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

QUALIFICATION WORK

on the topic: « **Investigation of potential anti-inflammatory agents among 4-amino-1,2,4-triazole-3-thione acetamide derivatives»**

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ANNOTATION

On the basis of the literature data, acetamide derivatives of 4-amino-1,2,4-triazole-3-thione were generated as potential anticonvulsants using the concept of modern drug design. The ADMET and drug-like parameters were determined, and the toxicity of the generated ligands was predicted. The feasibility of synthesis and in vivo study was substantiated by molecular docking into the active site of cyclooxygenase 2 inhibitor. Based on the results of a comprehensive study, promising anti-inflammatory agents, 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]thio]-N-arylacetamides, were identified.

The work consists of an introduction, 4 chapters, conclusions and a list of references containing 72 sources. The work is presented on 40 pages and contains 1 table, 14 figures, 7 schemes.

Key words: 4-amino-1,2,4-triazole, molecular docking, toxicity, cyclooxygenase, anti-inflammatory activity

АНОТАЦІЯ

За основі літературних даних реалізуючи концепцію сучасного drug-design були згенеровані ацетамідні похідні 4-аміно-1,2,4-тріазол-3-тіону як потенційні антиконвульсанти. Визначено параметри АДМЕТ та лікоподібності, а також спрогнозовано токсичність згенерованих лігандів. Доцільність синтезу та дослідження *in vivo* обгрунтовано молекулярним докінгом в активний сайт інгібітора циклооксигенази 2. За результатами комплексного дослідження визначені перспективні протизапальні агенти – 2-[[4-аміно-5-(2-піридил)-1,2,4-триазол-3-іл]тіо]-N-арилацетамиди.

Робота складається зі вступу, 4 розділів, висновків і списку використаної літератури, що містить 72 джерел. Робота викладена на 40 сторінках і містить 1 таблиць,14 рисунків,7 схему.

Ключевые слова: 4-аміно-1,2,4-триазол, молекулярний докинг, токсичність, циклооксигеназа, противовоспалительная активность

CONTENTS

LIST OF ABBREVIATIONS

ADMET	absorption, distribution, metabolism, excretion, and toxicity
APIs	active pharmaceutical ingredients
COX	cyclooxygenase
GIT	gastrointestinal tract
DMSO	Dimethyl sulfoxide
NSAIDs	non-steroidal anti-inflammatory drugs
in silico	Research methods using mathematical calculation methods
in vitro	Research methods using cell cultures
in vivo	Methods of study in a living organism
SPhU	State Pharmacopoeia of Ukraine
WHO	World Health Organization

INTRODUCTION

Relevance of the topic. The leading pathogenetic link in a significant number of diseases and pathological processes is inflammation, a typical physiological process that develops in vascularized organs and tissues in response to damage. In recent decades, the number of both acute inflammatory processes, which take a protracted course, and the number of chronic inflammatory diseases has been increasing.

In the complex therapy of acute and chronic diseases, to control pain and reduce the severity of the inflammatory response many drugs, mainly from the group of non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticosteroids, are used.

As for NSAIDs, along with favourable pharmacological action and undoubted clinical efficacy, their use has its limitations because, even short-term use of these drugs in small doses can lead to the development of side effects, which generally occur in about 25% of cases, and in 5% of patients can be seriously life-threatening. The issue of safety of NSAIDs is of particular importance also because these drugs are usually used on a long-term basis, in outpatient settings, and are widely used for self-medication. The risk of side effects is particularly high in the elderly, who account for more than 60% of NSAID users. In addition, it should be noted that many diseases require long-term use of these drugs.

The leading side effect of almost all NSAIDs is the development of adverse reactions from the gastrointestinal tract. According to statistics for 2008 in the United States of America, the mortality rate from NSAID-induced gastrointestinal lesions is the same as from AIDS and higher than from melanoma, bronchial asthma, cervical cancer or lymphogranulomatosis. Taking NSAIDs causes gastroduodenopathy, dyspeptic disorders, erosions and ulcers of the stomach and duodenum, bleeding and perforations of the GI tract, which is associated with both the local damaging effect of NSAIDs (most of them are organic acids) on the gastric and intestinal mucosa, and inhibition of cyclooxygenase-1 isoenzyme as a result of systemic action of the drugs.

In connection with the above, there is no doubt about the relevance of the search for highly effective and selective to cyclooxygenase-2 active pharmaceutical

ingredients that suppress inflammation and have minimal adverse reactions, which determined the need for this study.

Considering the convenience of structure modification and the possibility of introduction of various substituents, wide potential of pharmacological activity, 1,2,4-triazole-3-thione is very suitable and promising for research in this direction.

The purpose of the study. Realization of the concept of modern drug-design for creation of potential anti-inflammatory agents in a series of 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione derivatives.

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

- analyze scientific and patent primary sources regarding the modern approach to the search for new substances with anti-inflammatory activity among derivatives of 1,2,4-triazol-3-thione;
- apply the principles of modern "drug design" to design potential anti-inflammatory compounds;
- calculate the parameters of lycopodibility and ADMET, predict the level of toxicity to identify a therapeutically relevant biotarget and use *in silico* tools to calculate the degree of affinity of the generated structures to the anti-inflammatory target;
- study optimal conditions and obtain target arylacetamides 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione;
- conduct pharmacological screening for anti-inflammatory activity of the synthesized compounds;
- establish some qualitative characteristics of the structure-activity relationship.

The object of the study. Design, construction and study of potential antiulcer agents.

The subject of the study. Arylacetamide derivatives of 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione: structure design; prediction and discussion of anti-inflammatory activity, toxicity and ADMET parameters.

The methods of the study. Organic synthesis of 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione derivatives, physical, docking studies - AutoDock Vina, BIOVIADraw 2017R2, Chem3D, HyperChem 7.5, Discovery Studio Visualizer 2017/R2, drug likeness prediction - SwissADME, ProTox, standard pharmacological screening techniques.

The practical value of the results. The prospectivity of searching for potential anti-inflammatory agents in the series of alkylated derivatives of 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione was confirmed by virtual screening methods. Correlation by in silico and in vivo studies was established. A new promising structure was identified for further in-depth study of anti-inflammatory action. The results of physicochemical studies and pharmacological screening for anti-inflammatory activity of novel arylacetamide derivatives 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione have practical significance for scientists who are engaged in a targeted search for active pharmaceutical ingredients.

Elements of scientific research. Arylacetamide derivatives 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione were designed for the first time as potential anti-inflammatory agents, their ADMET parameters and toxicity were studied, and in vivo studies on the model of paw edema in rats were carried out.

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 40 pages and contains 1 table, 14 figures, 7 schemes. The list of the used literary sources contains 72 titles.

CHAPTER 1
SYNTHESIS AND BIOLOGICAL PROPERTIES OF 1,2,4-TRIAZOL-3-THIONE DERIVATIVES
(Literature review)

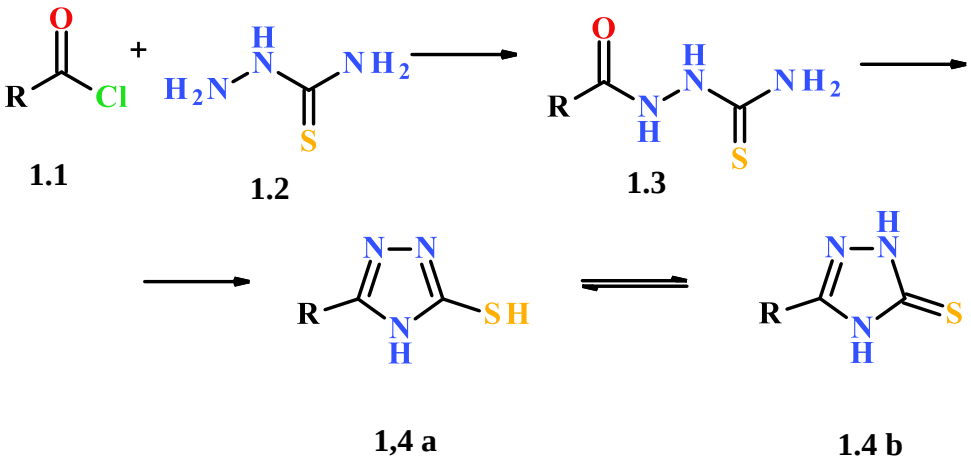
1.1 Approaches to the formation of the cycle of 1,2,4-triazole and its derivatives

The keen interest of specialists in the field of pharmaceutical and medicinal chemistry in 1,2,4-triazole derivatives is explained, among other reasons, by the fact that the formation of this heterocyclic system is quite simple and allows the introduction of a wide variety of substituents at positions 3,4,5 at this stage [1]. Varying the substituents makes it easy to modify the structures of the most promising substances in pharmacological terms. In addition, there are many approaches to the formation of this heterocyclic system, which allows to choose the most suitable and preparative one in each case and gives a choice for the manufacturer of active pharmaceutical ingredients (APIs). The unwavering interest of scientists in 1,2,4-triazole derivatives as potential APIs for use in medical and veterinary practice, agrochemicals, plasticizers, and metal corrosion inhibitors has been confirmed by the consistently high number of scientific publications over the past 20 years.

There are several classical methods for the formation of a heterocyclic ring with a thiol substituent at the 3-position that have been successfully used to date [2]. Synthetic chemists use them in different versions – traditional or modified in accordance with modern technical capabilities. These modifications are usually associated with microwave irradiation and the use of ultrasound, as well as the testing of modern catalysts.

One of the classical methods for the formation of a 1,2,4-triazole ring is the cyclization of acyl thiosemicarbazides 1.3 obtained by acylation of thiosemicarbazides 1.2 with acyl chlorides (Scheme 1.1). The product of the reaction is the corresponding 3-mercapto-1,2,4-triazole or its tautomer (1.4 a, b).

