Ф А 2.2.1-32-367

MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
pharmaceutical faculty
department of pharmaceutical chemistry

QUALIFICATION WORK

on the topic: « **INVESTIGATION OF POSSIBLE DIRECTIONS OF BIOTRANSFORMATION AND TOXICITY OF A NEW ANTICONVULSANT AGENT PYRIMIDINE-4-THIONE DERIVATIVE»**

Prepared by: higher education graduate of group
Фм20(4,10д) англ 01
specialty 226 Pharmacy, industrial pharmacy
educational and professional program Pharmacy
Mouad TALAL
Supervisor: professor of higher education institution of department of pharmaceutical chemistry, DSc, professor
Hanna SEVERINA
Reviewer: professor of higher education institution of general chemistry department, DSc, professor
Serhii KOLISNYK

Kharkiv 2025

ANNOTATION

An *in silico* study of the metabolism and toxicity of the new anticonvulsant Epirimil was conducted. Biotransformation pathways involving CYP450 were identified: sulfur oxidation, N-oxidation, acetamide hydrolysis, O-dealkylation, and glucuronidation, with no toxic metabolites predicted. The compound belongs to toxicity class IV. The thesis consists of 4 chapters, general conclusions, and a reference list (71 sources), presented on 44 pages and containing 11 figures.

Keywords: pyrimidine, anticonvulsant, metabolism, in silico, CYP450, toxicity.

АНОТАЦІЯ

Проведено *in silico* дослідження метаболізму та токсичності нового антиконвульсанта Епірімілу. Виявлено шляхи біотрансформації за участі CYP450: окиснення Сульфуру, N-окиснення, гідроліз ацетаміду, O-деалкілування, глюкуронування, а також відсутність токсичних метаболітів. Сполука відноситься до IV класу токсичності. Робота: 44 стор., 11 рисунків, 71 джерело. Робота складається з 4 розділів, загальних висновків та списку використаної літератури (71 джерело), викладена на 44 сторінках, містить 11 рисунків.

Ключові слова: піримідин, антиковульсант, метаболізм, *in silico*, CYP450, токсичність

CONTENTS

LIST OF ABBREVIATIONS

ADMET	Absorption, distribution, metabolism, excretion, and toxicity
AEDs	Antiepileptic Drugs
AI	Artificial Intelligence
APIs	Active pharmaceutical ingredients
CYP	Cytochrome P450
DL	Deep learning
EEG	Electroencephalography
FDA	Food And Drug Administration
HLM	Human Liver Microsomes
in silico	Research methods using mathematical calculation methods
in vitro	Research methods using cell cultures
in vivo	Methods of study in a living organism
GST	Glutathione S-Transferases
ML	Machine Learning
MRI	Magnetic Resonance Imaging
RMSE	Root Mean Square Error
tSOM	Site of metabolism
SULT	Sulfotransferase
UGT	UDP-Glucuronosyltransferases

INTRODUCTION

Relevance of the topic. Epilepsy remains one of the most prevalent neurological disorders worldwide, with more than 50 million people affected globally, according to WHO estimates. Despite the availability of a wide range of antiepileptic drugs (AEDs) with diverse mechanisms of action, effective seizure control is achieved in only 65–70% of patients [1]. This underscores the need for the development of novel multitarget compounds with improved efficacy and safety profiles. At the National University of Pharmacy, a promising anticonvulsant molecule — N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide — was synthesized. It demonstrated broad-spectrum activity in various seizure models along with favourable pharmacological properties and low toxicity.

The investigation of metabolic pathways of novel bioactive compounds is a crucial step in the preclinical development of pharmaceuticals, as metabolism significantly affects a drug's pharmacokinetics, bioavailability, efficacy, and safety. Inadequate understanding of biotransformation mechanisms can lead to the emergence of undesirable toxic effects or insufficient therapeutic outcomes during clinical trials, often resulting in the discontinuation of drug development.

Therefore, early prediction of metabolic transformations – especially using *in silico* methods – enables the identification of potentially harmful metabolites, the assessment of involvement of key enzymes such as CYP450 isoforms, and the evaluation of the need for molecular structure optimization before initiating costly *in vitro* and *in vivo* experiments. This approach improves the likelihood of successful progression through further development stages, optimizes resource utilization, and contributes to the creation of safer and more effective therapeutic agents.

To reduce the risk of failure at the clinical trial stage due to unfavourable metabolic characteristics, it is essential to apply reliable methods for predicting biotransformation, including *in silico*, *in vitro*, and *in vivo* approaches. Given that experimental techniques often require substantial time, resources, and specialized equipment, the importance of *in silico* prediction as an initial screening tool is steadily

increasing. A deep understanding of the enzymatic modifications a molecule undergoes is fundamental to the rational design of safe and efficacious drug candidates.

The purpose of the study Prediction of possible biotransformation pathways and toxicological profile of a potential anticonvulsant N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide (Epirimil).

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

- To analyze the scientific literature on the epidemiology of epilepsy, side effects of existing AEDs, as well as the main mathematical, statistical methods and web resources used to predict possible pathways of xenobiotics metabolism in the human body.
- Based on the results of the analysis, select the most effective web tools for in silico studies of drug metabolism and toxicological profile.
- To perform computer predictions of possible biotransformation pathways of the anticonvulsant Epirimil.
- Determine the toxicity of the anticonvulsant Epirimil by in silico method and compare with the results in vivo.
- Systematize the data obtained from different programs and formulate conclusions about the main directions of Epirimil biotransformation

The object of the study. Prediction of chemical biotransformation of a potential anticonvulsant agent.

The subject of the study. Metabolites of N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide (Epirimil).

The methods of the study. Analysis and sorting of scientific literature on the research topic. BioviaDraw2021 was used to visualize the structure and create Smiles string. Online programs XenoSite were used to predict the direction of Epirimil metabolism. The ProTox program was used to determine the toxicity of the anticonvulsant Epirimil. Visualization of the predicted metabolic pathways was performed using BioviaDraw2021.

The practical value of the results. The *in silico* prediction of possible biotransformation pathways and toxicity profile of the promising anticonvulsant Epirimil was performed. The results of the prediction show that the -(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide (Epirimil) can be biotransformed by the cytochrome P450 enzyme system without the formation of toxic metabolites – epoxides. The identified potential metabolites will be the basis for further in vivo studies and will help in the interpretation of the data on metabolic transformations of Epirimil.

Elements of scientific research. For the first time, in silico calculation and prediction of the directions of chemical biotransformation of a new aniconvulsant with a multifactorial mechanism of action and low toxicity were performed.

Structure and scope of the qualification work.

The paper consists of an introduction, four sections, conclusions, and a list of references. The work is presented on 44 pages and contains 11 figures, The list of the used literary sources contains 71 titles.

CHAPTER 1
EPILEPSY AND ITS EPIDEMIOLOGY. DRUG METABOLISM

(Literature review)

1.1. Epilepsy and its epidemiology

Epilepsy is a neurological disorder that can occur in people of any age, regardless of their geographic location, social status, or ethnic background. Information about epileptic seizures has been known since ancient times – they are mentioned in Mesopotamian writings and the sacred Indian texts, the Vedas. It is one of the oldest known medical conditions, with descriptions dating back as far as 4000 BC. The term "epilepsy" comes from the Greek word meaning "sudden attack" or "to be seized." An epileptic seizure can present with various symptoms – motor, sensory, psychic, or autonomic, and sometimes a combination of them. A seizure is a temporary disruption in a person's condition caused by excessive electrical activity in the brain. Seizures are classified as either provoked or unprovoked [1].

The revised 2014 operational definition of epilepsy by the International League Against Epilepsy (ILAE) outlines the following criteria:

1. The occurrence of two or more unprovoked (or reflex) seizures spaced more than 24 hours apart.
2. A single unprovoked (or reflex) seizure with a high likelihood of recurrence – estimated at 60% or more – over the next 10 years, which is similar to the recurrence risk following two unprovoked seizures.
3. A confirmed diagnosis of an epilepsy syndrome [2].

Epilepsy is considered resolved in individuals who:

- Previously had an age-related epilepsy syndrome but have now surpassed the age range in which it typically occurs,
- Or who have been free from seizures for at least 10 years, including at least the last 5 years without the use of anti-seizure medications [2].

A recent study reports that around 70 million people worldwide are living with epilepsy, with nearly 90% of those cases occurring in low- and middle-income countries. The average prevalence rate in rural areas of these regions is about 1.54%, while in urban settings it's slightly lower at 1.03%. Each year, approximately 2.4 million new epilepsy cases are diagnosed globally [3].

In wealthier nations, between 30 and 50 people per 100,000 develop epilepsy annually. However, in less affluent countries, this rate can be up to twice as high. Factors contributing to this include a higher risk of infections like malaria and neurocysticercosis, more frequent road and birth-related injuries, and limited access to quality healthcare and prevention programs [4].

According to World Bank classifications, about 90% of people with epilepsy live in areas with restricted medical resources. In developed countries, epilepsy affects between 4 and 10 individuals per 1,000 people. In contrast, research from developing and tropical regions shows significantly higher rates — ranging from 14 to 57 cases per 1,000 — which may partly be due to differences in research methods. However, in some parts of the world, infections such as neurocysticercosis are common causes of epilepsy [5].

In high-income countries, epilepsy rates follow a U-shaped distribution, with the highest incidence in young children and older adults. Meanwhile, in developing nations, the condition is most frequently diagnosed in early adulthood.

1.2 Difficulties of epilepsy therapy

The treatment of epilepsy is a complex and multifaceted process influenced by numerous factors. The main challenges in epilepsy therapy include:

1. Individual characteristics of the disease
   Epilepsy can present in various forms (focal, generalized, syndromic, etc.), each requiring a specific therapeutic approach. Additionally, the severity, frequency, and type of seizures vary significantly between patients [6].

2. Selection of antiepileptic drugs (AEDs). Not all patients respond the same way to a particular medication. Often, several drugs or combinations must be tried before achieving effective seizure control. Around 30% of patients have drug-resistant epilepsy, meaning their seizures are not controlled even after trials of two or more appropriately chosen and dosed AEDs [6].
3. Side effects of AEDs. Antiepileptic medications can cause adverse effects such as drowsiness, mood disturbances, cognitive impairment, liver dysfunction, or bone marrow suppression, often requiring dose adjustments or drug changes.
4. Comorbid conditions. Epilepsy is frequently accompanied by other disorders such as depression, anxiety, cognitive impairment, or somatic illnesses, which complicates both diagnosis and treatment [7].
5. Social and psychological aspects. Fear of seizures, social stigma, loss of employment, difficulties in education or driving - all significantly impact patients' quality of life and require a comprehensive approach beyond pharmacological treatment [8].
6. Access to treatment. In low- and middle-income countries, access to quality healthcare, modern medications, and diagnostic tools (such as MRI or EEG) is often limited, reducing the effectiveness of treatment.
7. Surgical treatment. For a certain group of patients who do not respond to medication, surgical intervention may be considered. However, this requires specialized evaluation, careful patient selection, and access to experienced neurosurgical centres [9].

Therefore, epilepsy therapy demands an individualized approach, interdisciplinary collaboration, and often a prolonged period to find the most effective treatment strategy.

Antiepileptic drugs can have a variety of side effects, which depend on the specific drug, dose, duration of treatment, and individual sensitivity of the patient. The main side effects can be classified into general, cognitive and psychiatric, organ toxicity, and allergic/immunologic effects [9].

1. General side effects:

- Drowsiness, fatigue;
- Dizziness, unsteadiness while walking;
- Nausea, vomiting, loss of appetite;
- Visual disturbances (double vision, blurred vision);
- Tremor, coordination problems [9].

2. Cognitive and psychiatric side effects:
   - Decreased concentration and memory;
   - Slowed thinking;
   - Depression, irritability;
   - Anxiety;
   - In rare cases, suicidal thoughts or behaviour (especially with levetiracetam, topiramate, etc.) [10].
3. Organ toxicity (damage to internal organs):
   - Hepatotoxicity (liver damage) – typical for valproic acid, carbamazepine, phenytoin;
   - Hematological disorders – reduced leukocyte or platelet count, or development of aplastic anaemia;
   - Nephrotoxicity (kidney damage) – can occur with long-term use of some drugs;
   - Pancreatitis – rare but possible (especially with valproates) [10, 11].
4. Allergic and immune reactions:
   - Skin rashes, hives;
   - Stevens-Johnson syndrome or toxic epidermal necrolysis – life-threatening conditions (especially with lamotrigine, carbamazepine);
   - Hypersensitivity reactions, including fever, liver damage, swollen lymph nodes (e.g., DRESS syndrome) [11].
5. Metabolic disorders:
   - Reduced bone density, osteoporosis (especially with long-term use of phenytoin, phenobarbital, carbamazepine);
   - Changes in body weight — weight gain (valproates), weight loss (topiramate);
   - Endocrine disturbances (e.g., menstrual disorders, gynecomastia) [10,11].

6. Teratogenicity.

Some drugs, especially valproic acid, can cause serious birth defects if used during pregnancy [11].

1.3 Characteristics of drug pharmacokinetics

Pharmacokinetics is a branch of pharmacology that examines the changes in drug concentration within the body's biological fluids, considering the processes of absorption, distribution, metabolism, and excretion. Among these, metabolism and elimination play a crucial role, as they largely determine both the duration and effectiveness of a drug's action [12,13].

