ряду похідних 1-бензил-3-арил-тієно[3,2-d]піримідин-2,4-діону	Design of molecules with antimicrobial properties in the series of derivatives of 1-benzyl-3-aryl-thieno[3,2-d]pyrimidine-2,4-dione derivatives	проф. Северіна Г.І.	проф. Подольський І.М.

Ректор

Вірно: Секретар

ФА2.8-03-317

ВИСНОВОК

експертної комісії про проведену експертизу
щодо академічного плагіату у кваліфікаційній роботі

здобувача вищої освіти

«07» травня 2025 р. № 331139422

Проаналізувавши кваліфікаційну роботу здобувача вищої освіти Мірі Дуніа, групи Фм20(4,10д) англ 01, спеціальності 226 Фармація, промислова фармація, освітньої програми «Фармація» навчання на тему: «Дизайн молекул з антимікробними властивостями в ряду похідних 1-бензил-3-арил-тієно[3,2-d]піримідин-2,4-діону / Design of molecules with antimicrobial properties in the series of derivatives of 1-benzyl-3-aryl-thieno[3,2-d]pyrimidine-2,4-dione derivatives», експертна комісія дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

Голова комісії,
проректор ЗВО з НПР,
професор **Інна ВЛАДИМИРОВА**

Ф А 2.2.1-32-353

REVIEW

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

Dounia MIRI

on the topic: «Design of molecules with antimicrobial properties in the series of derivatives of 1-benzyl-3-phenyl-thieno[3,2-d]pyrimidine-2,4-dione derivatives»

Relevance of the topic. The subject of the master's thesis, "Design of Molecules with Antimicrobial Properties in the Series of Derivatives of 1-Benzyl-3-Phenyl-thieno[3,2-d]pyrimidine-2,4-dione," is highly relevant to current pharmaceutical research priorities. The growing threat of antimicrobial resistance and the limited efficacy of conventional drugs call for innovative approaches to antibiotic development. The selection of the thienopyrimidine scaffold is justified by its established pharmacological versatility and potential to serve as a core structure for new antibacterial agents, particularly targeting resistant strains of *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*. The work aligns with global scientific trends aimed at identifying novel antimicrobial mechanisms, including targeting bacterial TrmD enzymes.

Practical value of conclusions, recommendations and their validity. The practical value of the thesis lies in the rational design and synthesis of a novel series of thienopyrimidine derivatives, followed by their in silico evaluation against TrmD, an essential bacterial enzyme absents in humans. The study successfully identified compound 4.1 as a promising TrmD inhibitor with high predicted affinity, supported by molecular docking results. The conclusions and recommendations are well-substantiated and based on validated computational methods, including structure–activity relationship analysis and molecular modeling. These findings serve as a

solid foundation for further *in vitro* and *in vivo* studies and may contribute to the development of multitarget antibacterial therapies.

Assessment of work. The thesis is well-organized, coherent, and demonstrates a high level of academic and scientific competence. The candidate has shown thorough knowledge of medicinal chemistry, microbiology, and molecular modeling techniques. The literature review is comprehensive, the objectives are clearly defined, and the applied methods are appropriate and systematically implemented. The experimental section includes detailed synthetic protocols and analytical data. The results are well interpreted and presented with clarity. The student has demonstrated strong research skills, independence, and analytical thinking. The work fully meets the requirements for a master's qualification paper.

General conclusion and recommendations on admission to defend. The qualification work of Dounia MIRI is performed at a high level with scientific novelty and practical significance of the results obtained. In terms of relevance, level of implementation and validity of conclusions, the work meets the requirements for graduate qualification works and can be submitted for defense in the Examination Commission.

Scientific supervisor _____ Hanna SEVERINA
«13 » May 2025 year

Ф А 2.2.1-32-356

REVIEW

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

Dounia MIRI

on the topic: «Design of molecules with antimicrobial properties in the series of derivatives of 1-benzyl-3-phenyl-thieno[3,2-d]pyrimidine-2,4-dione derivatives»

Relevance of the topic. The topic of the thesis is highly relevant given the global threat of antimicrobial resistance and the urgent need for new antibacterial agents with innovative mechanisms of action. The choice of thieno[3,2-d]pyrimidine derivatives as a chemical scaffold is scientifically justified due to their broad biological activity and potential to interact with bacterial targets, particularly the TrmD enzyme.

Theoretical level of work. The thesis demonstrates a high theoretical level. The author provides a comprehensive and well-structured literature review covering the antimicrobial potential of thienopyrimidines, their structural diversity, and pharmacophoric features. Theoretical aspects of microbial resistance and the rationale for selecting thienopyrimidine structures are clearly and convincingly presented.