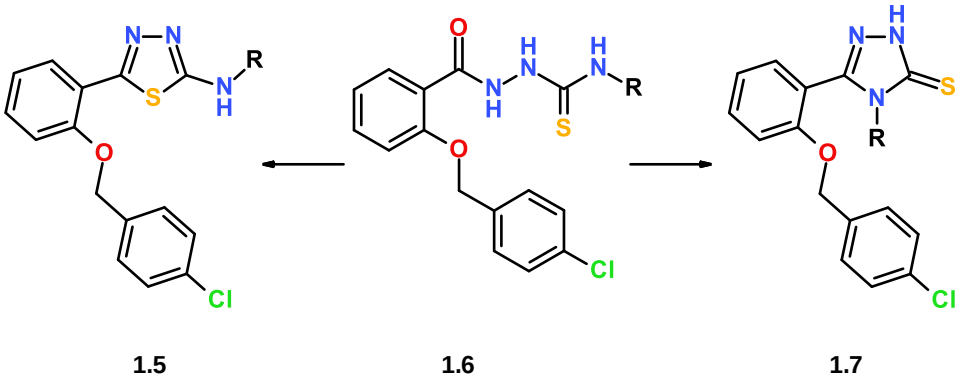
Scheme 1.1



In general, many works have been devoted to the study of the thion-thiol tautomerism of 1,2,4-triazole-3-thione derivatives [4, 5]. This is undoubtedly important, since in medicinal chemistry, affinity for a receptor is determined by the smallest changes in the structure of the molecule, and this can play a key role not only in the intensity of the pharmacological effect but also in its manifestation [6, 7].

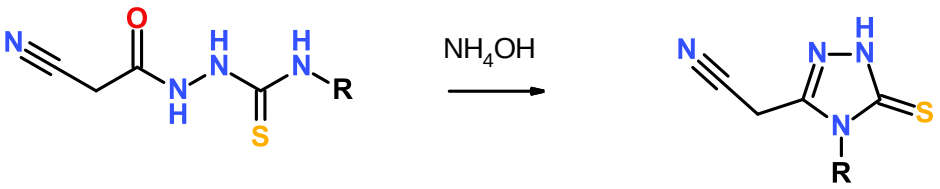
Oxidative cyclization is also possible [10]. In addition, it is known that 3-thioproducts of 1.7 are formed in an alkaline environment. When acid is added to acylthiosemicarbazide 1.6, the reaction product is the corresponding 3-amino derivatives of thiadiazole 1.5 (Scheme 1.2) [11]. Derivatives containing a tert-butyl residue at position 4 were synthesized in a similar way. The hydrolysis of these compounds by boiling for 3 hours with 30% sulfuric acid leads to the preparation of unsubstituted products 1.4 at position 4 [12].

Scheme 1.2



Cyanacetylthiosemicarbazides 1.8 were synthesized, which reacted with ammonia to give 5-cyanomethylsubstituted 1,2,4-triazol-3-thiones 1.9 (Scheme 1.3) [13].

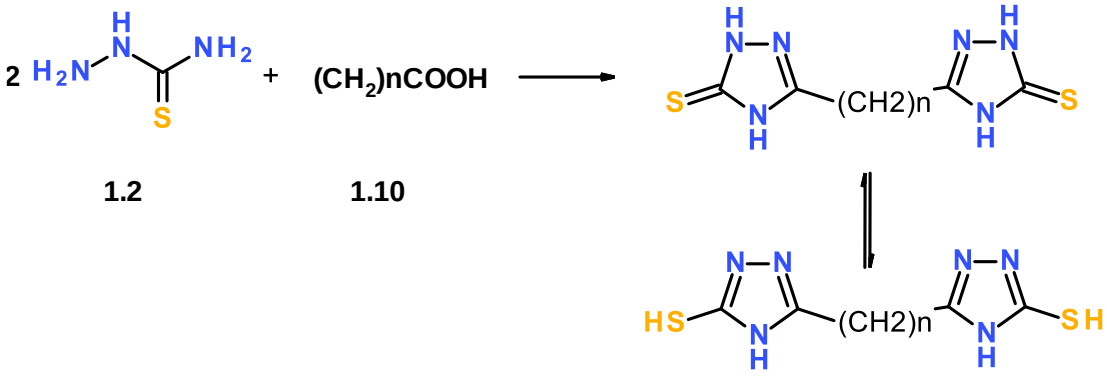
Scheme 1.3



1.8 **1.9**

The direct interaction of dicarboxylic acids 1.10 with thiosemicarbazide 1.2 leads to the corresponding bistriazoles 1.11, which also exist in two tautomeric forms (Scheme 1. 4) [14].

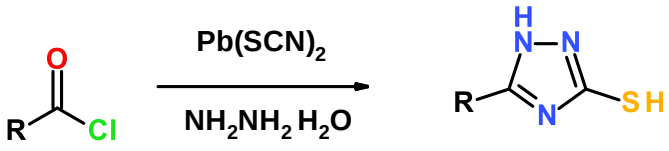
Scheme 1.4



1.2 **1.10** **1.11**

The interaction of acyl chlorides 1.1 with hydrazine hydrate and plumbum thiocyanate was also used to obtain 3-mercaptopotassium 1.4 (Scheme 1.5) [15].

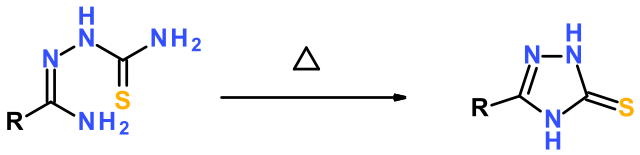
Scheme 1.5



1.1 **1.12**

The cyclization of substituted thiosemicarbazides can occur under the action of dimethyl acetals of dimethylformamide or dimethyl acetamide [16], as well as the corresponding carbamates [17,18]. The preparation of targeted triazole thiones 1.4 by thermolysis of thiosemicarbazone 1.13 (Scheme 1.6) has also been described [19]. A variation of this reaction is the interaction of thiosemicarbazone with amines [20].

Scheme 1.6



1.13 **1.4**

As can be seen from the above information, there are many options for the synthesis of 1,2,4-triazole derivatives and the choice of method depends on the desired substituents in the structure.

1.2 Pharmacological properties of derivatives of 1,2,4-triazole-3-thione

Among nitrogen-containing heterocyclic compounds, it is the triazoles that have a wide range of pharmacological applications. Due to their structural characteristics, 1,2,4-triazoles can have a wide range of substituents, which opens the way for the creation of a variety of new bioactive compounds [21]. Derivatives of 1,2,4-triazoles have a significant variety of pharmacological properties, including antimicrobial, antiviral, antituberculosis, anticancer, anticonvulsant, analgesic, antioxidant, anti-inflammatory and antidepressant activity and other types of activity [22, 23].

Among the derivatives of 1,2,4-triazol-3-thione, compounds possessing antibacterial [24, 25], antifungal [26], antiparasitic [27], antimalarial [28], antiviral [29, 30], antidepressant [31], analgesic, anti-inflammatory [32, 33], antitumor [34, 35], antiproliferative [36], antihistamine [37], antituberculosis [38, 39], anticonvulsant [40, 41] and other types of activity.

1.2.1 Medicinal products among 1,2,4-triazole derivatives

The 1,2,4-triazole nucleus is a structural fragment of many active pharmaceutical substances, which are included in pharmaceutical preparations and are widely used all over the world [42]. First, this is a group of antifungal drugs (Fig. 1.1).

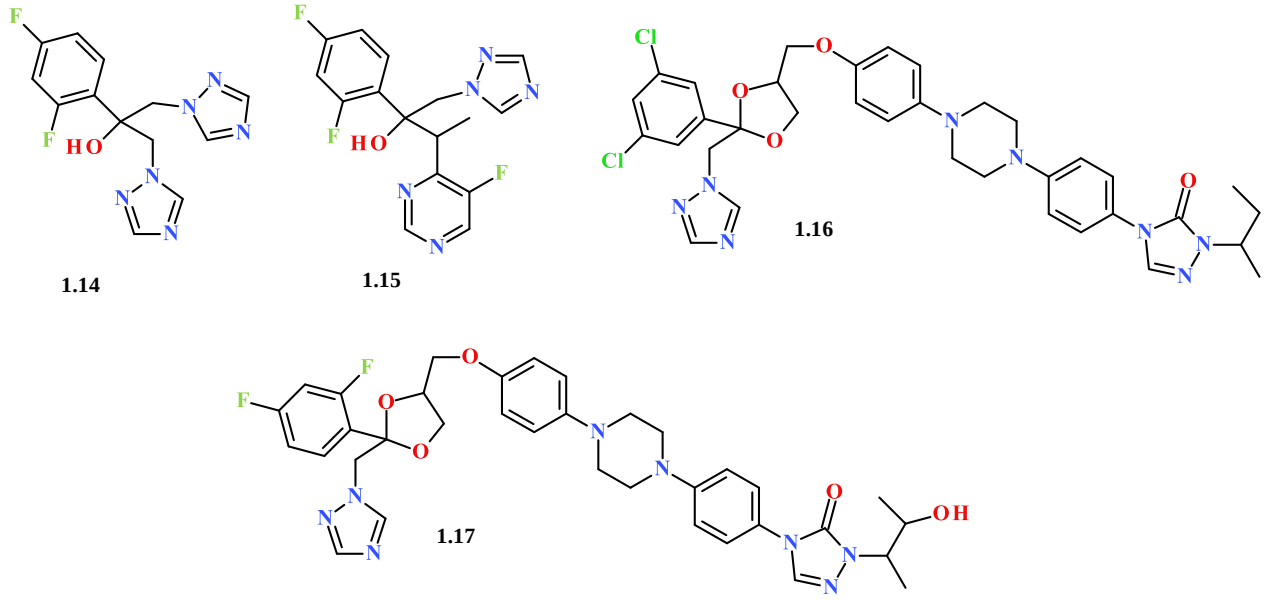


Figure 1.1 Antifungal drugs containing of 1,2,4-triazole

Antifungal agents inhibit ergosterol biosynthesis and reduce the permeability of the fungal cell membrane – fluconazole 1.14, voriconazole 1.15, intraconazole 1.16, petroconazole 1.17.