Drug metabolism involves a complex series of biochemical transformations through which xenobiotics (foreign substances to the body) are converted into more polar and water-soluble metabolites, facilitating their efficient elimination from the body [12,13]. The liver is the primary site of these metabolic conversions, where various enzyme systems operate, with the cytochrome P450 enzyme family playing a central role. However, other organs – such as the kidneys, lungs, gastrointestinal tract, skin, and placenta – also contribute to the metabolic process [14].

The resulting metabolites can vary in their biological activity – they may be inactive, retain pharmacological activity, or even become toxic. Thus, metabolism not only alters the chemical structure of the drug compound but also defines its pharmacological profile, duration of action, bioavailability, and potential for adverse effects. In some instances, it is the metabolite rather than the parent drug that exerts the therapeutic effect — such compounds are known as prodrugs [15].

Elimination represents the final phase of the pharmacokinetic process, responsible for removing drugs and their metabolites from the body. The major routes of elimination include renal (via urine), hepatobiliary (via bile), and pulmonary (via the respiratory tract), along with less prominent pathways such as perspiration, saliva, tears, and breast milk. The rate and efficiency of elimination depend on the compound's physicochemical properties, its degree of plasma protein binding, and the functional status of the liver and kidneys [16,17].

Disruptions in drug metabolism and elimination can lead to the accumulation of toxic compounds in the body, which may result in adverse effects, hepatic and renal toxicity, metabolic imbalances, and drug-induced intoxication [18]. Therefore, a thorough investigation of these processes is a mandatory component of the preclinical evaluation of novel drug candidates.

Notably, more than 70% of drugs used in clinical practice undergo metabolic transformation [19], highlighting the strategic importance of metabolism in pharmaceutical development. These metabolic considerations are critical during the design of new dosage forms, the development of prodrugs, and the assessment of potential drug–drug interactions [20].

In recent years, increasing attention has been given to the integration of artificial intelligence (AI) technologies in pharmacokinetic research. Modern AI systems, equipped with advanced analytical algorithms, are capable of processing large volumes of experimental and clinical data, simulating metabolic pathways, and predicting key pharmacokinetic parameters – including metabolic rates, enzyme involvement, and elimination routes [21].

Machine learning–based models enable rapid *in silico* screening of thousands of chemical compounds, allowing for the early identification of potentially hazardous or ineffective molecules before the onset of experimental testing. This approach significantly reduces research costs, shortens the preclinical development timeline, and enhances the overall success rate in drug discovery [21].

Furthermore, artificial intelligence offers the capability to simulate drug–drug interactions in polypharmacy scenarios, which are increasingly common in modern clinical practice. Predicting the metabolic behaviour of drugs while accounting for patient-specific characteristics paves the way for personalized pharmacotherapy – a cutting-edge direction in contemporary medicine [20].

Thus, the investigation of drug metabolism and elimination using innovative approaches, particularly AI-based methodologies, represents a crucial area of modern pharmaceutical science aimed at improving both the efficacy and safety of pharmacological treatments.

1.4 Characterization of drug metabolism

Drug metabolism encompasses a series of biochemical processes through which the body modifies xenobiotics for the purposes of detoxification, activation, or preparation for excretion. These processes play a central role in determining the pharmacokinetic properties of a drug, including its bioavailability, duration of action, pharmacological activity, and potential toxicity. Biotransformation facilitates the conversion of drug compounds into more hydrophilic metabolites, which promotes their subsequent elimination. In pharmacology, metabolism is generally divided into two main stages – Phase I and Phase II – which can occur sequentially, simultaneously, or even in reverse order, depending on the properties of the substrate [16, 23]. A generalized scheme illustrating the steps involved in Phase I and Phase II metabolism is presented in Figure 1.1.

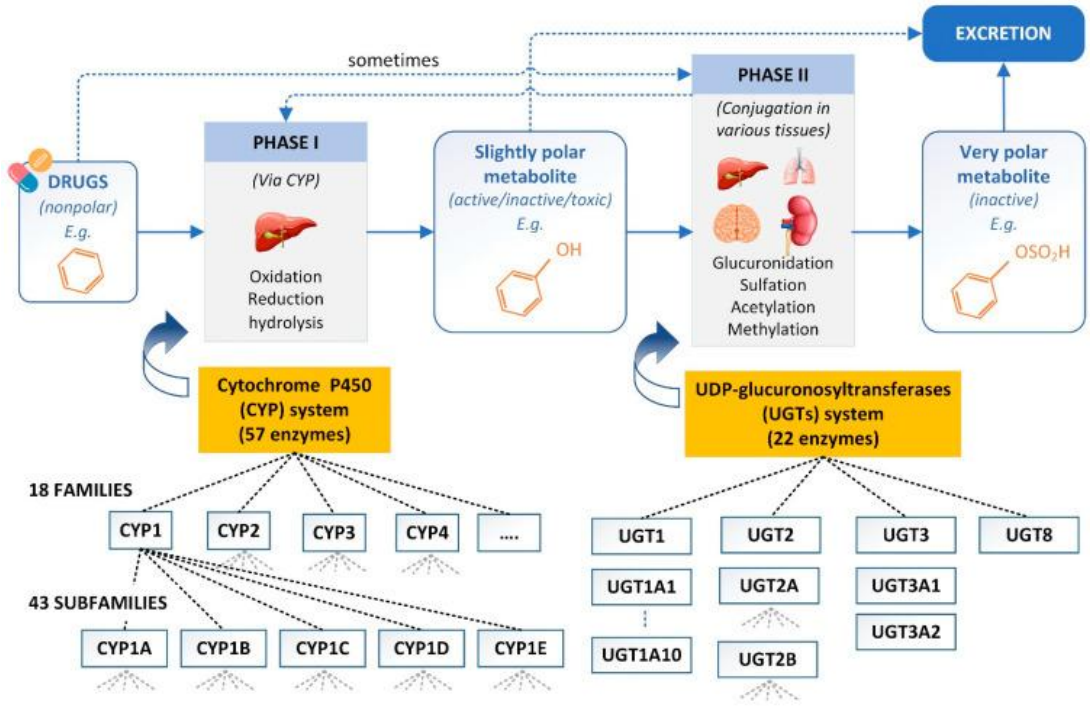


Fig. 1.1 Phase I and Phase II of drug metabolism

Phase I metabolism involves oxidation, reduction, and hydrolysis reactions that result in the chemical modification of drug molecules. These transformations typically produce more reactive metabolites, which may undergo further conjugation

in Phase II or, in some cases, acquire new pharmacological properties on their own. The resulting metabolites can be pharmacologically inactive, thereby reducing the drug's overall efficacy, or they may exhibit biological activity – occasionally even surpassing that of the parent compound. In certain instances, these metabolites can be toxic or induce adverse effects, making their identification and characterization essential for drug safety assessment [24].

The primary enzymes involved in Phase I metabolism are cytochrome P450 enzymes – a large family of hemoproteins predominantly localized in the liver, although they are also found in other tissues such as the intestine, lungs, kidneys, and brain. These enzymes mainly catalyze monooxygenation reactions, in which one atom of oxygen is incorporated into the substrate and the other is released as water.

It is known that 57 CYP isoforms are expressed in the human body, but only five (1A2, 2C9, 2C19, 2D6, and 3A4) account for approximately 95% of all drug metabolism. In particular, the CYP3A4 isoenzyme is the most active in the metabolism of a wide range of xenobiotics, including many commonly used pharmaceutical drugs, and is thus of particular interest in the study of drug interactions and individual variations in therapeutic responses [25].

Phase II of metabolism is characterized by conjugation reactions, where polar endogenous compounds, such as glucuronic acid, sulfate groups, amino acids, or glutathione, are attached to molecules that have already been modified in Phase I. These reactions serve as a detoxification mechanism, as conjugated metabolites are generally less toxic and more water-soluble than their precursors. Due to their increased hydrophilicity, these compounds are more easily excreted from the body, primarily via the kidneys (in urine) or through bile from the liver. Thus, Phase II plays a critical role in completing the biotransformation of xenobiotics, reducing the risk of their accumulation and toxicity [25].

The most significant enzymes of Phase II are UDP-glucuronosyltransferases (UGT), which catalyze the attachment of glucuronic acid, sulfotransferases (SULT), responsible for sulfation, and glutathione S-transferases (GST), which facilitate conjugation with glutathione. These enzymes have a broad substrate specificity and

are expressed in various tissues, including the liver, kidneys, gastrointestinal tract, and lungs. It is important to note that the effectiveness of Phase II reactions can be influenced by genetic polymorphisms, age, sex, health status, and interactions with other drugs, which may affect both the pharmacokinetics and pharmacodynamics of the drugs. In some cases, metabolites formed during conjugation may retain biological activity or even have their own pharmacological effect, which is also considered when developing new pharmaceutical drugs [26].

It should be noted that the rate and efficiency of metabolic processes can vary significantly depending on several factors, such as genetic variations, age, sex, liver function, comorbidities, diet, and the influence of other medications. Specifically, genetic polymorphisms in genes encoding CYP enzymes can lead to different phenotypes of metabolizes, including fast or slow metabolizes, which significantly affect the effectiveness and safety of pharmacotherapy [27].

A particular emphasis is placed on studying the inhibition and induction of metabolic enzymes, as this underlies numerous drug interactions. CYP inhibitors can decrease the metabolism rate of co-administered drugs, increasing their plasma concentration and the risk of toxicity. In contrast, inducers accelerate metabolism, which may reduce the therapeutic effectiveness of the drugs. These aspects are critically important in polypharmacy and in the development of new pharmacological agents [28].

To optimize the process of developing new drugs at the preclinical stage, enzymatic studies are actively used, which allow:

- determining the metabolic stability of molecules,
- quantitatively assessing and identifying the main metabolites,
- establishing the primary metabolic pathways,
- predicting potential drug interactions [29].

In modern conditions, particular attention is given to information technologies and in silico methods, specifically the use of artificial intelligence to predict metabolism. Applying these methods allows significantly reducing the time and

resources needed for investigating new compounds and also increases the accuracy of predictions of their pharmacokinetic properties [30, 31].

In particular, in silico prediction of drug metabolism is usually divided into three main areas [32]:

1. Prediction of the site of metabolism (SOM) – allows for the identification of atoms in a molecule most likely to undergo metabolic transformation.
2. Prediction of metabolite structures – is based on simulating phase I and II reactions, considering the enzymatic specificity involved.
3. Prediction of pharmacokinetic parameters, including clearance, half-life, and bioavailability, taking into account metabolic pathways [33].

Integrating these approaches into the preclinical development process helps create more effective, safer, and personalized treatment strategies, particularly for chronic and polyetiological diseases. [32, 33]

1.5 Predicting of drug metabolism sites

Site of Metabolism (SOM) refers to the specific position (atom or group) in a molecule where a metabolic transformation occurs, such as oxidation or conjugation. Predicting the site of metabolism (SOM) is a crucial step in xenobiotic research, as it allows for the prediction of potential metabolites that could form in the body. Identifying the likely site of metabolic transformation in a molecule's structure enables chemists to model potential metabolites based on the location of reactive atoms or functional groups [34]. The use of in silico methods to predict SOM and corresponding metabolite structures, especially in reactions mediated by CYP450 enzymes, is an important tool in the early stages of studying metabolic pathways. This, in turn, helps improve the safety and efficacy profile of drugs. Several specialized programs have been developed for this purpose, capable of modeling phase I and II metabolic transformations, including FAME, FAME 2, FAME 3, GLORY, GLORYx, BioTransformer, CypReact, CyProduct, PreMetabo, and Xenosite [35-40].

The web tool CypReact implements machine learning approaches to predict the likelihood of low-molecular-weight compounds interacting with nine key isoforms of the CYP system. Random forest models are used for seven isoforms, while ensemble models combining RF, support vector machines, logistic regression, and decision trees are used for 2C9 and 2D6. Substrate specificity prediction is based on the analysis of structural characteristics and physicochemical properties of the molecules. To improve the quality of the training dataset, the authors used 679 compounds from the XenoSite database and manually gathered an additional 1053 chemical compounds that do not interact with CYP. The dataset included drugs, food components, pesticides, environmental pollutants, endogenous metabolites, and other types of substances. The developed classifiers demonstrated high effectiveness, with AUC values ranging from 83–92% [41].

Another tool developed by the same research group is CyProduct — an in silico platform for predicting CYP-mediated metabolism products. It consists of three interrelated modules [42-43]:

1. CypReact – predicts whether a compound can undergo a reaction with a specific CYP isoform;
2. CypBoM Predictor – identifies the "binding site" of the reaction, i.e., the chemical bonds that are subject to transformation;
3. MetaboGen – generates structures of possible metabolites based on the predicted binding site [42-43].

CyProduct covers nine major CYP isoforms: 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4. A unique feature of CypBoM is the introduction of the concept of "metabolism bond" – an expanded version of the "site of metabolism," which identifies not only individual atoms but also entire chemical bonds that are modified during the reaction. A specialized dataset containing 1845 CYP-mediated phase I metabolic reactions was created for model training. The prediction accuracy of reactive bonds, assessed using the Jaccard index, ranged from 0.380 to 0.452 for all nine isoforms, indicating higher accuracy compared to alternative tools – specifically, 0.13 higher than FAME 2 and 0.12 higher than FAME3 [43].

In conclusion, CyProduct demonstrates superior performance in predicting metabolites, surpassing other well-known software products such as ADMET Predictor, GLORY, and BioTransformer. Specifically, for a test set of 68 CYP substrates, CyProduct's performance was on average 200% higher. Compared to BioTransformer, the advantage was about 30%. Thanks to its accuracy, innovative approach to determining sites of metabolism, and modern architecture, CyProduct is one of the newest and most effective tools for predicting metabolic transformations [43].