Author's suggestions on the research topic. The author proposed a rational design of new thieno[3,2-d]pyrimidine derivatives based on SAR analysis and pharmacophore modeling. A series of novel compounds was synthesized, and molecular docking studies were performed against the TrmD enzyme from *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*. Compound 4.1 was identified as the most promising inhibitor, which highlights the scientific novelty and research potential of the study.

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Practical value of conclusions, recommendations and their validity. The practical value of the study lies in the development of novel chemical structures with predicted antimicrobial activity against a validated bacterial target (TrmD), which is absent in human cells. The findings provide a strong basis for further experimental investigation and the development of multitarget antimicrobial agents. The conclusions are well-reasoned, substantiated by in silico data, and based on modern computational tools.

Disadvantages of work. Minor drawbacks include occasional stylistic inaccuracies and some inconsistencies in scientific terminology in the English text. In a few places, the language is overly technical, which may complicate readability.

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

Reviewer _____ prof. Illya PODOLSKY

«15» May 2025 year

Ф А2.2.1-91-287

ВИТЯГ

з протоколу засідання кафедри фармацевтичної хімії

№ 14 від 16 травня 2025 р.

Засідання проводилось з використанням ZOOM технологій з 12 год. 05 хв. по 12 год. 50 хв.
Чисельний склад кафедри: 16 науково педагогічних працівників, з них присутні – 16 осіб.
ПРИСУТНІ: зав.каф. проф. Георгіянц В.А., професори: Баюрка С.В., Перехода Л.О., Северіна Г.І., Сидоренко Л.В., доценти: Амжад Абу Шарк І., Бевз Н.Ю., Вислоус О.О., Головченко О. С., Гриненко В.В., Кобзар Н.П., Михайленко О.О., Петрушова Л.О., Рахімова М.В., Яременко В.Д., ас. Григорів Г.В.; аспіранти: Асмолов В. Є., Гончар О.О., Гуріна В. О., Коптєлов А. С., Куцанян А. А., Мураль Д. В., Сайфудінова Р. П., Сулейман Р. М., Суржиков І.О.
ПОРЯДОК ДЕННИЙ:
Звіт про стан виконання кваліфікаційної роботи здобувача вищої освіти фармацевтичного факультету, Фм20(4,10д) англ 01 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Дуніа МІРІ на тему: «Дизайн молекул з антимікробними властивостями в ряду похідних 1-бензил-3-феніл-тієно[3,2-d]піримідин-2,4-діону».
СЛУХАЛИ: доповідь здобувача вищої освіти здобувача вищої освіти фармацевтичного факультету, Фм20(4,10д) англ 01 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Дуніа МІРІ на тему: «Дизайн молекул з антимікробними властивостями в ряду похідних 1-бензил-3-феніл-тієно[3,2-d]піримідин-2,4-діону»., керівник – професор кафедри фармацевтичної хімії, д.фарм.н., проф. Ганна СЕВЕРІНА.
УХВАЛИЛИ: рекомендувати кваліфікаційну роботу Дуніа МІРІ до офіційного захисту в Екзаменаційній комісії.

Голова

зав. кафедри, доктор фарм. наук,

професор **Вікторія ГЕОРГІЯНЦ**

Секретар

доцент, канд. фарм. наук **Марина РАХІМОВА**

Ф А2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Дуніа МІРІ до захисту кваліфікаційної роботи за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньо-професійною програмою Фармація

на тему: «Дизайн молекул з антимікробними властивостями в ряду похідних 1-бензил-3-феніл-тієно[3,2-d]піримідин-2,4-діону»

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Микола ГОЛІК /

Висновок керівника кваліфікаційної роботи

Здобувачка вищої освіти Дуніа МІРІ виконала роботу на сучасному рівні. За період виконання кваліфікаційної роботи проявила високий рівень теоретичної підготовки. Кваліфікаційна робота викладена послідовно, грамотно, висновки коректні та логічні, витікають зі змісту роботи. Кваліфікаційна робота Дуніа МІРІ може бути рекомендована до захисту в Екзаменаційній комісії.

Керівник кваліфікаційної роботи

_____ Ганна СЕВЕРІНА

«13» травня 2024 р.

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувачка вищої освіти Дуніа МІРІ допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувачка кафедри
фармацевтичної хімії

_____ Вікторія ГЕОРГІЯНЦ

«16» травня 2025 року

Qualification work was defended

of Examination commission on

« __ » __June__ 2025 year

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Volodymyr YAKOVENKO /