Widely used drugs with antidepressant activity - alprazolam, triazolam (Fig. 1.2) – are anxiolytics of medium duration of action used to treat panic disorders, anxiety neuroses, such as anxiety disorder or sociophobia.

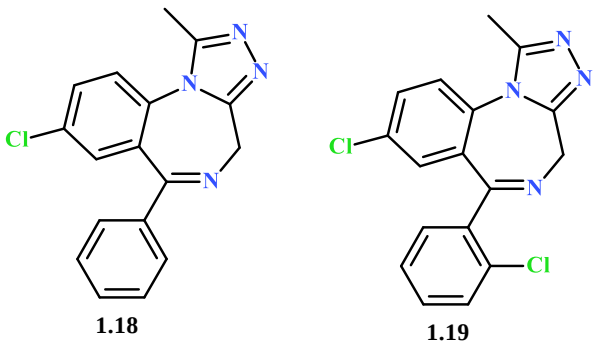


Figure 1.2 Anxiolytic drugs containing 1,2,4-triazole

Anastrozole 1.20 and letrozole 1.21, non-steroidal aromatase inhibitors that are used to treat breast cancer, have antitumor effects (Figure 1.3).

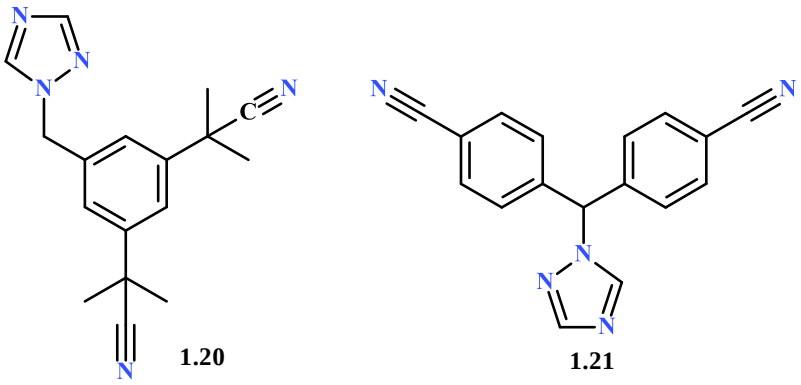


Fig. 1.3 Antitumor drugs derivatives of 1,2,4-triazole

Scientists of Zaporizhzhya State Medical University [43] under the guidance of Professor I.A. Mazur developed a unique drug – thiotriazolin 1.22 –a cardiological agent with anti-ischemic, membrane-stabilizing, antioxidant, and immunomodulatory effect (Fig. 1.4). Currently, Ukraine produces thiotriazolin substance and its dosage forms: tablets, solutions for injection, ointment, suppositories, eye drops [44].

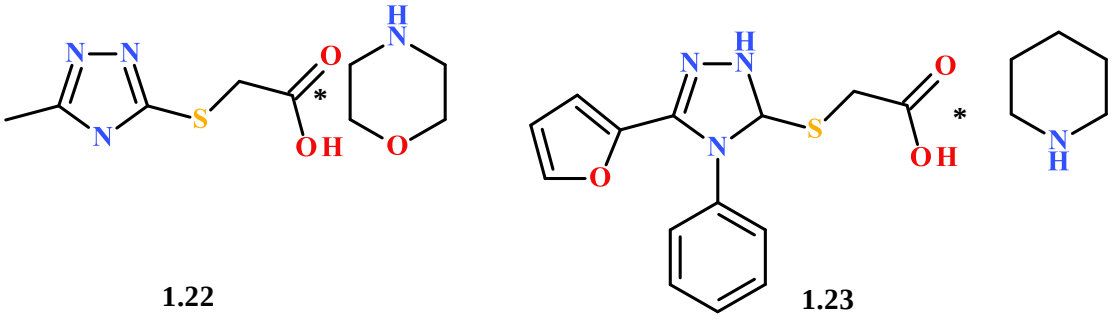


Figure 1.4 Developments of Ukrainian scientists – thiotriazolin and trifuzol

Ukrainian scientists in 2015 patented and introduced into veterinary practice the drug – Trifuzol 1.23 – a new generation antiviral drug with hepatoprotective, cardioprotective, antioxidant, immunomodulatory, anti-inflammatory, detoxifying, wound healing effect intended for all kinds of animals, as well as birds [45].

The antiviral drug Ribavirin 1.24 is also world-renowned and is widely used to treat diseases caused by respiratory syncytial virus and hepatitis C virus (Figure 1.5).

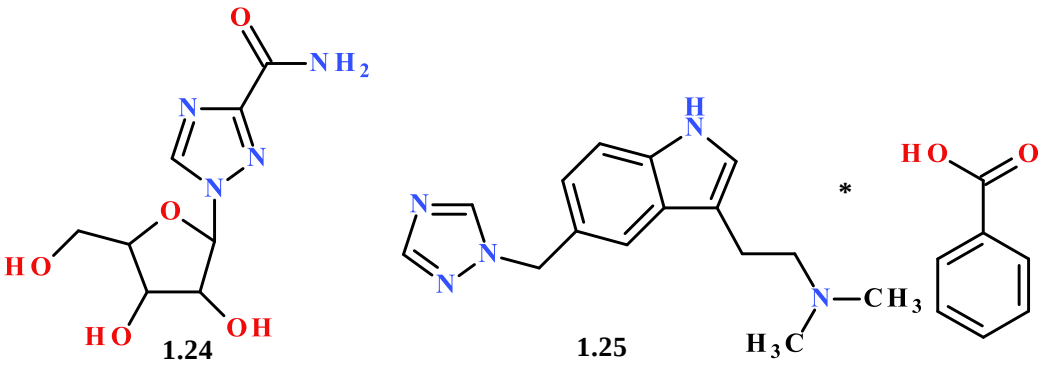


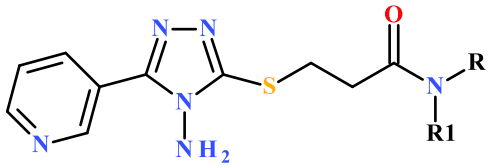
Fig.1.5 1,2,4-triazole derivatives - ribavirin and rizatriptan

The serotoninergic drug rizatriptan 1.25, whose active ingredient is N,N-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-ethanamine benzoate (Figure 1.5), exhibits antimigraine action by selective excitation of serotonin 5-NT 1B/1D receptors, constricts intracranial vessels dilated during migraine, and inhibits neuropeptide release.

1.2.2 Pharmacological potential of pyridine-substituted 1,2,4-triazole derivatives

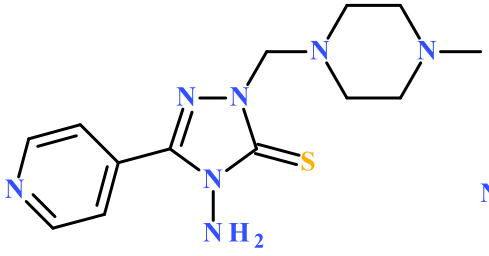
The pharmacological potential of pyridine-substituted derivatives of 1,2,4-triazol-3-thiones is significant. Large-scale studies are being conducted worldwide to search for new APIs among compounds of this class. The most significant results are the search for antimicrobial, antifungal and anticancer agents. The majority of scientific articles are devoted to such studies.

Researchers from India have revealed antitubercular properties of derivatives 5-(N-substituted carboxamidoethylthio)-3-(3'-pyridyl)-4-amino-1,2,4-triazole of general formula 1.26 [46]. All the investigated substances in in vitro tests at a concentration of 10 μg/mL inhibit the growth of Mycobacterium tuberculosis H37RV and exceed the standard drug Amikacin in antitubercular activity.



1.26

4-Pyridine-substituted derivatives of 1,2,4-triazol-3-thione were tested for antibacterial activity by agar diffusion method [47]. Among them, compounds of formula 1.27 and 1.28 were highly active *against E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, and *B. serovus* strains.



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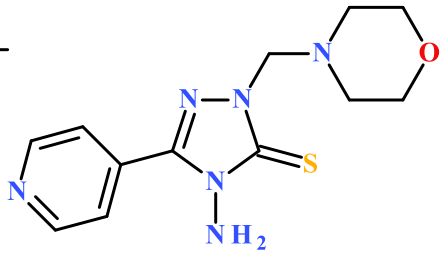
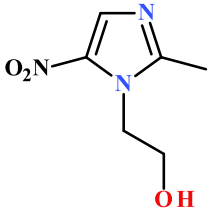


1.28

Among new derivatives of metranidazole 1.29, its 1,2,4-triazole-3-thio-substituted derivatives were synthesized, which have high antiparasitic activity against *E. histolytica* and *G. intestinalis* and Gram-positive anaerobic culture of *C. sporogenes*. It was found that pyridine-substituted derivatives 1.30 and 1.31 have the highest activity, which exceeds the activity of the reference drug metronidazole by 1.6 times [48].



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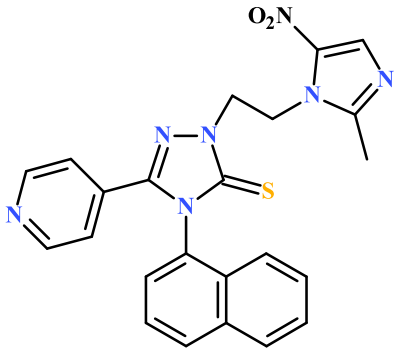

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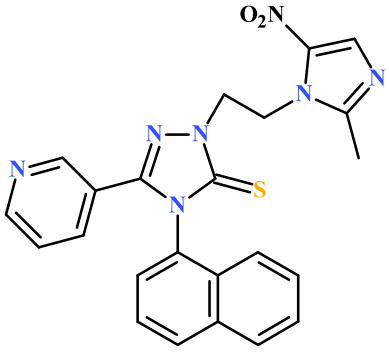

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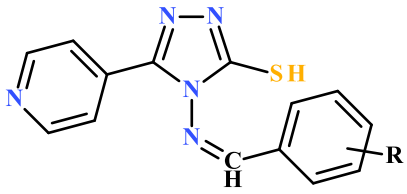
Schiff bases obtained by interaction of 4-amino-3-thio-1,2,4-triazole derivatives with aromatic aldehydes deserve special attention, since compounds with diverse pharmacological properties were found among them [49, 50]. Thus, among

the Schiff bases of 4-amino-3-mercapto-5-(pyridin-4-yl)-1,2,4-triazole (1.32), highly effective antifungal agents with a wide spectrum of activity were found [51].