The latest version of the software tool, GLORYx, has significantly improved previous approaches to predicting drug metabolism by integrating the determination of sites of metabolism (SOM) with reaction rules to predict both phase I and phase II transformations. Unlike previous versions, GLORYx implements a comprehensive strategy that combines machine learning with expert knowledge of biotransformation reactions. To identify the most likely sites of metabolism, the FAME 3 model is used, trained based on machine learning and working with a SOM dataset that includes 1748 parent molecules sourced from the MetXBioDB database [40, 42].

The FAME 3 model is based on the Random Forest method and uses circular atomic descriptors combined with 15 key two-dimensional descriptors from the Chemistry Development Kit to accurately predict the likelihood of a metabolic reaction at a specific position in the molecule. As a result, GLORYx can generate potential metabolites based on the predicted sites, taking into account typical biotransformation patterns [39].

The main reason for this is the limited number of publicly available and reliably annotated data on the metabolism of small molecules, which limits the accuracy and generalizability of predictions for a wide range of chemical structures. As a result, the further development of GLORYx is directly dependent on the enrichment of high-quality databases that reflect the diversity of metabolic pathways [40].

1.6 Prediction of cytochrome P450 inhibition

Inhibitors of cytochrome P450 enzymes are critical in regulating drug metabolism by inhibiting the activity of specific CYP isoforms involved in the biotransformation of substrates. This alteration in enzyme activity can lead to changes in pharmacokinetic parameters such as bioavailability, half-life, and clearance, and may also impact the pharmacodynamic properties of drugs, potentially leading to adverse effects or toxicity. Additionally, the rate and extent of CYP-mediated metabolism are influenced by several factors, including the use of other medications, genetic variations, age, sex, diet, and the overall health status of the individual [46].

Due to the complexity of these interactions, predicting whether a compound will act as a substrate or an inhibitor of CYP is a challenging task, requiring a comprehensive analysis of its structural, physicochemical, and biological properties. Despite the development of numerous in silico models, the accuracy of these predictions remains variable and often requires experimental verification [46].

In recent years, significant research has been devoted to predicting inhibitory activity against specific CYP isoforms, with many studies showing high accuracy rates. Focus has been placed on five major CYP isoforms which are responsible for metabolizing most drugs. Several tools have been developed, including DeepCYP [44], SuperCYPsPred [45], CYPlebrity [44], iCYP-MFE [46], VirtualRat [33], and others which offer effective predictions of small molecule interactions with these isoforms [47, 48].

1.7 Predicting of drug elimination

Drug elimination is a key process in pharmacokinetics, involving the removal of active substances from the body either as the unchanged drug or its metabolites [17]. This is a complex, multi-step process that includes several primary elimination pathways. The most significant pathway is renal, responsible for the excretion of predominantly water-soluble compounds. Another important mechanism is biliary excretion, which eliminates substances that are poorly absorbed in the gastrointestinal tract. Although the contribution from other routes such as the intestines, saliva, sweat, breast milk, or lungs is relatively minor, it can have clinical significance. For example,

volatile anaesthetics may be actively eliminated through the respiratory system, and drugs that pass into breast milk may potentially affect infants [17].

When developing new drugs, elimination parameters must be considered, as they play a crucial role in determining the safety and effectiveness of therapy. This includes toxicological validation, preliminary risk assessment for humans, analysis of potential drug interactions, and dosimetry for clinical studies.

Key pharmacokinetic parameters that characterize the elimination process include clearance and half-life ($t_{1/2}$). Clearance (Cl) is defined as the volume of plasma from which a drug is removed per unit of time and is measured in l/hour or ml/min [49]. Total clearance includes the sum of hepatic, renal, and extra-organ clearance. Its value depends on several physiological and pharmacological factors: cardiac output, body weight, body surface area, liver and kidney function, the degree of drug binding to plasma proteins, concomitant therapies, and the expression levels of metabolizing enzymes [50].

Since clearance influences bioavailability, dosing, and frequency of drug administration, it is one of the most important parameters both in preclinical stages and in clinical practice [59].

As a result, numerous tools for predicting ADMET parameters have been developed, including the FP-ADMET software, which employs machine learning algorithms to predict various types of clearance: intrinsic, renal, metabolic intrinsic, and human liver microsomal clearance [52]. This tool is built upon the Random Forest (RF) algorithm, using molecular fingerprints and databases that include thousands of compounds from previous studies [53-55]. For instance, when predicting renal clearance, 244 compounds were used, and the FP-ADMET model demonstrated better accuracy compared to the Chen et al. model on the same data [56].

AstraZeneca, based on a large internal dataset of 73,620 compounds, applied the SVM algorithm and achieved an RMSE of 0.377 [57]. Modern approaches are also actively incorporating deep learning. For example, combining machine learning methods with DeepSnap-DL enabled the creation of a new clearance prediction model

based on 1545 compounds, achieving an AUC of 94.3% and an accuracy of 87.4% [58], which outperforms individual ML or DL models.

Thus, the development and improvement of clearance prediction models, particularly through the integration of classical ML algorithms and modern DL architectures, opens up new opportunities for more efficient preclinical drug evaluation and optimization of their pharmacokinetic properties

1.8 Databases for metabolism prediction

Selecting the appropriate database is a crucial step in developing accurate and reliable AI-based metabolism and drug elimination prediction models. Achieving high efficiency requires considering not only the quantity but also the quality, relevance, and completeness of the data. In this context, providing access to reliable information is essential for analyzing metabolic pathways, predicting biotransformation, and optimizing the pharmacokinetic properties of drugs. Below are some of the most widely used databases actively employed in metabolism research and drug development:

- HMDB 5.0 – a large database of low-molecular-weight metabolites found in the human body, including information on their chemical and physical properties, metabolic pathways, and clinical biomarkers. HMDB contains data on over 220,000 metabolites and 8,500 protein sequences [59].
- METLIN – f metabolite database containing information on more than 960,000 compounds. It includes molecular formulas, chemical structures, and biological activities of metabolites. METLIN also offers MS/MS data for different collision energy values in both positive and negative ionization modes [60].
- MetaCyc – database of metabolic pathways and enzymes for various organisms. It includes information on 3,085 pathways, 18,785 metabolites, and 18,391 reactions involved in metabolite biotransformation. It can be used to build metabolic models for specific organisms.
- MetXBioDB – database of metabolic pathways and enzymes for a range of organisms, including bacteria, archaea, and eukaryotes. MetXBioDB contains

data on more than 2,000 biotransformations, including information on enzyme structure and function, as well as reactions and pathways involved in metabolite biotransformation [42].

- Metabolights – a database that includes information on metabolites, metabolic pathways, and metabolic networks of over 27,500 compounds. Metabolights also provides tools for data analysis and visualization, as well as resources for sharing and reusing metabolic data [61].
- KEGG Pathway – a database containing maps and diagrams of metabolic networks, as well as information on enzymes and metabolites. It includes information on over 17,000 metabolic pathways and more than 22,000 enzymes [62].
- HumanCyc – database of human metabolic pathways, enzymes, and the human genome. HumanCyc contains data on reactions and biotransformation pathways of metabolites, as well as enzymes and genes involved in these processes. It includes data on 28,783 genes and their products, as well as the metabolic processes and pathways they catalyze [63].
- DrugBank – database of drugs and their targets, including information on drug metabolism and pharmacokinetics, as well as enzymes involved in drug biotransformation. It contains data on over 500,000 drugs, related targets, pathways, and metabolic processes [64].
- ChEMBL – a atabase of biologically active molecules, including drugs and drug candidates, with information on their activity, targets, and metabolic pathways. It contains data on over 2.3 million compounds and their associated activity and targets [65].
- PubChem – publicly accessible database of chemical structures and their associated biological activity, containing information on over 114 million compounds, as well as tools for data analysis and visualization [66].
- OCHEM – a platform for developing and validating predictive models for chemical and biological data. OCHEM includes tools for data preprocessing,

feature selection, and model training, as well as a library of pre-trained models. It contains over 3.7 million entries for 689 properties [67].

- OpenFDA – database of FDA-approved drugs, including information on drug labeling, side effects, and clinical trial data. OpenFDA includes tools for data analysis and visualization, as well as an API for accessing FDA data [68].

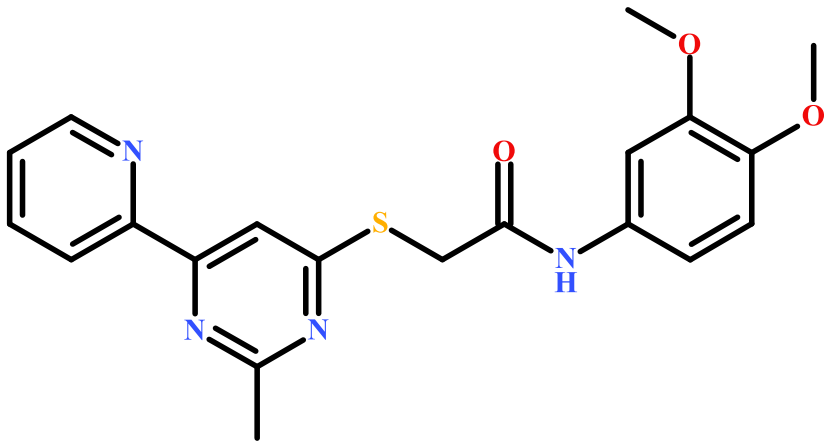
Conclusions for Chapter 1

The development and implementation of advanced methods for predicting drug metabolism is a crucial aspect of modern pharmaceutical science and industry. Through artificial intelligence technologies, particularly machine learning and deep learning, significant progress has been made in predicting pharmacokinetic properties such as clearance and metabolism. The integration of classical ML algorithms with modern DL architectures allows for the creation of more accurate and reliable models, opening new possibilities for optimizing the drug development process. A key stage in this process is the selection of the right databases, which provide essential information for analysing metabolic pathways, biotransformation, and interactions between drugs and their targets. Thus, the development of databases and prediction methods are essential steps in improving the effectiveness and safety of drugs. Thanks to advanced analysis and modelling tools, the pharmaceutical industry gains a powerful instrument for faster and more accurate evaluation of pharmacokinetic properties, which, in turn, accelerates the development of new drugs and reduces risks in clinical trials.

CHAPTER 2

MATERIALS AND METHODS OF RESEARCH OF N-(3,4-DIMETHOXYPHENYL)-2-[2-METHYL-6-(2-PYRIDYL)PYRIMIDIN-4-YL]SULFANYL-ACETAMIDE

As part of this study, the object of investigation was a new promising compound with anticonvulsant activity – N-(3,4-dimethoxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanyl-acetamide, which was provisionally named "Epirimil" (Fig. 2.1). The synthesis of this molecule was carried out in the laboratory of the Department of Pharmaceutical Chemistry at the National University of Pharmacy (NUPh) under the scientific supervision of Doctor of Pharmaceutical Sciences, Professor Hanna Severina [69].



Epirimil

The selection of this compound as the object of study was driven by its promising pharmacological profile, which includes strong anticonvulsant activity demonstrated in various *in vivo* seizure models and low toxicity. Its biological activity is attributed to the presence of biologically active fragments in the structure – pyrimidine derivatives and a thioacetamide group – which are known for their ability to modulate neurotransmitter system functions. Preliminary preclinical trials showed that Epirimil exhibited a high level of anticonvulsant activity compared to reference drugs such as phenobarbital, sodium valproate, and carbamazepine.

During the research, modern methods of pharmaceutical analysis, molecular docking, *in vitro* and *in vivo* models for testing anticonvulsant activity, as well as molecular modeling were used to assess the possible mechanisms of interaction of the compound with biological targets involved in the development of seizure conditions.

2.1 Synthesis of N-(3,4-dimethoxyphenyl)-2-((2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl)thio)acetamide

The synthesis of N-(3,4-dimethoxyphenyl)-2-((2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl)thio)acetamide was performed in three main stages.

Step 1. Synthesis of 2-methyl-6-(pyridin-2-yl)pyrimidin-4(3H)-one (2.3)

Ethyl 3-oxo-3-(2-pyridyl)propanoate (2.2) (1 mol) was dissolved in 100 mL of anhydrous methanol. To this solution, a freshly prepared solution of sodium methoxide (3 mol in 300 mL of methanol) was added. The reaction mixture was stirred for 30 minutes at room temperature (25 °C). Subsequently, amidine hydrochloride (2.1) (1.5 mol) was added portionwise over 30 minutes under continuous stirring. The mixture was then heated at 80 °C for 8 hours. After completion of the reaction, the mixture was cooled to 25 °C, and acetic acid (3 mol) was added to neutralize the reaction medium. Methanol was removed under reduced pressure using a rotary evaporator. The residue was diluted with 200 mL of distilled water and stirred for 30 minutes. The precipitate formed was filtered off, washed three times with 100 mL portions of water, and dried under vacuum. Yield: 95%.

Step 2. Synthesis of 2-methyl-6-(pyridin-2-yl)pyrimidin-4(3H)-thione (2.4)

2-methyl-6-(pyridin-2-yl)pyrimidin-4(3H)-one (2.3) (1 mol) was suspended in 300 mL of toluene, and Lawesson’s reagent (1.1 mol) was added. The reaction mixture was refluxed with vigorous stirring for 5 hours. After cooling to 25 °C, the formed precipitate was filtered, washed with toluene, recrystallized from isopropanol, and dried under vacuum. Yield: 84%.

Step 3. Synthesis of N-(3,4-dimethoxyphenyl)-2-((2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl)thio)acetamide (2.5)

2-methyl-6-(pyridin-2-yl)pyrimidin-4(3H)-thione (2.4) (1 mol) was dissolved in 100 mL of dimethylformamide (DMF). Triethylamine (1.1 mol) and 2-chloro-N-(3,4-dimethoxyphenyl)acetamide (1.1 mol) were added at 25 °C. The reaction mixture was stirred vigorously and heated at 60 °C for 5 hours. Upon completion, the mixture was cooled to room temperature, and 500 mL of water was added to precipitate the product. The solid was filtered, washed with water, and recrystallized from isopropanol. Yield: 92%. Melting point: 220–222 °C.

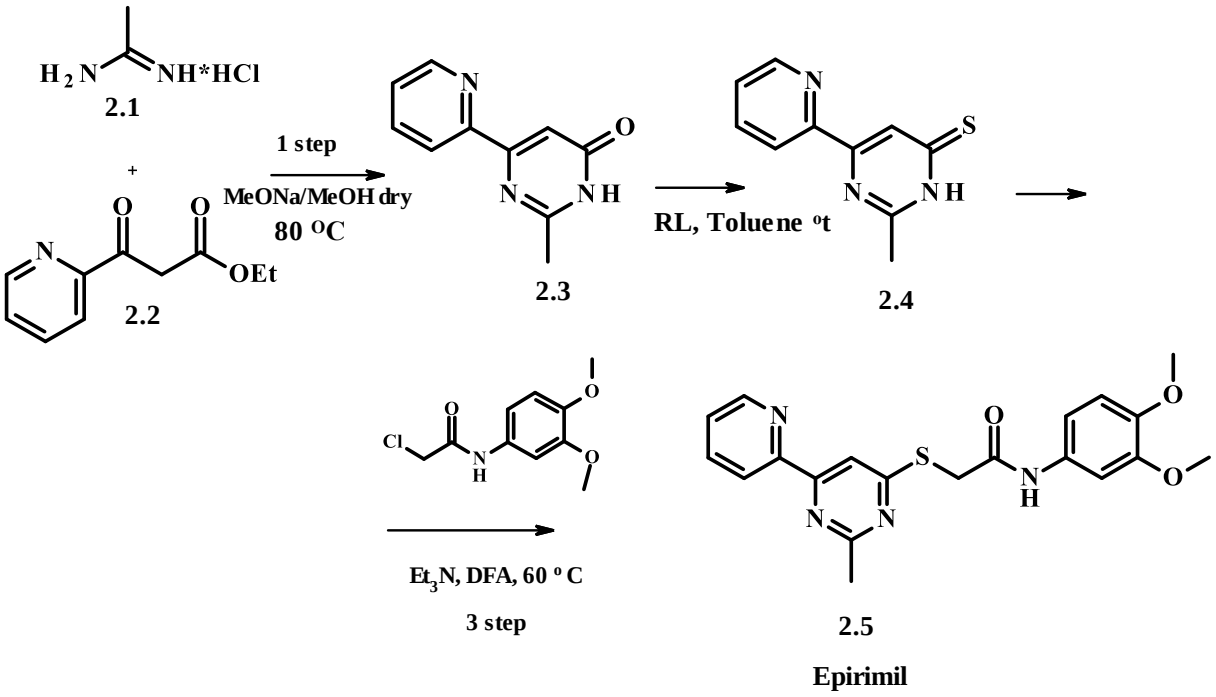


Figure 2.1 Scheme of Epirimil synthesis

2.2 Discussion of the results of pharmacological action of Epirimil

The anticonvulsant potential of N-(3,4-dimethoxyphenyl)-2-((2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl)thio)acetamide was assessed through extensive *in vivo* studies that adhered to all established guidelines for the preclinical evaluation of antiepileptic drugs (AEDs). Following international standards for the development of new AEDs – specifically, the Anticonvulsant Drug Development Program and the Epilepsy Therapy Screening Program – it is essential to investigate the compound's efficacy not only against pentylenetetrazole-induced seizures but also in a model of

primarily generalized clonic-tonic seizures provoked by electrical stimulation (the maximal electroshock seizure test). The seizure mechanism in the MES model involves depolarization of neuronal membranes due to sodium ion influx. Screening for anticonvulsant efficacy was therefore conducted using both pentylenetetrazole-induced and MES-induced seizure models [69].

Pentylenetetrazole-induced seizures. Experimental animals were administered a single intragastric dose of Epirimil (50 mg/kg) or reference compounds – lamotrigine ("Lamictal," GlaxoSmithKline) at 20 mg/kg and phenobarbital ("Phenobarbital IC," Interchem, Ukraine) at 20 mg/kg. Both Epirimil and the reference drugs were dissolved in Tween-80 and delivered via a gastric cannula at a volume of 0.5 mL/100 g body weight, one hour prior to seizure induction. Control animals received an equivalent volume of solvent. Pentylenetetrazole (Sigma, USA) was administered subcutaneously as an aqueous solution at a dose of 80 mg/kg. Following convulsant administration, each mouse was individually placed in a plastic cylindrical container (20 cm in diameter and 35 cm in height) and continuously observed for 60 minutes. If no seizures occurred during this period, the latent period was recorded as 60 minutes. Anticonvulsant effects were evaluated based on several parameters: latency to clonic or tonic seizures, severity of paroxysms (scored), duration of the convulsive episode, and mortality rate [69].

N-(3,4-dimethoxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]thio-acetamide (Epirimil) exhibited strong antiepileptic properties, completely preventing seizure onset in the treated animals and demonstrating efficacy comparable to that of the reference drug phenobarbital across all measured outcomes (Figure 2.2).

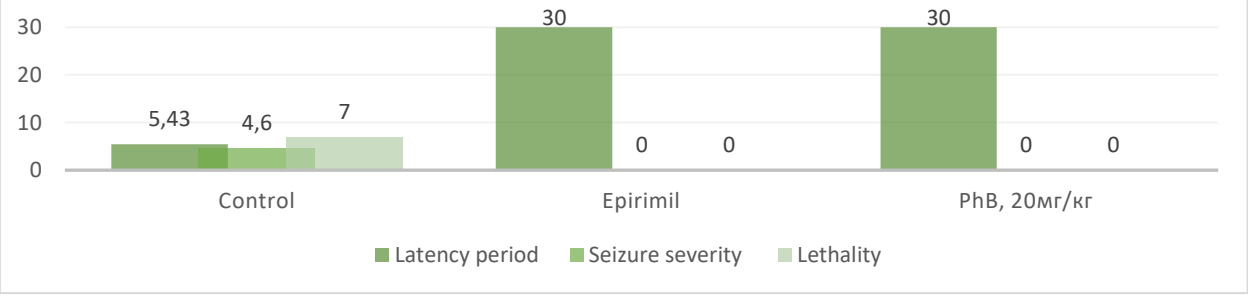


Fig. 2.2 Indicators of anticonvulsant activity of Epirimil

MES-induced seizures.

The study was conducted on 40 outbred mice of both sexes, weighing 25–28 g. The animals were divided into four groups, with 10 mice in each group. Group 1 served as the control. Animals in Groups 2, 3, and 4 received intragastric administration of compound 2.5 (50 mg/kg), lamotrigine (20 mg/kg) (GlaxoSmithKline Pharmaceuticals), and carbamazepine (15 mg/kg) (Novartis), respectively. The evaluation of anticonvulsant activity was performed one hour after compound administration. Electrically induced seizures were provoked using a Ugo-Basile ECT 57800 device (Italy) equipped with corneal electrodes. To reproduce the MES model, a 50 mA current with a frequency of 50 Hz and a duration of 0.2 seconds was applied using sinusoidal stimuli. Electrodes were moistened with a 0.9% sodium chloride solution ("ARTERIUM," Ukraine), and a 2% lidocaine hydrochloride solution ("EGIS," Hungary) was instilled into the conjunctival sac to minimize discomfort [69]. The following parameters were recorded: the number of mice exhibiting tonic seizures, total seizure duration, and mortality rate.

In the MES test, compound 2.5 demonstrated pronounced anticonvulsant activity (Figure 2.3), including a reduction in the incidence of electroshock-induced seizures to 10%, an 86.9% decrease in seizure duration, and complete prevention of hind limb tonic extension in 100% of animals ($p < 0.05$).

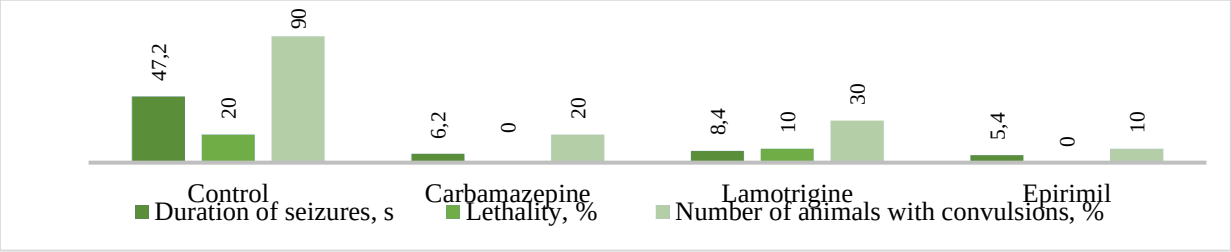


Fig. 2.3 Anticonvulsant activity of Epirimil in the MES model

The derivative 2.5 was not inferior in anticonvulsant effect to the comparison drug carbamazepine and slightly superior to lamotrigine in all parameters. On the background of compound 2.5 administration there were no dead animals, and the total duration of convulsive attack was 88.5% shorter ($p<0.05$) than in animals without pharmacological correction.

2.3 Study of acute toxicity

Acute toxicity (LD_{50} and its confidence interval) was assessed using the method of V.B. Prozorovsky as modified by T.V. Pastushenko [52]. The experiment involved 34 outbred white mice, standardized by body weight (24 ± 3 g), which were divided into five groups. The test groups received Epirimil orally at doses ranging from 100 mg/kg to 1000 mg/kg, dissolved in an appropriate amount of Tween-80. The animals were observed over a 14-day period. On the first day, continuous monitoring was conducted. Researchers recorded animal behavior and body weight, assessed clinical signs of intoxication – general condition, motor activity, respiratory pattern, condition of skin and fur, presence of convulsions, food and water intake—and noted the number of deaths in each group.

The results of the acute toxicity study of the compound are presented in Figure 2.4

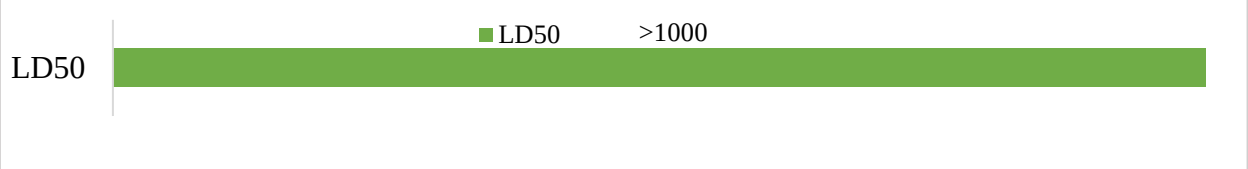


Fig. 2.4 LD_{50} of Epirimil

At higher doses, Epirimil exhibited pronounced hypnosedative effects, including respiratory depression and reduced locomotor activity, which progressed to a deep narcotic state resulting in animal death. Mortality was recorded within the first three days following administration of high doses. Surviving animals regained activity within 12 to 24 hours and remained clinically stable over the subsequent 14-day observation period. No significant differences in food intake or body weight were observed compared to the control group. Reflexes remained intact, and no behavioral abnormalities or clinical signs of intoxication were detected. The skin remained smooth and glossy, with no redness, scaling, cracking, or other visible changes. Furthermore, surviving mice showed no statistically significant deviations in body weight.

The calculated LD_{50} value for compound 2.5 administered orally in white mice was 522.0 mg/kg (confidence interval: 432 – 613 mg/kg). According to the Hodge and Sterner toxicity classification system, Epirimil falls under Class IV – low-toxicity substances.

Conclusions to Chapter 2

1. The methods for the synthesis of the studied compound, *N*-(3,4-dimethoxyphenyl)-2-((2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl)thio)acetamide (Epirimil), have been described and characterized in detail. The synthetic route allows for the efficient preparation of the target molecule with a satisfactory yield and purity, which is essential for further pharmacological evaluation.
2. The pharmacological potential of *N*-(3,4-dimethoxyphenyl)-2-((2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl)thio)acetamide has been substantiated, supporting its consideration as a promising active pharmaceutical ingredient (API). Preliminary biological studies indicate its anticonvulsant activity, combined with a low level of acute toxicity and a favourable safety profile. These findings justify the need for further in-depth pharmacological investigations to fully assess its therapeutic potential and mechanism of action.

CHAPTER 3

PREDICTION OF EPIRIMIL METABOLISM USING XENOSITE WEB TOOLS

3.1. List of computer programs used for biotransformation analysis

Several tools can be used to predict P450 metabolism sites and the metabolic properties of molecules. These tools help obtain forecasts that aid in understanding how a molecule may be metabolized.