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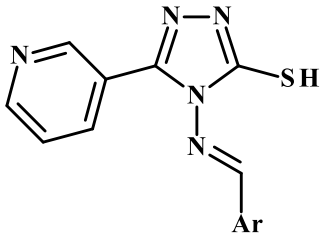
In comparison with standard antibiotics - ampicillin, amoxicillin, norfloxacin, benzylpenicillin - substituted derivatives of 5-(pyridin-3-yl)-1,2,4-triazole 1.33, 1.34 and 1.35 showed significant inhibition of growth zones of E. *coli, B. megaterium, S. substillis, P. vulgaris* and *A. niger fungi.* At the same time, these derivatives were inactive against *M. tuberculosis* strains [52].



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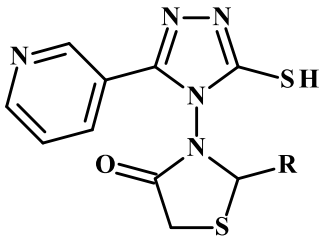

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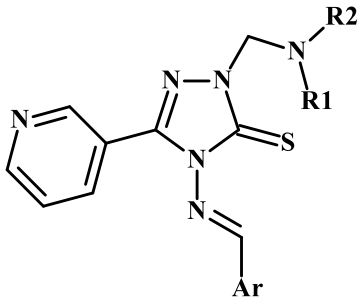

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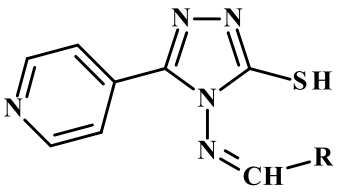
A series of derivatives of 4-[(arylmethylene)amino]-5-pyridin-4-yl-4H-1,2,4-triazol-3-thiols (1.36) and those obtained by interaction with primary and secondary amines of Mannich bases (1.37) showed high antibacterial activity against *Y. pseudotuberculosis, E. coli, P. aeruginosa, B.* and *S. aureus* strains [53].



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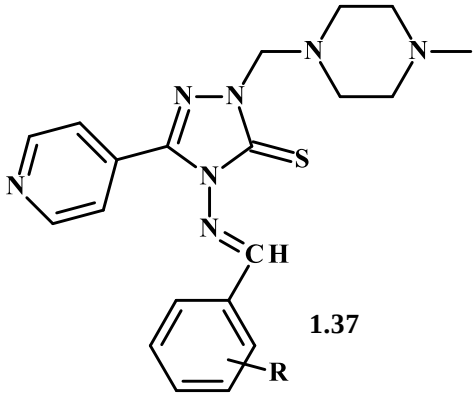
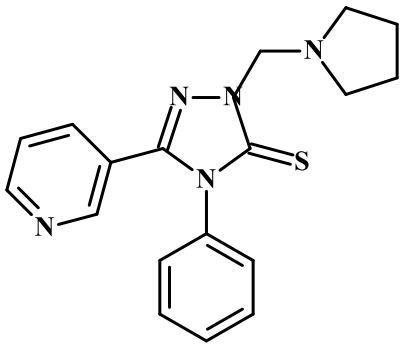


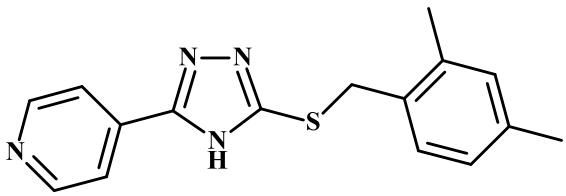
1.37

A series of Mannich bases of 1,2,4-triazol-3-thione have been synthesized and investigated for anticancer activity against 6 types of cancer. Only 5-(pyridin-3-yl)-4-phenyl-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione (1.38) exerted cytotoxic effects with growth inhibition of mammary epithelial tumor (T47D) [54].

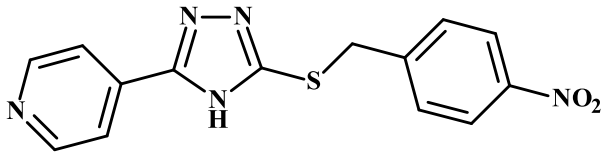


1.38

A new series of benzyl substituted thiogroup 1,2,4-triazoles were evaluated for analgesic and anti-inflammatory activity in mice and rats, respectively. Among all these derivatives, 1.39 showed superior anti-inflammatory activity and 1.40 showed significant analgesic activity [55].



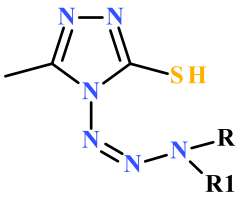
1.39



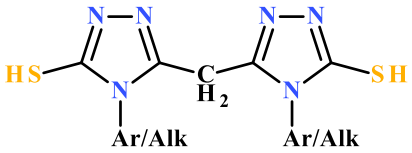
1.40

1.3 Anti-inflammatory activity of 1,2,4-triazole derivatives

Arylidene derivatives of 5-substituted 3-mercapto-4-amino-1,2.4-triazole 1.41 showed high anti-inflammatory activity at the level of ibuprofen in the carrageenan model of paw edema in rats [56]. Several bis-triazole derivatives of type 1.42 were evaluated for anti-inflammatory activity by carrageenan-induced paw edema in mice. All tested compounds showed high anti-inflammatory activity [57].

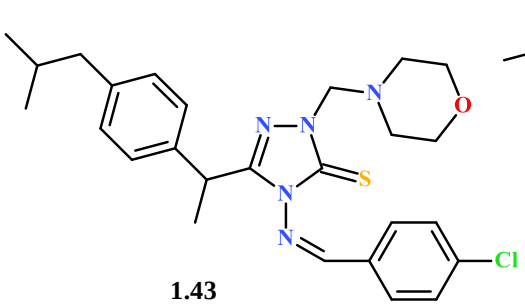


1.41

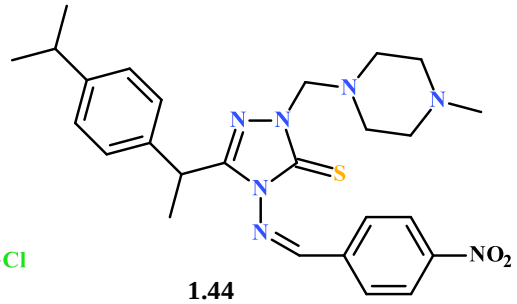


1.42

According to carrageenan screening of rat paw edema, 2-substituted derivatives of aminomethyl-4-arylideneamino-1,2,4-triazol-3-thione, all compounds showed higher anti-inflammatory activity than ibuprofen [58]. In addition, it was proved that morpholine (1.43) and N-methylpiperazine residues (1.44) contributed to the increased activity.

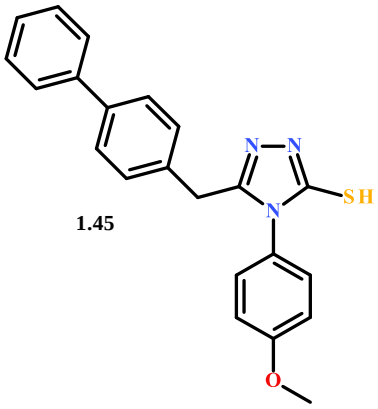


1.43

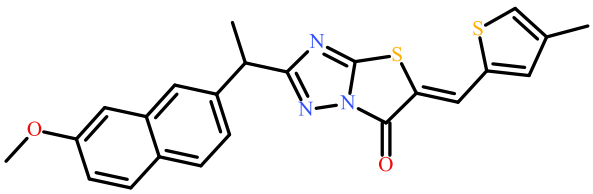


1.44

A new series of triazolothiones were investigated in vivo for their anti-inflammatory activity and found that compound 1.45 exhibited strong anti-inflammatory activity with 56.49% inhibition and analgesic activity was 65.24% compared to the control drug [59].



1.45

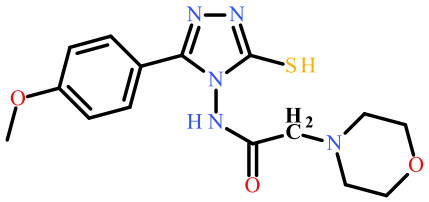


1.46

Sorigol et al. synthesized thiazolo[3,2-b]1,2,4-triazol-6(5H)-one derivatives and investigated their analgesic and anti-inflammatory activity in vivo. Compound

1.46 had the most selective inhibition to COX-2 of all tested compounds and significant analgesic and anti-inflammatory activity [60].

Upmanyu et al. reported that derivatives of 4-substituted ethanoylamino-3-mercapto-5-(4-methoxy-phenyl)-1,2,4-triazoles have pronounced anti-inflammatory and antinociceptive activities [61]. Compound 1.47 showed the highest percentage of inhibition of carrageenan edema.



1.47

Conclusions to the Chapter 1

In this section, we have analysed, structured, and summarized the data of scientific literature concerning the methods of synthesis and pharmacological activity of derivatives of 1,2,4-triazole-3-thione and its pyridine-substituted derivatives. Some regularities of the structure-anti-inflammatory activity relationship have been established and the expediency of further investigation of this class of compounds has been substantiated.

CHAPTER 2

RATIONAL DRUG DESIGN OF 1,2,4-TRIAZOLE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

2.1 Implementing the concept of “drug design”

For many years, the main and traditional method of searching for promising biologically active substances has been the synthesis of numerous series of organic compounds and their testing for various types of activity. Experimental studies were conducted on animals, which in fact always led to the death of a significant number of them. In addition, financial and time costs associated with the synthesis of unpromising substances determined the need to create a theoretical basis and methodology for the search for new biologically active substances. The modern scientific world has accumulated a significant database of experimental data and created enough tools to improve the efficiency of research on the synthesis of new active pharmaceutical ingredients. Nowadays, both the world's leading pharmaceutical companies and small research laboratories apply the latest “drug design” technologies, using a wide range of *in silico* methods, to search for new APIs and subsequently create drugs [61]. The use of this approach allows to significantly reduce financial and time costs, as well as to reduce the number of laboratory animals used [62]. The key point of all drug design approaches is the targeted synthesis of lead-compounds, drug-candidates and their subsequent optimization considering all ADME-Tox studies.

In our work we used “drug design” approaches to create promising APIs with as promising anti-inflammatory agents. The stages of the planned work are depicted in Fig. 2.1 and included the following actions: logical and structural analysis of literature data and selection of a privileged structure with which biological activity is associated more often than with other structures; compound design; molecular docking for activity prediction; conventional synthesis; pharmacological screening; and selection of a leader structure.

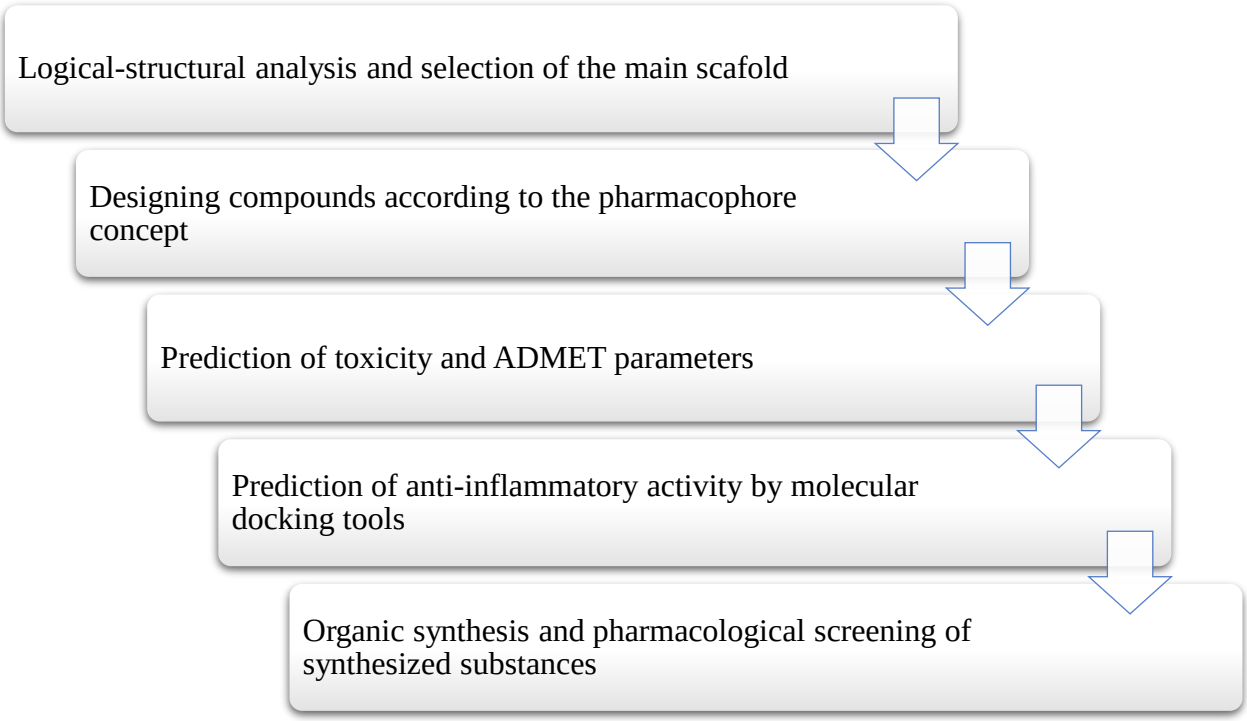


Fig. 2.1. Implementation of the drug-design concept used in the study

The first and main step at the planning stage of API synthesis is the choice of the basic structure - a fragment of the molecule, which will be associated with the presence of the desired pharmacological effect, and with which chemical modification will be carried out in order to introduce the necessary pharmacophore fragments. The theoretical justification of the choice of the basic structure is based on the accumulated empirical experience and logical-structural analysis.

The logical-structural approach is based on the systematization of already available data on the relationship between the chemical structure and biological activity of substances. A thorough study of the literature data on the pharmacological activity of 1,2,4-triazole derivatives allows us to assert with certainty that the presence of the heterocyclic system of 1,2,4-triazole in the structure of the substances determines their anti-inflammatory activity. Moreover, some studies suggest that the presence of this heterocycle causes selective inhibition of cyclooxygenase-2 (COX-2) [63]. Scientists of European countries refer the heterocyclic system of 1,2,4-triazole to the privileged structure, since most synthesized derivatives of this heterocycle show some pharmacological, in particular, anti-inflammatory activity.

2.2 Construction of targeted 4-amino-1,2,4-triazole-3-thione

1,2,4-triazole derivatives are low-toxic, fairly easy to obtain and highly reactive substances, which allows the introduction of various pharmacophore fragments into their structure. Triazoles undergo various types of reactions, for example, with thiourea derivatives, thioethers, easily form Mannich and Schiff bases, condense to form other heterocyclic systems: triazolothiadiazoles and triazolothiadiazines, triazolothiazines and triazolothiazepines [64]. In view of the above, we chose 4-amino-1,2,4-triazole-3-thione as the basic structure for the targeted synthesis of potential anti-inflammatory agents (Fig. 2.2).

The choice of 3-thiotriazole, since the presence of a sulfur atom in the structure of the substances, increases their lipophilicity and, consequently, their absorption and bioavailability. Therefore, in our opinion, it was reasonable to use 1,2,4-triazole-3-thione to achieve the goal. In addition, the presence of the thiogroup expands the possibilities for the introduction of additional pharmacophore moieties.

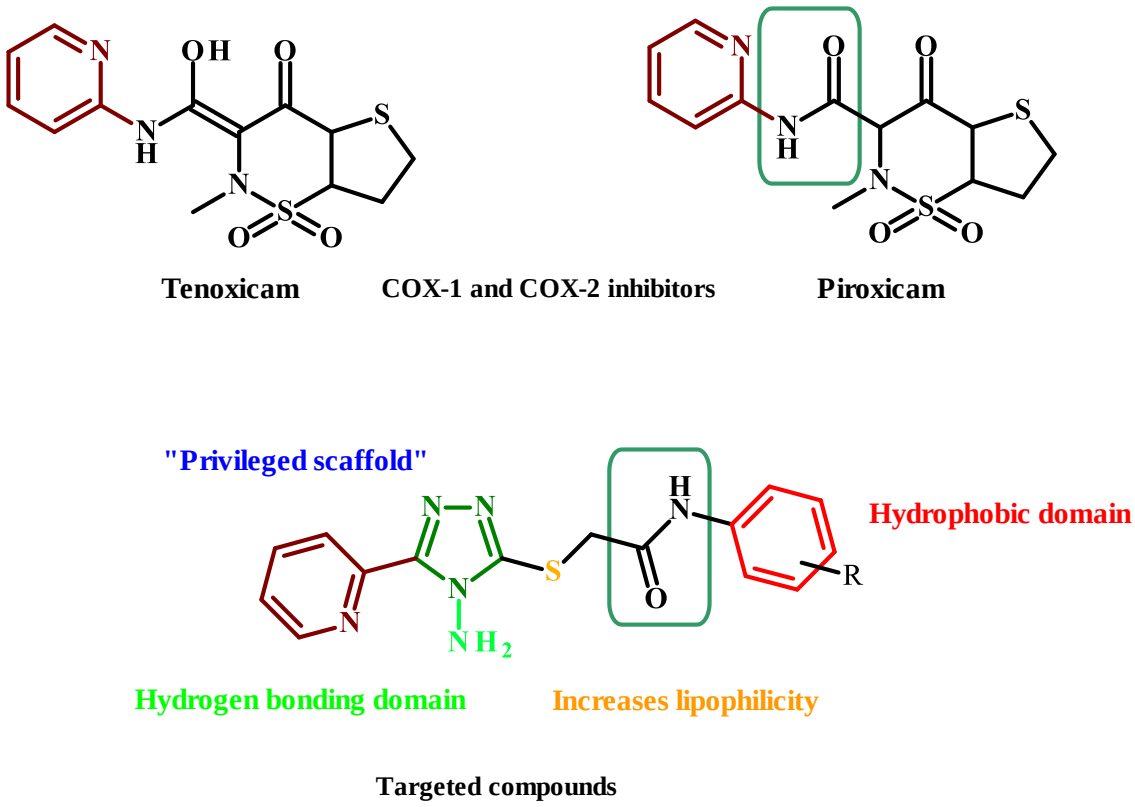


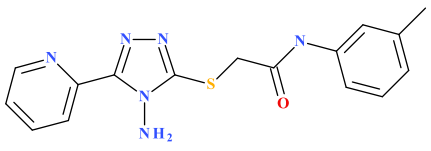
Figure 2.2. Directions for the design of target compounds

Analysis of the pharmaceuticals market has shown that drugs having an amino group in their composition are quite highly effective, in addition, the amino group is part of amino acids, which are extremely important in the organic world. The free amino group has an unshared pair of electrons, which can be an additional group for the formation of hydrogen bonds when binding to the receptor.

Literature data regarding the influence of substituents in the 5 position of the triazole cycle show that the presence of aryl and heteryl radicals increases the likelihood of anti-inflammatory activity. Most publications concern aryl-substituted derivatives of 1,2,4-triazole, while there are not many studies on the anti-inflammatory activity of heteryl-substituted derivatives.