1. XenoSite – one of the most popular tools for predicting sites of metabolism (SOMs) in molecules. It uses machine learning based on experimental data. XenoSite also allows for isoform-specific P450 predictions, which is useful for more accurate analysis when specific enzymes (e.g., CYP3A4, CYP2D6) are involved. XenoSite predicts which atoms in a molecule are likely to undergo metabolism by cytochrome P450 (CYP450) enzymes. It identifies SOMs – regions of the molecule most likely to be altered during metabolism – and can perform both general P450 metabolism predictions and predictions for specific isoforms.
2. GLORYx – is a computational tool used in cheminformatics and drug metabolism research to predict the formation of reactive metabolites. It simulates both Phase I and Phase II biotransformations of xenobiotics, such as drug candidates, by integrating tools like SMARTCyp and BioTransformer to identify likely sites of metabolism (SOMs) and generate potential metabolite structures. GLORYx is particularly useful for forecasting the formation of electrophilic or otherwise reactive species that could covalently bind to nucleophilic biological macromolecules like proteins or DNA – an important aspect in early drug development for assessing metabolic liability and toxicity risks.
3. MetaSite – Another powerful tool for predicting metabolic changes in molecules. It also utilizes a metabolism database and can forecast potential sites

of metabolism for cytochrome P450. It is particularly useful in the study of molecules with potential toxicity or metabolic stability issues.

4. ADMET Predictor – A comprehensive tool for predicting ADMET properties (absorption, distribution, metabolism, excretion, and toxicity), including P450 metabolism. This tool allows not only metabolism prediction but also a more detailed assessment of a molecule's potential toxicity.
5. PharmacoPy – Another tool for predicting molecular metabolism, helping to identify which regions of a molecule may undergo metabolic transformations as a result of P450 interactions. It can be used to detect potentially bioactive or toxic metabolites.
6. Derek Nexus – Specialized in toxicity and bioactivation prediction, particularly for molecules metabolized via P450 enzymes. It is valuable for identifying potentially hazardous metabolites at early stages of molecular development.

These tools not only predict metabolic sites but also assess other important characteristics of the molecules.

3.2 Results of predicting possible metabolic pathways of the anticonvulsant Epirimil using the XenoSite

Phase I enzymes play a key role in drug biotransformation, catalyzing over 90% of metabolic reactions associated with FDA-approved drugs. These enzymes facilitate a wide range of chemical transformations, resulting in metabolites with significant structural diversity.

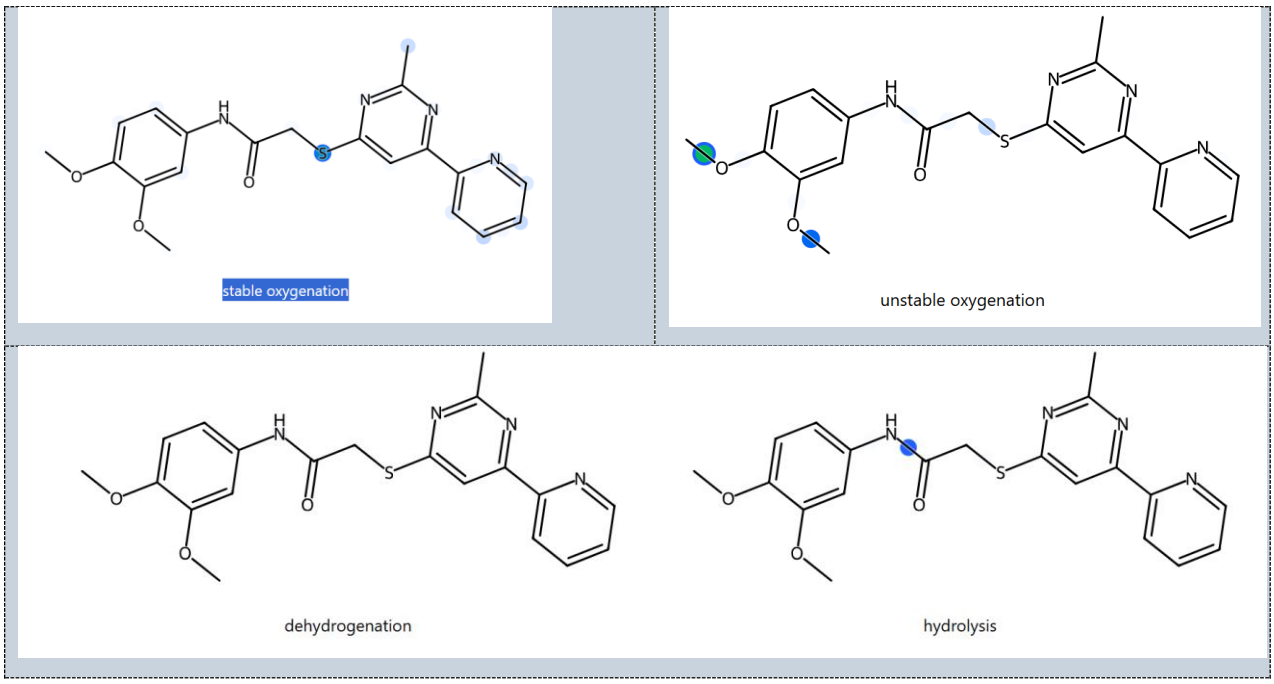
To systematize this diversity, a unified annotation scheme has been proposed, which simultaneously identifies the sites of metabolism (SOMs) and classifies the types of reactions. Within this scheme, all reactions are divided into five key categories:

1. Stable oxidation – A type of phase I metabolic reaction in which an atom or group of atoms in a molecule is oxidized without forming chemically unstable or reactive intermediates.

2. Unstable oxidation – A phase I metabolic reaction in which reactive or unstable intermediates are formed, which may be toxic or biologically hazardous.
3. Dehydrogenation – A process involving the removal of hydrogen atoms (H) from a molecule. This reaction is often catalyzed by cytochrome P450 enzymes.
4. Hydrolysis – A reaction that involves the cleavage of a chemical bond through the addition of water (H_2O).
5. Reduction – A metabolic reaction in which a molecule gains electrons or hydrogen atoms, resulting in the reduction of the oxidation state of certain functional groups.

These five classes encompass 21 types of phase I reactions, which collectively account for 92.3% of all documented metabolic transformations in our database.

The result of the XenoSite calculation is the fragments of the Epirimile molecule or other atomic sites of the molecule - the sites of metabolism (SOM) – that are primarily transformed by the P450 system. The results of the prediction for Epirimil shown in Fig. 3.1



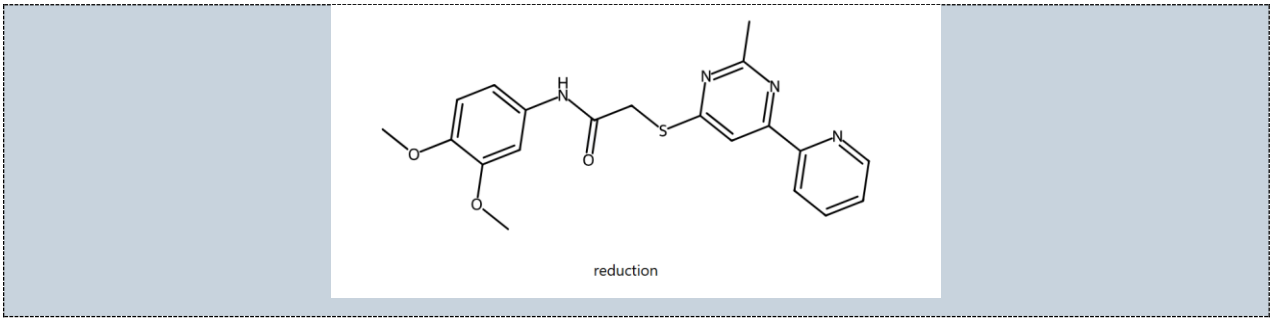


Fig. 3.1 Results of predicting possible pathways of Epirimil metabolism using the online service XenoSite

A high probability of stable oxidation of the sulfur atom in two steps was predicted: the formation of mono- (I) and disulfoxide (II) N-(3,4-dimethoxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfinyl-acetamide. In addition, stable oxidation is possible by the formation of N-oxide at the pyridine substituent at position 6 (III). The stable oxidation reaction leads to the formation of more polar and stable metabolites that are easily excreted from the body and do not cause toxicity, which is a key difference from unstable oxidation.

In the first phase of metabolism, unstable oxidation of the methoxy groups at the 3 and 4 positions of the phenyl radical is likely to occur through oxidative O-dealkylation catalyzed by oxidoreductases, resulting in the formation of O-dealkylated derivatives – N-(4-hydroxy-3-methoxy-phenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanyl-acetamide (IV) or N-(3,4-dihydroxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanyl-acetamide (V).

The conversion scheme of metabolism is shown in Fig. 3.2.

One of the most probable directions of metabolism is the hydrolysis reaction under the action of hydrolases to form two products - 2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanylacetic acid (VI) and 3,4-dimethoxyaniline (VII) (Fig. 3.2).

Dehydrogenation and reduction reactions are not predicted.

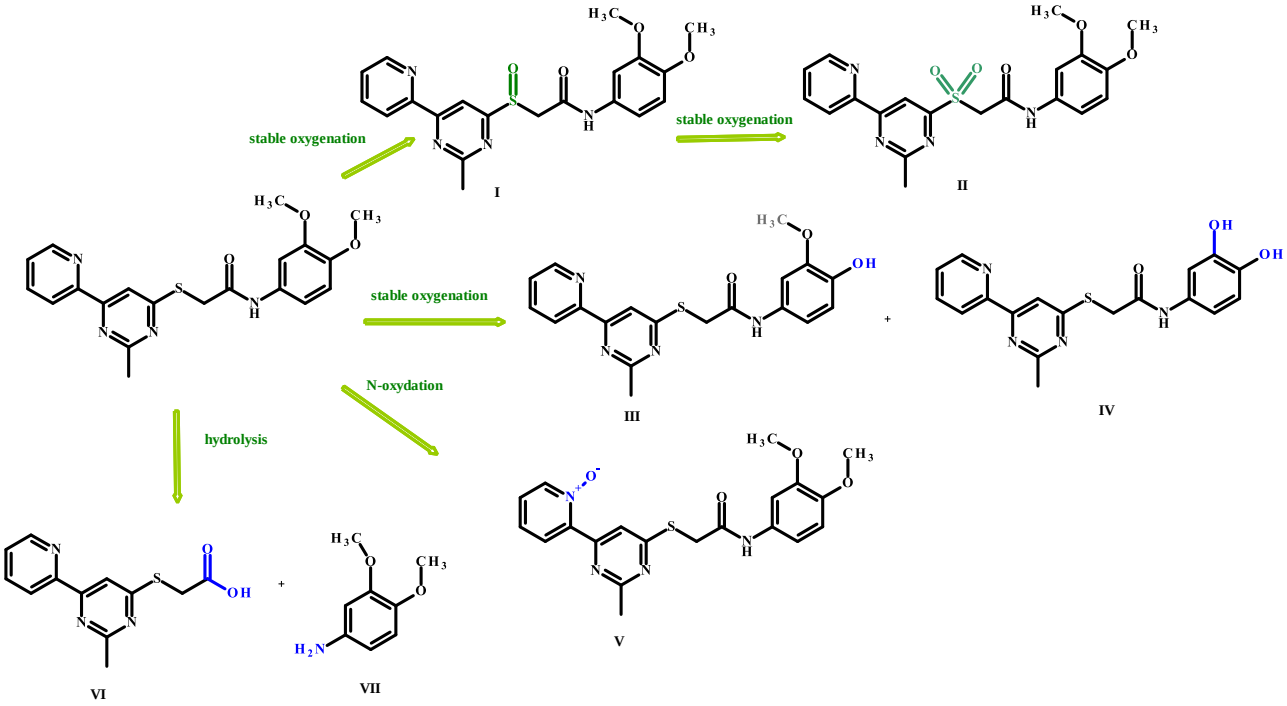


Fig. 3.2 Main directions of Epirimil metabolism predicted by XenoSite

Despite substantial resource investment, approximately 40% of drug candidates are terminated due to toxicity, frequently caused by interactions between electrophilic drugs or their metabolites and nucleophilic biological macromolecules such as DNA and proteins. For Epirimil, the formation of electrophilic metabolites capable of interacting with nucleophilic DNA is not predicted.

Glucuronide formation at the first position of the pyridine ring is also possible, catalyzed by uridine diphosphate glucuronosyltransferase (UGT) (Fig. 3.4).

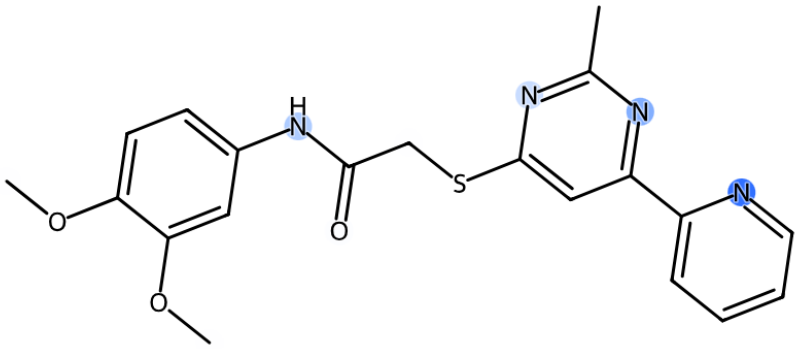


Fig. 3.4 SOM of UGT conjugation

It should be noted that no epoxidation sites were identified, and the likelihood of epoxide formation is low. Epoxides are common reactive metabolites formed by cytochrome P450 enzymes and are often associated with drug toxicity due to their ability to covalently bind to proteins.

Conclusions for Chapter 3

1. The metabolism of the anticonvulsant compound Epirimil was predicted using the XenoSite web tool, which provided insights into the most probable sites of metabolism (SOMs) catalyzed by cytochrome P450 enzymes.
2. The most likely metabolic transformations include stable oxidation of the sulfur atom (leading to sulfoxide and disulfoxide derivatives), N-oxidation at the pyridine ring, and hydrolytic cleavage of the acetamide bond.
3. Unstable oxidation, primarily oxidative O-dealkylation of methoxy groups on the phenyl ring, is also predicted, potentially forming catechol-like metabolites.
4. The formation of glucuronide conjugates via UGT enzymes is possible, indicating a potential phase II detoxification pathway.
5. No dehydrogenation or reduction reactions were predicted, and the formation of epoxides, which are commonly associated with drug toxicity, is considered unlikely. Importantly, no formation of electrophilic or DNA-reactive metabolites was predicted, suggesting a favorable metabolic safety profile for Epirimil.

CHAPTER 4
PREDICTING DIFFERENT TYPES OF EPIRIMIL TOXICITY

Assessment of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of a compound represents a critical phase in the drug development process. Before a candidate compound proceeds to clinical trials, its ADMET profile must be carefully evaluated [70]. Traditionally, toxicity assessments are carried out using animal experiments, as was done in our study for the investigational compound Epirimil (described in Section 2). However, in silico toxicity prediction provides a rapid and cost-effective alternative to animal testing, relying on known toxicity data to train models capable of forecasting the toxic potential of new compounds [71]. Despite these advances, mechanistic toxicity prediction remains an evolving field, and gaining such insights is crucial for drug development. A single compound may affect multiple toxicity endpoints. Moreover, off-target interactions with proteins can lead to binding with various targets of different affinities, potentially activating diverse signaling pathways or interfering with distinct biological functions. Disruption of these interconnected signaling or functional pathways may result in synergistic or antagonistic systemic effects, extending across organs, tissues, and cellular levels, and potentially contributing to severe toxicity profiles [72].

4.1 Determination of the Lethal dose of the anticonvulsant agent Epirimil

The ProTox platform integrates molecular similarity, fragment propensity, key structural features, and machine learning (including fragment-based CLUSTER cross-validation), encompassing 61 prediction models for endpoints such as acute toxicity, organ toxicity, molecular initiating events, metabolic effects, adverse outcomes (Tox21), and toxicity targets.

The novelty of the ProTox webserver lies in its multi-layered toxicity classification system, which includes oral toxicity (e.g., acute toxicity in rodents), organ toxicity (e.g., hepatotoxicity), classical toxicological endpoints (e.g.,

mutagenicity, carcinogenicity, cytotoxicity, immunotoxicity via B-cell proliferation inhibition), molecular initiating events (MOEs), adverse outcome pathways (AOPs), and toxicity targets. This offers valuable insights into the possible molecular mechanisms underlying toxic responses.

According to the ProTox-predicted toxicity for the antiepileptic agent "Epirimil," the estimated oral toxicity was found to be 1000 mg/kg, which correlates with the experimentally determined toxicity in animals. The results are shown in Figure 4.1.

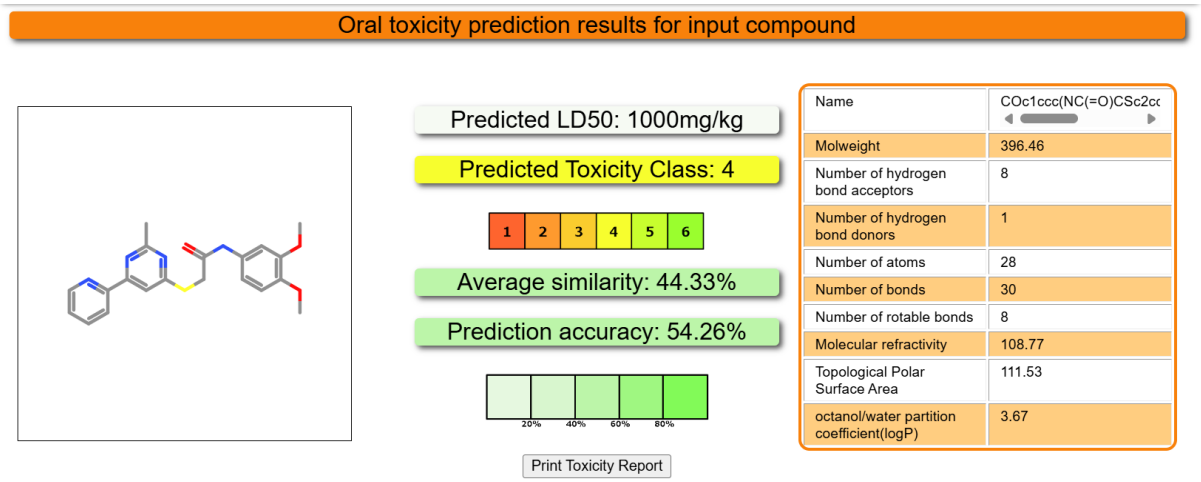


Fig. 4.1 Results of toxicity of the anticonvulsant agent Epirimil

The obtained results prove the possibility of using this program as a preliminary toxicity prediction, since it does not give overly false results.

4.2 Prediction of Epirimil toxicity by various biological or toxicological parameters

The ProTox-II Radar Plot tool allows the creation of a toxicity radar chart, which is used for visual representation of predicted toxicity of a chemical compound across various biological or toxicological parameters. In the radar chart, each axis corresponds to a specific type of toxicity or biological activity (e.g., hepatotoxicity, carcinogenicity, mutagenicity, etc.). It displays the probability (in percentages or via color gradients/vector lengths) of a positive toxicity outcome compared to the average toxicity level within the corresponding compound class. The closer a point is to the edge of the radar, the higher the likelihood of toxicity for that parameter. Conversely,

the further away to the center, the lower the probability of toxic effects. This tool can be used for rapid toxicity risk assessment during in silico analysis, especially in the screening of novel bioactive compounds in pharmacology and chemistry.

The toxicity radar chart of Epirimil is visualised to the figure 4.2

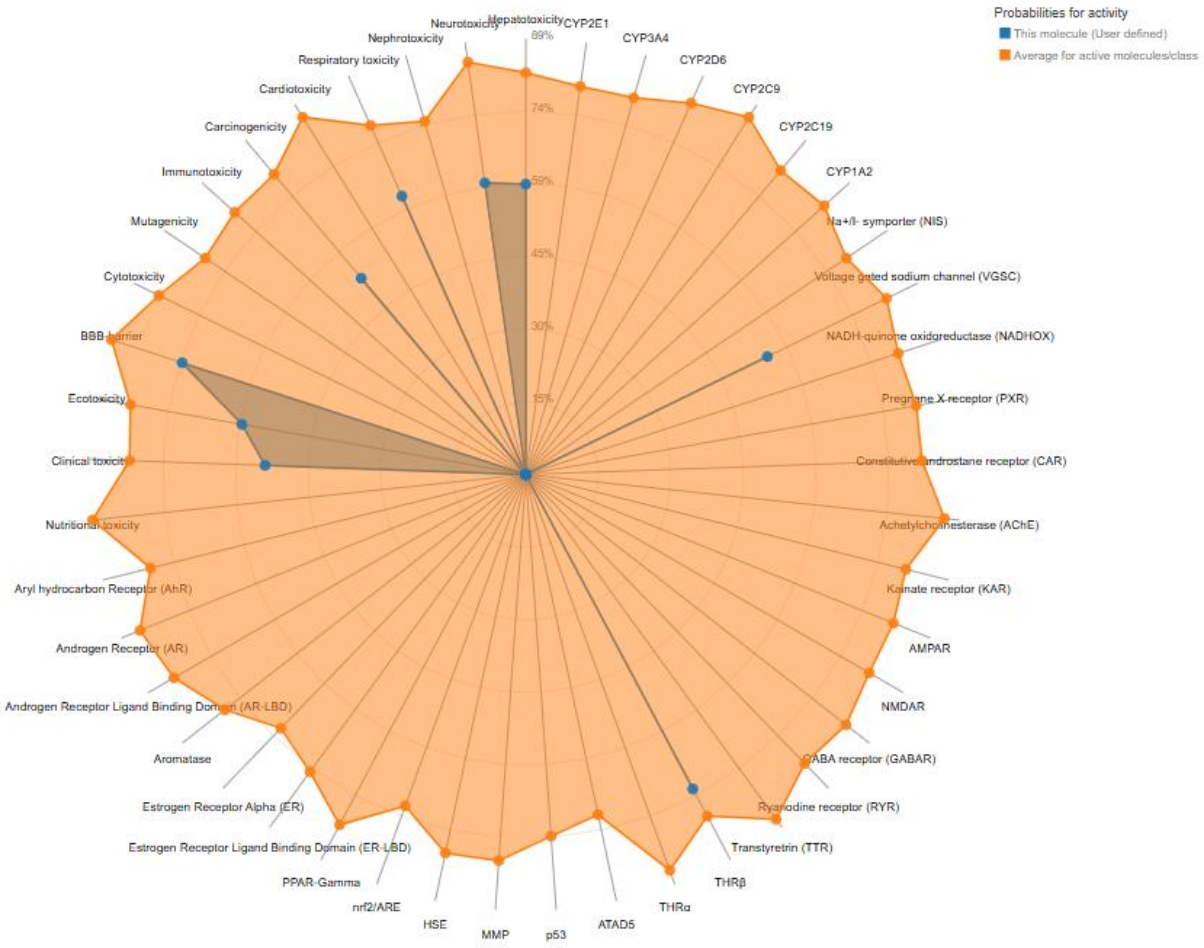


Fig. 4.2 The toxicity radar chart of Epirimil

Describing the diagram, it is worth noting that the blue line (with dots) represents the molecule under study; the orange zone is the average toxicity values for the class of active molecules. Accordingly, the further away from the center a point is located, the higher the probability of toxic activity.

As seen from the toxicity radar, none of the parameters exceed the diagram's boundaries. Parameters with a high probability of toxicity (60% and above) include: Hepatotoxicity – 59%, with a likely inhibition or induction of the enzyme CYP2E1 (64%), which may affect drug metabolism. The probability of negative impact on the

nervous system is Neurotoxicity – 49%. Potential interaction with voltage-gated sodium channels (VGSC) – ~45% and NADH-quinone oxidoreductase (NADHOX) – ~40% is also observed. BBB permeability – 60%, which is essential for a drug targeting the central nervous system (CNS).

Low probability of toxicity (<30%) is noted for carcinogenicity, Mutagenicity, Immunotoxicity, and cytotoxicity, suggesting a low risk of carcinogenic, mutagenic, or cytotoxic effects.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.59
Organ toxicity	Neurotoxicity	neuro	Active	0.60
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.53
Organ toxicity	Respiratory toxicity	respi	Active	0.62
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.73
Toxicity end points	Carcinogenicity	carcino	Active	0.52
Toxicity end points	Immunotoxicity	immuno	Inactive	0.77
Toxicity end points	Mutagenicity	mutagen	Inactive	0.83
Toxicity end points	Cytotoxicity	cyto	Inactive	0.68
Toxicity end points	BBB-barrier	bbb	Active	0.73
Toxicity end points	Ecotoxicity	eco	Active	0.58
Toxicity end points	Clinical toxicity	clinical	Active	0.53
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.72
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.84
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.90
Tox21-Nuclear receptor signaling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.97
Tox21-Nuclear receptor signaling pathways	Aromatase	nr_aromatase	Inactive	0.91
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.88
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.96
Tox21-Nuclear receptor signaling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.83
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.95
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.95
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.77
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.82
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.90
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.88
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Active	0.72
Molecular Initiating Events	Transthyretin (TTR)	mie_ttr	Inactive	0.65
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.83
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.87
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.98
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Acheylcholinesterase (AChE)	mie_ache	Inactive	0.89
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.98
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.75
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.93
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Active	0.54
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.92
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.76
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.79
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.65
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.64
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.52
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.99

Fig.4.3 Toxicity model report of Epirimile

Therefore, the molecule shows a moderate probability of hepatotoxicity and neurotoxicity, as well as potential interaction with key metabolic enzymes (CYPs).

The risks of carcinogenicity, cytotoxicity, and mutagenicity are low, which is certainly a positive aspect.

Conclusions for Chapter 4.

1. The *in silico* toxicity prediction of the investigational compound Epirimil using the ProTox-II platform provided valuable insights into its potential biological safety profile. The predicted oral toxicity (LD_{50}) of 1000 mg/kg corresponds well with experimental data, supporting the reliability of the ProTox model as a preliminary screening tool.
2. The radar toxicity plot indicated no extreme toxicity risks, with most parameters remaining within acceptable probability ranges. Notably, Epirimil demonstrated a moderate likelihood of hepatotoxicity (59%) and neurotoxicity (49%), with a potential effect on metabolic enzymes, particularly CYP2E1 (64%). These findings may suggest the need for careful monitoring of liver function and CNS-related effects during further preclinical and clinical development.
3. Importantly, the molecule exhibited low predicted risks for carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity – key indicators for drug safety – thus enhancing its potential as a therapeutic candidate.

CONCLUSIONS

1. The *in silico* prediction of Epirimil metabolism using the XenoSite web tool identified key metabolic pathways involving cytochrome P450 enzymes, including stable sulfur oxidation, N-oxidation of the pyridine ring, and hydrolytic cleavage of the acetamide bond.
2. Potential phase I transformations also include oxidative O-dealkylation of methoxy groups on the phenyl ring, possibly leading to catechol-type metabolites, while phase II conjugation via glucuronidation is likely, indicating possible detoxification routes.
3. No dehydrogenation, reduction, or formation of electrophilic or DNA-reactive metabolites were predicted, and the risk of toxic epoxide formation appears minimal, suggesting a favorable metabolic safety profile.
4. Toxicity prediction using the ProTox-II platform estimated an oral LD_{50} of 1000 mg/kg for Epirimil, which corresponds well with available experimental data and supports the reliability of the model for preliminary risk assessment.
5. The radar toxicity analysis revealed no parameters exceeding critical thresholds; however, moderate probabilities of hepatotoxicity (59%) and neurotoxicity (49%) were identified, along with a potential effect on CYP2E1 activity (64%).
6. Epirimil demonstrated low predicted risks for carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity, which are key indicators of drug safety and support its further development as a potential therapeutic agent.