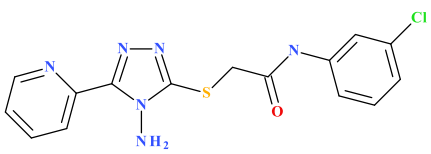
As shown in Fig. 2.2, modern anti-inflammatory drugs from the oxicam group - tenoxicam and piroxicam - contain a pyridine substituent in their structure. A few authors [65] confirm the effect of the pyridine radical on the inhibition of cyclooxygenase-1 and 2. Therefore, we decided to use 5-pyridinyl-substituted 4-amino-1,2,4-triazole-3-thione as the initial intermediate and basic structure for the synthesis of potential anti-inflammatory agents (Fig. 2.2).

We planned to obtain S-acetamide derivatives of 5-(4-pyridinyl)-3-thio-4-amino-1,2,4-triazole because of the known effect of the acetamide residue in enhancing anti-inflammatory activity. In addition, the acetamide moiety is an additional distal site capable of forming hydrogen bonds while stabilizing the receptor-ligand conformation, which may increase affinity to the biotarget.

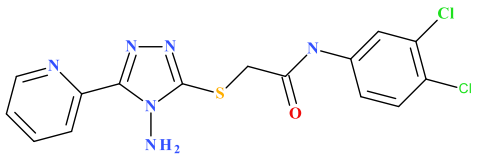


AS 111

2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl] sulfanyl]-N-(m-tolyl)acetamide
Cc1cccc(NC(=O) CSc2nnc(c3ccccn3)n2N)c1



2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]sulfanyl]-N-(3-chlorophenyl)acetamide
Nn1c(SCC(=O)Nc2cccc(Cl)c2)nnc1c3ccccn3



AS 113

2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]sulfanyl]-N-(3,4-dichlorophenyl)acetamide

Nn1c(SCC(=O)Nc2ccc(Cl)c(Cl)c2)nnc1c3ccccn3

2.3 Determination of drug-like parameters of the designed compounds

The modern era of artificial intelligence is characterized by advances in deep learning technologies and methods for its implementation, which have demonstrated their benefits in many areas, including drug development. ADMET (absorption, distribution, metabolism, excretion, and toxicity) describes the pharmacokinetics and pharmacodynamics of a drug molecule. The ADMET profile of a biologically active compound can affect its efficacy and safety. Moreover, efficacy and safety are considered to be among the main reasons for clinical failures in the development of new chemical entities [66]. All data (lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), unsaturation (INSATU) and flexibility (FLEX)) are displayed on the SwissADME bioavailability radar (Figure 2.3) [66].

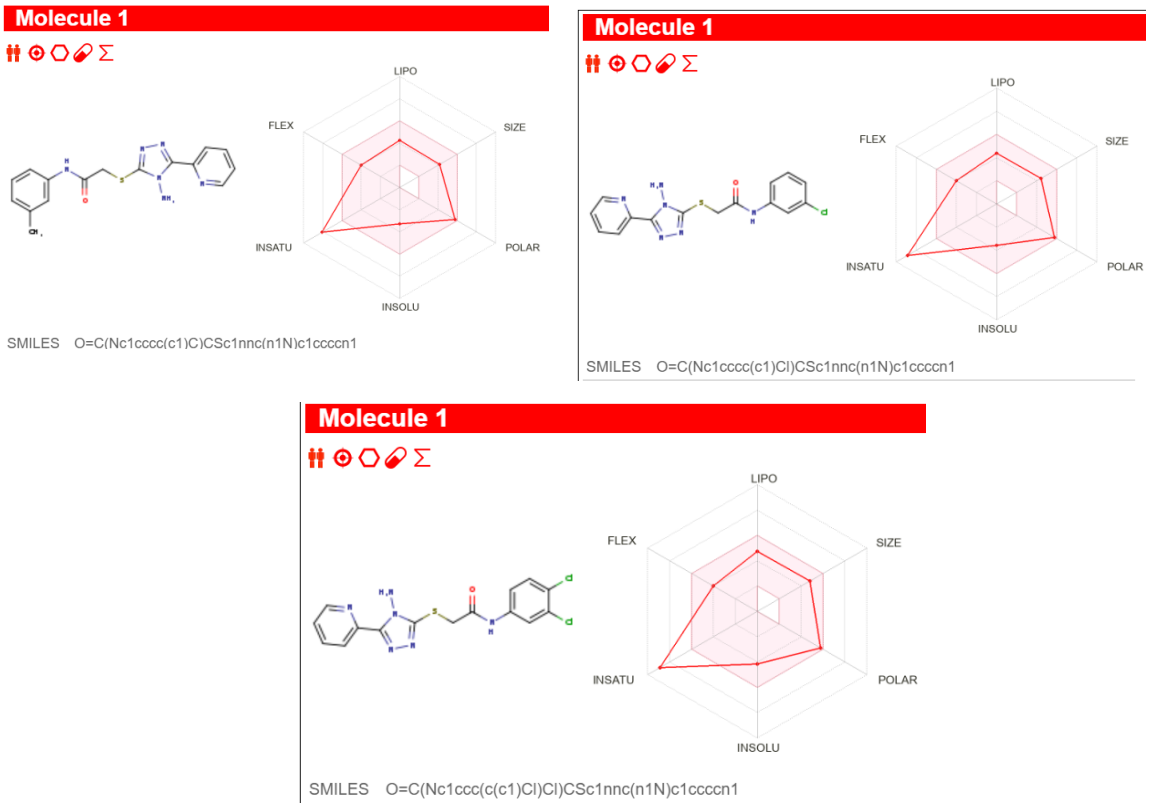


Fig. 2.3 Visualization of the SwissADME bioavailability radar of the studied derivatives

As can be seen from the bioavailability radar image (Fig. 2.3), insufficient water solubility is predicted for all substances, but this parameter is not critical and can be corrected during the synthesis process by the formation of salt forms of these substances.

2.4 Calculation of toxicity parameters of generated structures

Discovering new drugs is an expensive and time-consuming process that takes 6 to 12 years and costs billions of dollars. To improve efficiency, researchers are looking for ways to reduce costs and speed up the search for promising candidates. Predicting toxicity early in development can save resources and prevent potentially harmful compounds from being studied. Toxicity assessment helps to identify compounds that may cause unwanted effects in humans. Toxicity prediction allows researchers to prioritize safe compounds for further study. It also reduces the need for animal testing, which is ethical and regulatory compliant.

We used a machine learning based method, ProTox, which assesses toxicity using molecular fingerprints and predicts toxicity with 72% accuracy. It is being integrated into virtual screening protocols to filter out potentially toxic or difficult to synthesize candidates.

The results of the toxicity prediction for the generated 1,2,4-triazole derivatives are shown in Fig. 2.4. The lowest toxicity values were predicted for 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]sulfanyl]-N-(m-tolyl)acetamide AS111 – class 4 toxicity LD50 1000 mg/kg. For both chloro-substituted derivatives AS112 and AS113, a lower average lethality dose of 500 mg/kg was predicted, but the toxicity class is also 4.

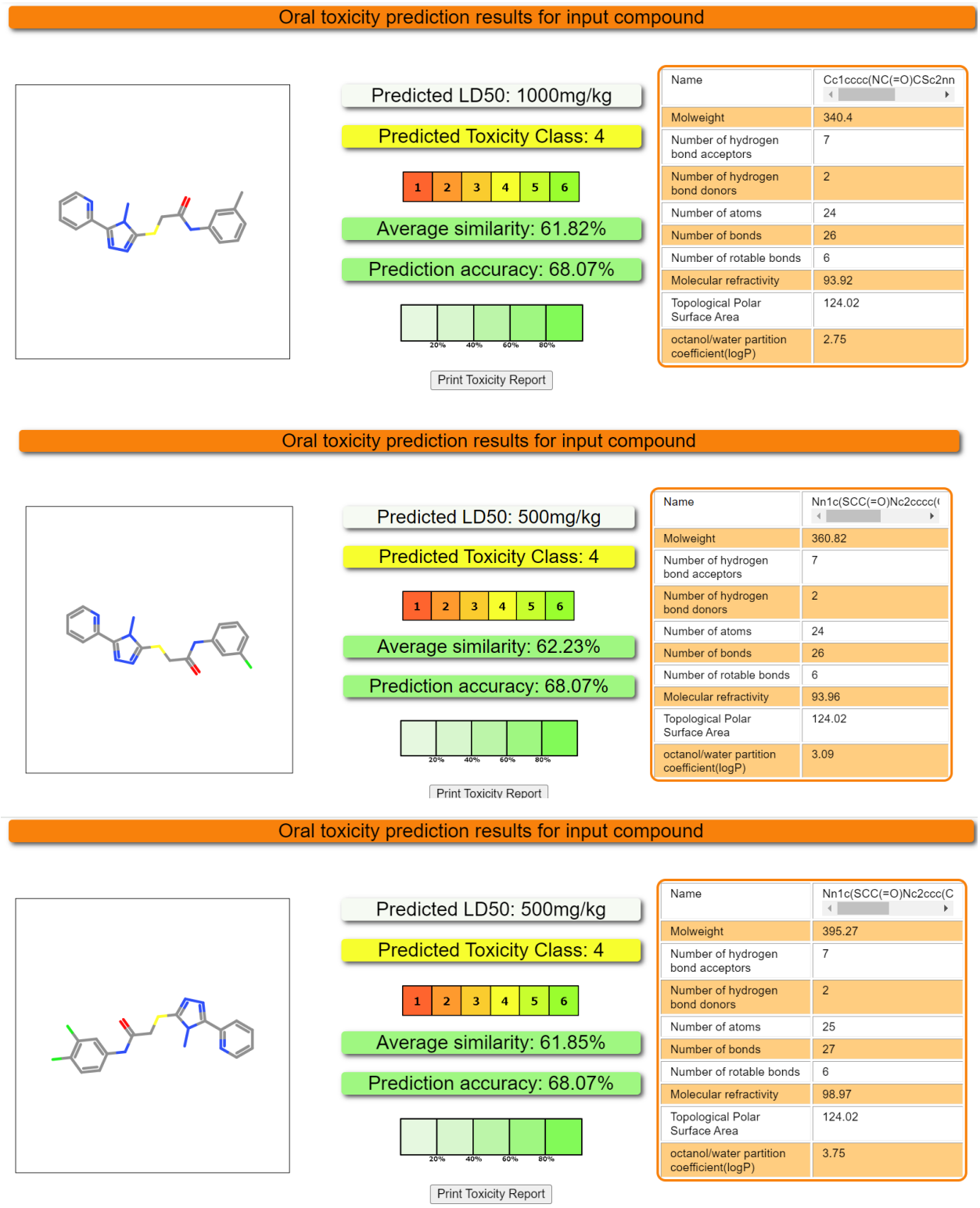


Fig. 2.4 Visualization of toxicity prediction data for AS111, AS112, AS113 derivatives

Conclusions to the Chapter 2

1. By logical-structural analysis of the literature data of the structure-anti-inflammatory activity relationship, the basic structure was determined

and the substances were designed as potential anti-inflammatory agents.