REFERENCES

1. Singh A., Trevick S. The epidemiology of global epilepsy. *Neurol Clin*. 2016. Vol. 34(4). P. 837-847.
2. ILAE classification and definition of epilepsy syndromes with onset in neonates and infants: Position statement by the ILAE task force on nosology and definitions / S. M. Zuberi et al. *Epilepsia*. 2022. Vol. 63(6). P. 1349-1397.
3. Newton C. R., Garcia H. H. Epilepsy in poor regions of the world. *Lancet*. 2012. Vol. 380(9848). P. 1193–201.
4. A systemic review of the frequency neurocysticercosis with a focus on people with epilepsy / P. Ndimubanzi et al. *PLoS Negl Trop Dis*. 2010. Vol. 4. P. 1–17.
5. Carpio A., Hauser W. A. Epilepsy in the developing world. *Curr. Neurol. Neurosci. Rep*. 2009. Vol. 9(4). P. 319–26.
6. Abou-Khalil B. W. Update on antiepileptic drugs 2019. *Continuum (Minneap Minn)*. 2019. Vol. 25(2). P. b508-536.
7. Keezer M. R. Epilepsy Comorbidities. *Continuum (Minneap Minn*). 2025. Vol. 31(1). P. 232-246. DOI: 10.1212/CON.0000000000001529.
8. Elucidating the Potential Side Effects of Current Anti-Seizure Drugs for Epilepsy / E. Akyüz et al. *Curr. Neuropharmacol*. 2021. Vol. 19(11). P. 1865-1883.
9. Psychiatric and behavioral side effects of antiepileptic drugs in adults with epilepsy / B. Chen et al. *Epilepsy Behav*. 2017. Vol. 76. P. 24-31.
10. Cutaneous adverse effects of antiepileptic drugs / F. Zgolli et al. *Therapie*. 2024. Vol. 79(4). P. 453-459.
11. Adverse cutaneous drug reactions associated with Old- and New- generation antiepileptic drugs using the Japanese Pharmacovigilance Database / K. Hosohata et al. *Clin Drug Investig*. 2019. Vol. 39(4). P. 363-368
12. Lettieri M., Rodda M., Carlucci V. Machine learning in early prediction of metabolism of drugs. *Methods Mol. Biol*. 2025. Vol. 2834. P. 275-291.
13. Dudas B., Miteva M. A. Computational and artificial intelligence-based approaches for drug metabolism and transport prediction. *Trends Pharmacol. Sci*. 2024. Vol. 45(1). P. 39-55.

14. Talevi A., Bellera C. L. Drug excretion. *ADME Processes in Pharmaceutical Sciences* / ed. by A. Talevi, P. Quiroga. Cham, 2018. P. 78.

15. Ha C.–E., Bhagavan N. V. Chapter 35—Drug metabolism. *Essentials of Medical Biochemistry*. 3rd ed. Cambridge, 2023. P. 795–805.

16. Susa S. T., Preuss C. V. Drug Metabolism. *Treasure Island*. FL, 2022. P. 2.

17. Barreto E. F., Larson T. R., Koubek E. J. Drug Excretion. Amsterdam : Elsevier, 2021. 7 p.

18. Recent advances in the translation of drug metabolism and pharmacokinetics science for drug discovery and development / Y. Lai et al. *Acta Pharm. Sin. B.* 2022. Vol. 12(6). P. 2751–2777.

19. Predicting drug metabolism: Experiment and/or computation? / J. Kirchmair et al. *Nat. Rev. Drug Discov.* 2015. Vol. 14. P. 387–404.

20. Mackenzie P. I., Somogyi A. A., Miners J. O. Advances in drug metabolism and pharmacogenetics research in Australia. *Pharmacol. Res.* 2017. Vol. 116. P. 7–19.

21. Smith G. F. Artificial intelligence in drug safety and metabolism. *Artif. Intell. Drug Des.* 2022. Vol. 56. P. 483–501.

22. Gad S. C., Spainhour C. B. Nonclinical drug administration: formulations, routes and regimens for solving drug delivery droblems in animal model systems. FL, 2017. 17 p.

23. Phang–Lyn S., Llerena V. A. Biochemistry, biotransformation. *Treasure Island*. FL, 2022. 34 p.

24. Current trends in drug metabolism and pharmacokinetics / Y. Li et al. *Acta Pharm. Sin. B.* 2019. Vol. 9. P. 1113–1144.

25. Guengerich F. P. Cytochrome P450 and chemical toxicology. *Chem. Res. Toxicol.* 2008. № 21. P. 70–83.

26. Testa B., Pedretti A., Vistoli G. Reactions and enzymes in the metabolism of drugs and other xenobiotics. *Drug Discov. Today.* 2012. Vol. 17. P. 549–560.

27. Site of metabolism prediction for six biotransformations mediated by cytochromes P450 / M. Zheng et al. Bioinformatics. 2009. Vol. 25. P. 1251–1258.

28. Zhang Z., Tang W. Drug metabolism in drug discovery and development. *Acta Pharm. Sin. B.* 2018. № 8. P. 721–732.

29. Mann B., Melton R., Thompson D. Drug Metabolism. Drug metabolism in drug discovery and preclinical development. London, 2021. P. 14–17.

30. In silico approaches and tools for the prediction of drug metabolism and fate: A review / S. R. Kazmi et al. *Comput. Biol. Med.* 2019. Vol. 106. P. 54–64.

31. Litsa E. E., Das P., Kavraki L. E. Machine learning models in the prediction of drug metabolism: Challenges and future perspectives. *Expert Opin. Drug Met.* 2021. Vol. 17. P. 1245–1247.

32. Recent applications of deep learning and machine intelligence on in silico drug discovery: Methods, tools and databases / A. S. Rifaioglu et al. *Brief. Bioinform.* 2019. Vol. 20. P. 1878–1912.

33. Hsiao Y., Su B. H., Tseng Y. J. Current development of integrated web servers for preclinical safety and pharmacokinetics assessments in drug development. *Brief Bioinform.* 2021. Vol. 67. P. 22.

34. Concepts of Artificial Intelligence for Computer-Assisted Drug Discovery / X. Yang et al. *Chem. Rev.* 2019. Vol. 119. P. 10520–10594.

35. Cypreact: A Software Tool for in Silico Reactant Prediction for Human Cytochrome P450 Enzymes / S. Tian et al. *J. Chem. Inf. Model.* 2018. Vol. 58. P. 1282–1291.

36. GLORY: Generator of the Structures of Likely Cytochrome P450 Metabolites Based on Predicted Sites of Metabolism / K. C. Bruyn et al. *Front Chem.* 2019. Vol. 7. P. 402.

37. FAst MEtabolizer (FAME): A rapid and accurate predictor of sites of metabolism in multiple species by endogenous enzymes / J. Kirchmair et al. *J. Chem. Inf. Model.* 2013. Vol. 53. P. 2896–2907.

38. FAME 2: Simple and effective machine learning model of cytochrome P450 regioselectivity / M. Sicho et al. *J. Chem. Inf. Model.* 2017. Vol. 57. P. 1832–1846.

39. FAME 3: Predicting the Sites of Metabolism in Synthetic Compounds and Natural Products for Phase 1 and Phase 2 Metabolic Enzymes / M. Sicho et al. *J. Chem. Inf. Model.* 2019. Vol. 59. P. 3400–3412.

40. GLORYx: Prediction of the Metabolites Resulting from Phase 1 and Phase 2 Biotransformations of Xenobiotics / Kops C. Bruyn et al. *Chem. Res. Toxicol.* 2021. Vol. 34. P. 286–299.

41. Zaretzki J., Matlock M., Swamidass S. J. XenoSite: Accurately predicting CYP-mediated sites of metabolism with neural networks. *J. Chem. Inf. Model.* 2013. Vol. 53. P. 3373–3383.

42. Biotransformer: A comprehensive computational tool for small molecule metabolism prediction and metabolite identification / Y. Djoumbou–Feunang et al. *J. Cheminform.* 2019. Vol. 11. P. 2.

43. Cyproduct: A Software Tool for Accurately Predicting the Byproducts of Human Cytochrome P450 Metabolism / S. Tian et al. *J. Chem. Inf. Model.* 2021. Vol. 61. P. 3128–3140.

44. CYPlebrity: Machine learning models for the prediction of inhibitors of cytochrome P450 enzymes / W. Plonka et al. *Bioorg. Med. Chem.* 2021. Vol. 46. P. 11.

45. Ridder L., Wagener M. SyGMa: Combining expert knowledge and empirical scoring in the prediction of metabolites. *ChemMedChem Chem. Enabling Drug Discov.* 2008. № 3. P. 821–832.

46. Prediction of human cytochrome P450 inhibition using a multitask deep autoencoder neural network / X. Li et al. *Mol. Pharm.* 2018. Vol. 15. P. 4336–4345.

47. Prediction of human cytochrome P450 inhibition using bio-selectivity induced deep neural network / H. Park et al. *Bulletin Korean Chem. Soc.* 2022. Vol. 43. P. 261–269.

48. Computational prediction of the isoform specificity of cytochrome P450 substrates by an improved bayesian method / H. Dai et al. *Res. Sq.* 2019. Vol. 87. P. 209–231.

49. Wade K. C. Fanaroff and Martin's Neonatal–Perinatal Medicine. *Pharmacokinetics in neonatal medicine*. Philadelphia : Elsevier, 2020. P. 722–734.

50. Clearance in drug design / D. A. Smith et al. *J. Med. Chem.* 2019. Vol. 62. P. 2245–2255.

51. Data curation can improve the prediction accuracy of metabolic intrinsic clearance / T. Esaki et al. *Mol. Inform.* 2019. Vol. 38. P. 1800086.

52. ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties / G. Xiong et al. Nucleic Acids Res. 2021. Vol. 49. P. 5–14.

53. Chemi-net: A molecular graph convolutional network for accurate drug property prediction / K. Liu et al. *Int. J. Mol. Sci.* 2019. Vol. 20. P. 3389.

54. Hsiao Y. W., Fagerholm U., Norinder U. In silico categorization of in vivo intrinsic clearance using machine learning. *Mol. Pharm.* 2013. № 10. P. 1318–1321.

55. In silico prediction of human renal clearance of compounds using quantitative structure-pharmacokinetic relationship models / J. H. Chen et al. *Chem. Res. Toxicol.* 2020. Vol. 33. P. 640–650.

56. In silico prediction of human intravenous pharmacokinetic parameters with improved accuracy / Y. C. Wang et al. *J. Chem. Inf. Model.* 2019. Vol. 59. P. 3968–3980.

57. Mamada H., Nomura Y., Uesawa Y. Prediction model of clearance by a novel quantitative structure-activity relationship approach, combination deepsnap-deep learning and conventional machine learning. *ACS Omega.* 2021. № 6. P. 23570–23577.

58. Sohlenius–Sternbeck A.–K., Terelius Y. Evaluation of ADMET predictor in early discovery drug metabolism and pharmacokinetics project work. *Drug Metab. Dispos.* 2022. Vol. 50. P. 95–104.

59. Montenegro–Burke J. R., Guijas C., Siuzdak G. Metlin: A tandem mass spectral library of standards. *Comput. Methods Data Anal. Metab.* 2020. Vol. 2104. P. 149–163.

60. MetaboLights: A resource evolving in response to the needs of its scientific community / K. Haug et al. *Nucleic Acids Res.* 2020. Vol. 48. P. 440–444.

61. Kanehisa M. The Kegg Database. Wiley Online Library. *«In Silico» Simulation of Biological Processes* : Novartis Foundation Symposium. New York, 2002. № 247. P. 91–103.

62. Beyond the genome (btg) is a (pgdb) pathway genome database: HumanCyc / M. Trupp et al. Genome Biol. 2010. Vol. 11. P. 1.

63. Bigg models 2020: Multi–strain genome-scale models and expansion across the phylogenetic tree / C. J. Norsigian et al. *Nucleic Acids Res.* 2020. Vol. 48. P. 402–406.

64. DrugBank 5.0: A major update to the drugbank database for 2018 / D. S. Wishart et al. *Nucleic Acids Res.* 2018. Vol. 46. P. 1074–1082.

65. ChEMBL: Towards direct deposition of bioassay data / D. Mendez et al. *Nucleic Acids Res.* 2019. Vol. 47. P. 930–940.

66. PubChem 2019 update: Improved access to chemical data / S. Kim et al. *Nucleic Acids Res.* 2019. Vol. 47. P. 1102–1109.

67. Artificial intelligence foundation for therapeutic science / K. Huang et al. *Nat. Chem. Biol.* 2022. Vol. 18. P. 1033–1036.

68. OpenFDA: An innovative platform providing access to a wealth of fda's publicly available data / T. A. Kass–Hout et al. *J. Am. Med. Inform. Assoc.* 2016. Vol. 23. P. 596–600.

69. Северіна Г. І. Синтез та фізико–хімічні характеристики цнс–агентів серед похідних піримідин-4-ону та піримідин-2(4)-тіону : автореф. дис. … д–ра фармацевт. наук : 15.00.02 / НФаУ. Харків, 2021. 47 с.

70. Collaborative development of predictive toxicology applications / B. Hardy et al. *J. Cheminform.* 2010. Vol. 2(1). P. 7.

71. Raies A. B. BajicV. B. In silico toxicology: computational methods for the prediction of chemical toxicity. *Wiley Interdiscip. Rev. Comput. Mol. Sci*. 2016. Vol. 6. P. 147–172.

APPLICATION

To evaluate overall drug-likeness, a series of rule-based filters were applied, including Lipinski's Rule of Five, and criteria proposed by Ghose, Veber, Egan, and Muegge. All compounds satisfied most of these filters, supporting their potential as drug-like candidates. However, based on the Brenk structural alert system, a common undesirable feature was identified across all molecules – the hydantoin fragment, which is associated with a potential increase in toxicity risk. Although this moiety is present in several pharmacologically active compounds, its presence warrants caution and further experimental validation regarding safety.

Conclusions. The calculated the ADMET profiling results allowed us to identify the ten most promising substances for further study of affinity for acetylcholinesterase and NMDA glutamate receptors, organic synthesis and in vitro and in vivo studies as potential biologically active substances for the treatment of Alzheimer's disease.

PREDICTION OF THE DIRECTION OF BIOTRANSFORMATION OF A NEW ANTICONVULSANT AGENT OF PYRIMIDINE DERIVATIVE

Mouad Talal
Scientific supervisor: Severina H.I.
National University of Pharmacy, Kharkiv, Ukraine
mouad.freind@gmail.com

Introduction. Epilepsy remains one of the most common neurological disorders worldwide – with over 50 million people affected, according to WHO estimates. Despite the availability of a wide range of antiepileptic drugs (AEDs) with diverse mechanisms of action, adequate seizure control is achieved in only 65–70% of patients. This highlights the need for the development of novel, effective multitarget agents with an optimal safety profile. At the National University of Pharmacy, a promising anticonvulsant compound – N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide – was synthesized. This compound demonstrated a broad spectrum of activity in seizure models of various etiologies, as well as favorable pharmacological characteristics and low toxicity.

The study of metabolism of new biologically active compounds is a crucial stage in the preclinical development of drugs. Metabolic transformations significantly affect the pharmacokinetics, bioavailability, efficacy, and safety of a pharmaceutical substance. Insufficient understanding of biotransformation pathways can lead to unpredictable toxic effects or poor therapeutic outcomes during clinical trials—one of the most frequent reasons for discontinuing drug development. Therefore, early prediction of metabolism – especially using *in silico* methods – makes it possible to identify potentially harmful metabolites, assess the involvement of key enzymatic systems (particularly CYP450 isoforms), and determine whether structural modifications are needed before moving on to expensive *in vitro* and *in vivo* studies. This approach increases the likelihood of successful progression through subsequent stages of preclinical and clinical development, optimizes research resources, and contributes to the creation of safer and more effective medications.

Aim. Prediction of possible pathways of biotransformation of the potential anticonvulsant N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide.

Materials and methods. BioviaDraw 2021 was used to visualize the structure and create Smiles strings. XenoSite was used for online prediction of metabolic direction (https://xenosite.org/).

Results and discussion. XenoSite is a tool for predicting which specific atoms in a molecule are likely to undergo metabolism by cytochrome P450 enzymes (CYP450). It identifies sites of metabolism (SOMs) – regions of the molecule most likely to be modified during metabolic processes. Moreover, it can be used for both general P450 metabolism prediction and for individual isoforms (e.g., CYP3A4, CYP2D6, etc.). The tool is based on machine learning and experimental data, which helps anticipate which parts of the molecule are most "vulnerable" to metabolism. An important feature is its utility in optimizing molecules to potentially enhance their stability in the body. It also provides insights into possible bioactivation pathways or toxicity risk.

According to the results of the prediction, the following transformations are most likely to occur in the first phase of metabolism: stable oxygenation — a high probability of oxidation of the sulfur atom in the acetamide fragment, leading to the formation of N-(3,4-dimethoxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfonyl-acetamide; unstable oxygenation — a high probability of O-demethylation, resulting in the formation of two hydroxyl groups: N-(3,4-dihydroxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanyl-acetamide; a high probability of amide hydrolysis, producing two metabolites: 2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanylacetic acid and 3,4-dimethoxyaniline.

Dehydrogenation and reduction processes are not predicted, nor is glucuronidation involving *Uridine diphosphate glucuronosyltransferases (UGTs)* as a catalyst. It is also worth noting that no site of epoxidation (SOE) was identified, and the probability of epoxide formation is low. Epoxides are common reactive metabolites formed by cytochrome P450 enzymes and often lead to drug toxicity due to their ability to covalently bind to proteins.

Conclusions. Thus, possible metabolic pathways for N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide were predicted. The highest likelihood of occurrence is associated with hydrolysis, sulfur atom oxidation, and O-demethylation reactions. The formation of reactive metabolites is not anticipated. Therefore, the compound is recommended for the next stage of *in vitro* studies.

DETERMINATION OF THE PROSPECTS OF NEW 2-METHYLTHIENO[2,3-D]PYRIMIDINE DERIVATIVES AS AGENTS FOR MODULATION OF NEURODEGENERATION

[1]Saifudinova R.P., [2]Vlasov S.V.
Scientific supervisor: Severina H.I.
National University of Pharmacy, Kharkiv, Ukraine
[1]Cherkasy Medical Academy, Cherkasy, Ukraine
[2]Taras Shevchenko National University, Kyiv, Ukraine
rita0587@ukr.net

Introduction. Neurodegenerative diseases, particularly Alzheimer's disease, Parkinson's disease, and other forms of dementia, represent a serious medical and social challenge in an aging global population. The lack of etiotropic therapy, the availability of only symptomatic treatments, and the complex pathogenesis of these disorders necessitate the search for new active molecules capable of modulating the key pathogenic mechanisms of neurodegeneration—such as protein aggregation (tau, α-synuclein), oxidative stress, neuroinflammation, and disturbances in cholinergic transmission.

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

Mouad TALAL

1. Topic of qualification work: «Investigation of possible directions of biotransformation and toxicity of a new anticonvulsant agent pyrimidine-4-thione derivative», 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: The qualification work is devoted to the prediction of the chemical biotransformation of the potential anticonvulsant N-(3,4-dimethoxyphenyl)-2-([2-methyl-6-(pyridin-2-yl)pyrimidin-4-yl]thio)acetamide (Epirimil).

4. Contents of the settlement and explanatory note (list of questions that need to be developed): • To analyze the scientific literature on the epidemiology of epilepsy, side effects of existing AEDs, as well as the main mathematical, statistical methods and web resources used to predict possible pathways of xenobiotics metabolism in the human body; based on the results of the analysis, select the most effective web tools for in silico studies of drug metabolism and toxicological profile; to perform computer predictions of possible biotransformation pathways of the anticonvulsant Epirimil; determine the toxicity of the anticonvulsant Epirimil by in silico method and compare with the results in vivo; systematize the data obtained from different programs and formulate conclusions about the main directions of Epirimil biotransformation.

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.	Epilepsy and its epidemiology. Drug metabolism (Literature review)	**September-November 2024**	**done**
2.	Materials and methods of research of N-(3,4-dimethoxyphenyl)-2-[2-methyl-6-(2-pyridyl)pyrimidin-4-yl]sulfanyl-acetamide	**October-November 2024**	**done**
3.	Prediction of Epirimil metabolism using Xenosite web tools	**November 2024 – December 2024**	**done**
4.	Predicting different types of Epirimil toxicity	**January 2025– February 2025**	**done**
6.	Preparation of qualification work and submission to the Examination Commission	**February-April 2025**	**done**

An applicant of higher education _____ Mouad TALAL

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**				
Талал Муад	Дослідження можливих напрямків біотрансформації та токсичності нового протисудомного агента похідного піримідин-4 тіону	Investigation of possible directions of biotransformation and toxicity of a new anticonvulsant agent pyrimidine-4-thione derivative	проф. Северіна Г.І.	проф. Колісник С. В.

Ректор

Віріо, секретар

ФА2.8-03-317

ВИСНОВОК

експертної комісії про проведену експертизу
щодо академічного плагіату у кваліфікаційній роботі

здобувача вищої освіти

«06» травня 2025 р. № 331123388

Проаналізувавши кваліфікаційну роботу здобувача вищої освіти Талал Муад, групи Фм20(4,10д) англ 01, спеціальності 226 Фармація, промислова фармація, освітньої програми «Фармація» навчання на тему: «Дослідження можливих напрямків біотрансформації та токсичності нового протисудомного агента похідного піримідин-4 тіону / Investigation of possible directions of biotransformation and toxicity of a new anticonvulsant agent pyrimidine-4-thione derivative», 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

Mouad TALAL

on the topic: « Investigation of possible directions of biotransformation and toxicity of a new anticonvulsant agent pyrimidine-4-thione derivative »

Relevance of the topic. The master's thesis addresses an important and timely issue in modern pharmaceutical science – the study of metabolic pathways and toxicity of a novel anticonvulsant compound based on pyrimidine-4-thione. Given the high prevalence of drug-resistant epilepsy and the limitations of current antiepileptic drugs (AEDs), the search for new multitarget agents with favorable pharmacokinetic and safety profiles is of great importance. The work aligns with global priorities in drug development and rational design of safer active pharmaceutical ingredients.

Practical value of conclusions, recommendations and their validity The thesis presents *in silico* predictions of biotransformation pathways and potential toxicity of the compound Epirimil using modern cheminformatics tools such as XenoSite and ProTox-II. The study offers a valid, evidence-based interpretation of possible metabolic transformations, identifies key interactions with CYP450 enzymes, and confirms the absence of toxic epoxide or electrophilic metabolites. These findings can serve as a scientific basis for further in vivo studies and optimization of the molecule's structure. The conclusions are well-supported by data and contribute to early-stage safety screening of new drug candidates.

Assessment of work. The thesis is well-structured, logically coherent, and written in clear scientific language. It contains a comprehensive literature review, a justified choice of research tools, detailed results interpretation, and informative illustrations. The candidate has demonstrated a high level of academic competence, critical thinking, and the ability to apply advanced in silico methodologies for pharmacokinetic and toxicological evaluation. The research meets all the

methodological, scientific, and practical requirements expected from a master's qualification work.

General conclusion and recommendations on admission to defend. The qualification work of Mouad TALAL 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

Mouad TALAL

on the topic: «Investigation of possible directions of biotransformation and toxicity of a new anticonvulsant agent pyrimidine-4-thione derivative»

Relevance of the topic. The chosen topic is highly relevant and addresses one of the key challenges in contemporary pharmaceutical research – the development of safer and more effective antiepileptic drugs. Given the significant proportion of patients with drug-resistant epilepsy and the complex metabolism of CNS-active agents, studying the biotransformation and toxicity profiles of new compounds at the early stages of development is both timely and essential.

Theoretical level of work. The master`s thesis demonstrates a solid theoretical foundation. The author provides a comprehensive literature review, critically analyzing data on epilepsy pathophysiology, limitations of existing therapies, and modern approaches to predicting ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties. The work reflects a good understanding of molecular pharmacology, enzymology, and computational toxicology.

Author's suggestions on the research topic. he author has proposed a clear research objective – to evaluate the potential metabolic pathways and toxicological risks of the compound Epirimil using in silico tools. The selection and application of specific computational models (XenoSite, ProTox-II) were justified and appropriate for the study objectives. The suggested metabolic transformations and interpretation of predicted toxicity risks are well substantiated and align with current scientific principles.

Practical value of conclusions, recommendations and their validity. The conclusions obtained from the study have practical value for further preclinical development of the investigated compound. The identification of likely metabolic

routes, absence of epoxidation, and low predicted toxicity provide a favorable safety profile for Epirimil. These findings can inform future in vivo studies and structure optimization strategies. The recommendations and conclusions are valid and supported by well-documented results.

Disadvantages of work. The work is generally well-executed; however, some minor shortcomings are noted. The thesis could benefit from a more detailed comparative discussion of the advantages and limitations of the applied prediction tools. There are a small number of grammatical errors and typos in the work, which generally does not affect the value of the results obtained.

General conclusion and assessment of the work. The qualification work of Mouad TALAL 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. Serhii KOLISNYK

«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» Муад ТАЛАЛ на тему: «Дослідження можливих напрямків біотрансформації та токсичності нового протисудомного агента похідного піримідин-4-тіону».
СЛУХАЛИ: доповідь здобувача вищої освіти здобувача вищої освіти фармацевтичного факультету, Фм20(4,10д) англ 01 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Муад ТАЛАЛ на тему: «Дослідження можливих напрямків біотрансформації та токсичності нового протисудомного агента похідного піримідин-4-тіону», керівник – професор кафедри фармацевтичної хімії, д.фарм.н., проф. Ганна СЕВЕРІНА.
УХВАЛИЛИ: рекомендувати кваліфікаційну роботу Муад ТАЛАЛ 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 Муад Талал до захисту кваліфікаційної роботи за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Дослідження можливих напрямків біотрансформації та токсичності нового протисудомного агента похідного піримідин-4 тіону»

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Микола ГОЛІК /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Муад Талал виконав роботу на сучасному рівні. За період виконання кваліфікаційної роботи проявив високий рівень теоретичної підготовки. Кваліфікаційна робота викладена послідовно, грамотно, висновки коректні та логічні, витікають зі змісту роботи. Кваліфікаційна робота Муад Талал 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. Здобувач вищої освіти Муад Талал 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 /