2. The generated database of compounds was analyzed by ADMET parameters: bioavailability radar, physicochemical parameters, lipophilicity, water solubility, pharmacokinetic parameters and drug-like parameters: satisfactory drug-like parameters were determined, except for poor water solubility.
3. The toxicity of the generated compounds was predicted by the ProTox program and it was determined that all 1,2,4-triazole-3-thione derivatives belong to the 4th class of toxicity with LD50 of 500 and 1000 mg/kg.

CHAPTER 3

MOLECULAR DOCKING OF GENERATED DERIVATIVES OF 1,2,4-TRIAZOL-3-THIONE

3.1 Selection of target for molecular docking

Back in 1994, J. Vane put forward a hypothesis according to which the anti-inflammatory, analgesic and antipyretic effect of NSAIDs is associated with their ability to inhibit cyclooxygenase-2 (COX-2) [67], while the most common side effects (impaired blood flow in the kidneys, digestive tract damage like NSAID-induced gastropathy, inhibition of platelet aggregation and hemorrhages) are associated with inhibition of cyclooxygenase-1 activity. This assumption became a theoretical prerequisite for the development of new NSAIDs and selection of agents with the highest selectivity to COX-2, the treatment of which would be highly effective and at the same time maximally safe. This assumption has by now been confirmed by numerous experimental data, and the structure of the enzyme and active sites of selective inhibitors have been established by X-ray structural analysis, which allows their use in in silico docking studies [69].

3.2 Determination of affinity of generated compounds to cyclooxygenase 2

To predict the affinity of the designed ligands AS111, AS112 and AS113 to COX-2 and to justify the feasibility of in vivo study for anti-inflammatory activity, docking into the active site of celecoxib was performed. Celecoxib is a selective high-affinity inhibitor of COX-2. The crystal structure of the enzyme in conformation with celecoxib (PDB ID-3LN1) is a homotetrameric protein, each homodimer of which contains an active site of the inhibitor [68]. According to experimental data, the selectivity of celecoxib is due to twelve strong and short hydrophobic bonds with the following amino acid residues: arginine Arg106, 499, phenylalanine Phe504, serine Ser339, valine Val335, 509, leucine Leu345, 370, methionine Met508, tyrosine Tyr341, tryptophan Trp373, alanine Ala513.

Reference docking confirmed the high affinity of celecoxib to COX-2: the calculated binding energy was -12.1 kcal/mL (Table 2.1).

Table 2.1.

Results of docking of 4-amino-1,2,4-triazol-3-thione derivatives to the active site of cyclooxygenase-2

Ligand	Binding energy kcal/mol	Hydrophobic interaction	Hydrogen bonds
Celecoxib	-12,1	VAL335(2), SER339, VAL509(2), LEU370, LEU345, LEU517, TYR371, TRP373, ALA513(2)	ARG106, ARG499, GLN178, LEU338, SER339
AS111	-8,8	VAL509(2), HIS75, LEU338, SER339, LEU370, MET508, TRP373 (2), PHE504, ARG499, ALA502	SER516(2), LEU338
AS112	-8.7	VAL509(2), HIS75 (2), LEU338, SER339, GLY512, ALA513, LEU370, MET508, TRP373 (2), PHE504, ALA513, ARG499, ALA502	SER516, LEU338
AS113	-8.3	VAL509(2), TYR371, TYR341 GLY512, ALA513, LEU370, VAL509, PHE367, TRP373, PHE504, ALA513, ALA502	SER516(2)

.

For all compounds planned for synthesis, higher binding energy values are predicted than those of the reference ligand: -8.8 (**AS111**), -8.7 (**AS112**) and -8.3 (**AS113**) kcal/mol, versus -12.5 kcal/mol, respectively. The affinity level is quite high according to generally accepted molecular docking criteria and the probability of exhibiting an inhibitory effect on cyclooxygenase-2 is highly probable. The highest binding energy value (lowest affinity) was demonstrated by 3,4-dichlorosubstituted triazole (**AS113**).

For all compounds, the formation of hydrogen bonds between the distal zone-acetamide moiety and the amino acid residues of serine SER516 and leucine

LEU338 is predicted to occur, which was planned when the compounds were designed and stabilizes the conformation in the active site (Figures 3.1-3.3).

For the compound 2-[[4-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3-methylphenyl)acetamide (**AS111**), the formation of 12 hydrophobic interactions with fixation of all fragments of the molecule is predicted.

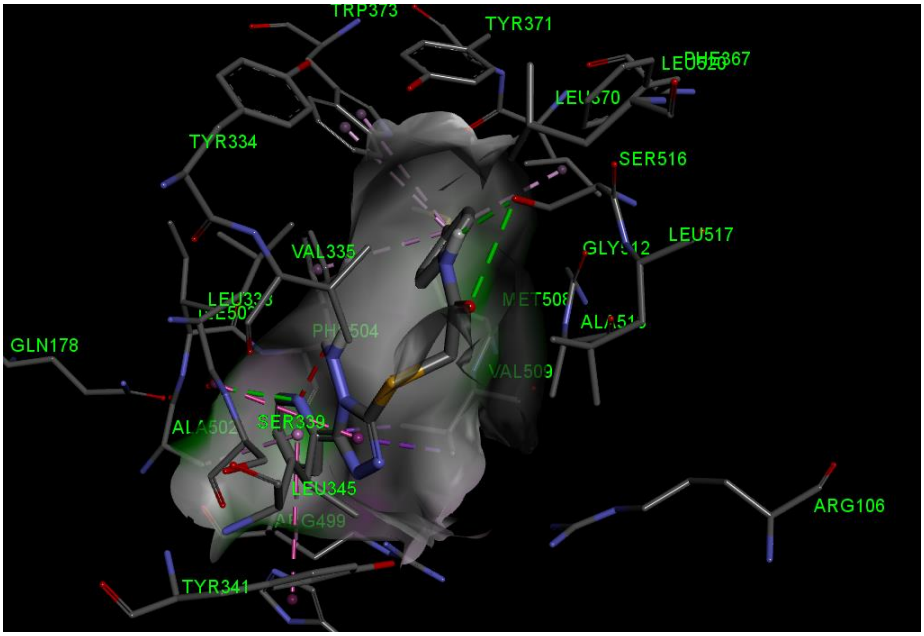


Figure 3.1 Interaction of 2-[[4-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3-methylphenyl)acetamide (**AS111**) with COX-2 active site amino acids

For the compound 2-[[4-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3-chlorophenyl)acetamide (AS112), the formation of 12 hydrophobic interactions with fixation of all fragments of the molecule is also predicted, in particular tetrahedral hydrophobic stabilization of the pyridine cycle and the 4-chlorophenyl fragment (Figure 3.2).

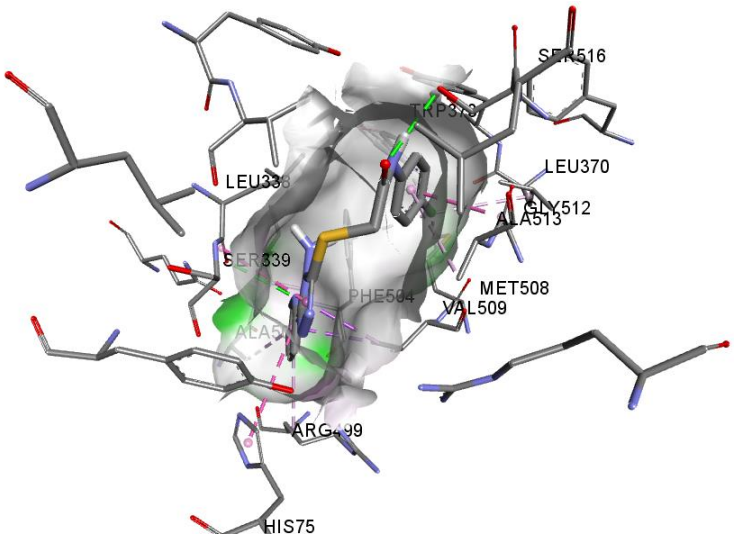


Fig. 3.2. 3D visualization of the reference interaction of 2-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3-chlorophenyl)acetamide (**SA112**) with COX-2 active site amino acids

For the compound 2-[[4-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3,4-dichlorophenyl)acetamide (**AS113**), the lowest affinity compound, 11 hydrophobic interactions are predicted to form, but less rigid fixation of the edge aromatic rings is noted (Figure 3.3).

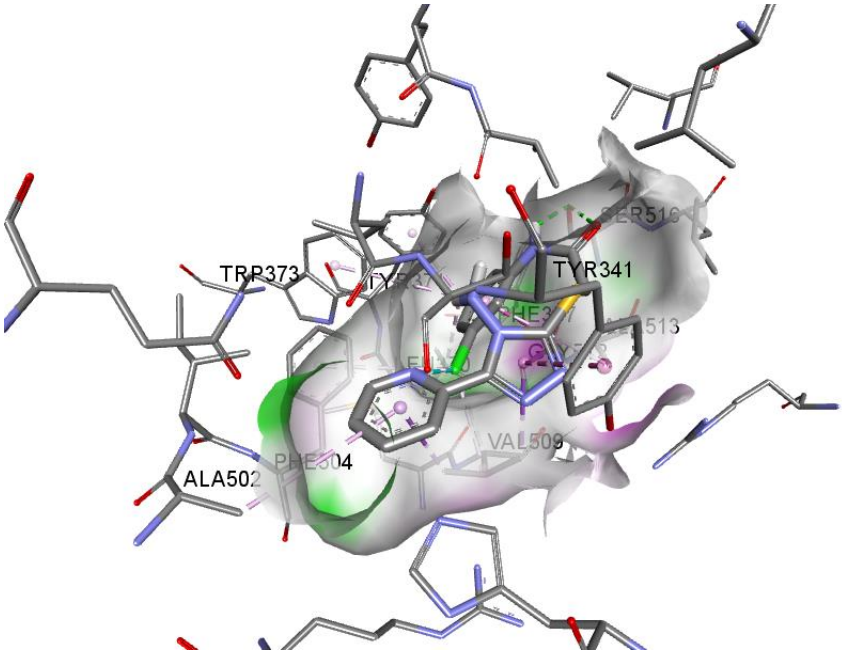


Fig. 3.3 3D visualization of the reference interaction of 2-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3,4-dichlorophenyl)acetamide (**AS113**) with the amino acids of the COX-2 active site.

When the investigated ligands (**AS111, AS112, AS113**) and reference celecoxib are co-located in the active site, the absolute identity of conformation in space becomes evident (Fig. 3.4). Structural fragments overlap, including aromatic radicals.

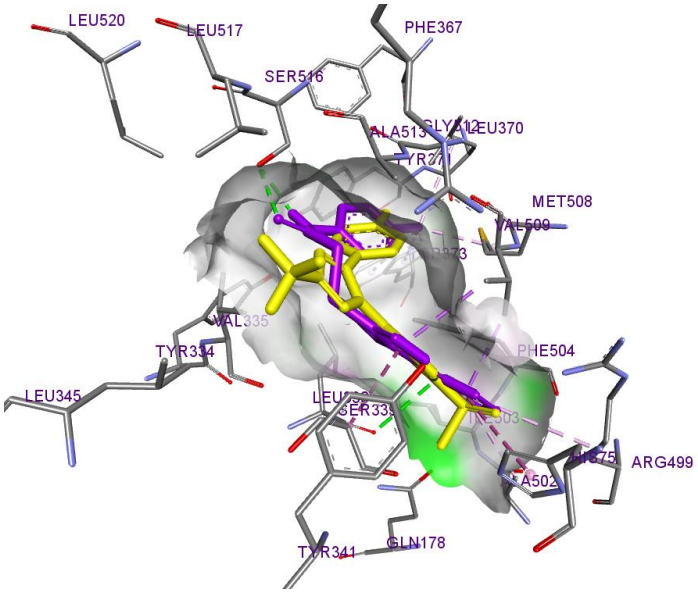


Fig. 3.4 Joint conformation of reference celecoxib (yellow molecule) and 2-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-(3-methylphenyl)acetamide (**AS111**, purple molecule) in the COX-2 active site

According to the results of molecular docking, all designed ligands have a high degree of affinity to the cyclooxygenase-2 inhibitor site and, therefore, are able to exhibit anti-inflammatory activity in an in vivo experiment.

Experimental part

Flexible molecular docking was performed using AutoDock Vina and AutoDockTools1.5.6 programs. Macromolecules were downloaded from Protein Data Bank [70]: COX-2 - PDB ID 3LN1. Structures were designed using BIOVIADraw 2021 program, mol format. Structures were optimized by Chem3D program using MM2 molecular mechanical algorithm, saved in .pdb format, converted to .pdbqt using AutoDockTools-1.5.6 [71].

Discovery Studio Visualizer 2021 was used to remove the solvent and native ligand from the crystal. The proteins were saved in .pdb format. AutoDockTools-1.5.6 added polar hydrogens to the protein structure and saved in .pdbqt format.

Grid box size and coordinates of its centers were determined using native ligands: COX-2 (PDB ID 3LN1): x=18.84, y=-52.89, z=53.81; size x=22, y=18, z=20;

Docking with the AutoDock Vina program. Interpretation of the results

interpretation of the docking results – Discovery Studio 2021.

Conclusion to the Chapter 3

1. Macromolecules were analyzed in Protein Data Bank and the target for molecular docking, cyclooxygenase 2 in conformation with the selective inhibitor celecoxib, was selected.
2. Molecular docking was performed, a high level of affinity of candidate structures to the cyclooxygenase-2 inhibitor site was predicted and the feasibility of their synthesis and in vivo studies was confirmed.

CHAPTER 4

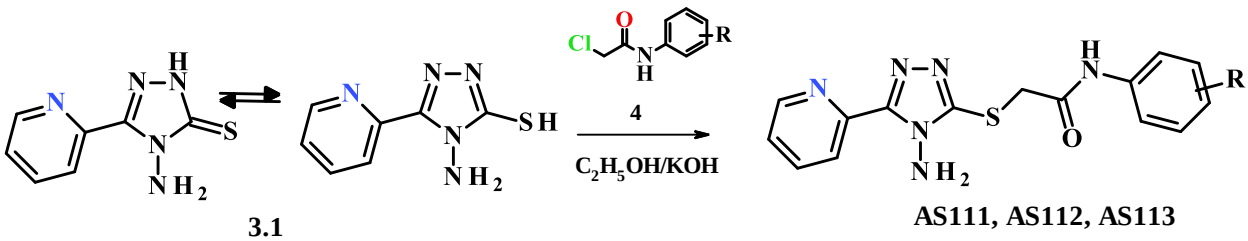
DISCUSSION OF THE METHODOLOGY OF SYNTHESIS AND RESULTS OF STUDYING THE ANTI-INFLAMMATORY ACTIVITY OF THE INVESTIGATED SUBSTANCES

4.1 Discussion of methodology for the synthesis of 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]thio]-N-arylacetamides

Among the alkylated derivatives of 1,2,4-triazole-3-thione, compounds with diverse pharmacological properties have been found [3]. Therefore, a considerable amount of work has been devoted to the study of the conditions of electrophilic substitution/reaction, the presence of thione-thiol and azole-azole tautomerism, which are characteristic of 1,2,4-triazole-3-thione.

The starting 4-amino-3-thio-5-(pyridin-2-yl)-1,2,4-triazole (4.1) was synthesized at the Department of Pharmaceutical Chemistry, National University of Pharmacy earlier. The alkylation reaction of 1,2,4-triazole-3-thione was carried out under the supervision of Prof. Anna Severina, and ethyl alcohol as a solvent alkalinized with potassium hydroxide was used to synthesize the target S-acetamides (**AS111, AS112, AS113**). The reaction for the preparation of acetamide derivatives of 5-(pyridin-2-yl)-4-amino-1,2,4-triazol-3-thione (**AS111, AS112, AS113**) is presented in Scheme 4.1.

Scheme 4.1



R= AS111 3-Me, AS112 3-Cl, AS113 - 3,4-diCl

N-arylsamidation of α-chloroacetamides were used as alkylating agents. Although the addition of alkali shifts the equilibrium towards the thiol form and increases the probability of the reaction proceeding on the sulfur atom, however, it

does not exclude the possibility of the alkylation reaction proceeding also on the nitrogen atom in the second position of the triazole cycle, which is also a nucleophilic center and the addition of potassium hydroxide can lead to its activation. Accordingly, under these conditions both S- and N-acetanilides of 4-amino-3-thio-5-(pyridin-4-yl)-1,2,4-triazole can still be formed, and also formation of their mixture is probable.

The alkylation of 4-amino-5-(2-pyridyl)-1,2,4-triazole-3-thione (3.1) was carried out by its interaction with N-arylsamidazenimine α-chloroacetamidamide (Scheme 4.1). The reaction mixture of the initial triazole 5 with the corresponding acetanilide was boiled with reflux condenser for one hour in ethyl alcohol in the presence of KOH. The target acetanilides AS111, AS112, AS113 were obtained in satisfactory yields.

General procedure for the synthesis of 4-amino-5-(pyridin-2-yl)-1,2,4-triazol(4H)-3-ylthioacetamides (AS111, AS112, AS113).

To a solution of 0.002 mol of thioaminotriazole (3.2) in 20 mL of ethanol 20 mL of 0.002 M potassium hydroxide aqueous solution was added. To the resulting solution a solution of 0.002 mol of the corresponding N-arylsamidated α-chloroacetamide was added under stirring. The reaction mixture was heated with reflux condenser for 1 h and then poured into 200 mL of water. The precipitate was filtered off, washed with water, dried, crystallized from ethyl alcohol.

4.1 Results of animal studies of anti-inflammatory activity of the synthesised compounds

Logical-structural analysis in the choice of research objects and the results of predictive research methods of the synthesized thioaminotriazole derivatives obtained in Section 2-3 confirmed and substantiated the expediency of experimental pharmacological studies on anti-inflammatory activity.

Anti-inflammatory activity was studied on the experimental model of formalin-induced paw edema in rats under the supervision of Doctor of

Pharmaceutical Sciences, Professor Anna Olegovna Syrova, Head of the Department of Medical and Bioorganic Chemistry, Kharkov National Medical University.

To study the anti-exudative activity, the research was carried out to compare the effect of synthesized arylacetamide derivatives of 4-amino-5-(2-pyridinyl)-1,2,4-triazole-3-thione with the reference drug - diclofenac sodium.

Selection of doses and methods of studies were carried out according to the existing recommendations in Ukraine [72]. Anti-exudative effect of the synthesized substances **AS111, AS112, AS113** was studied on white male rats. Animals were divided into 5 groups of 6 animals in each group. Animals of the 1st group were the control group, they were injected once intragastrically with 3% starch glue (2 ml per 200 g of animal body weight). Animals of groups 2-4 were administered acetamide derivatives of 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione **AS111, AS112, AS113** at the rate of 10 mg/kg once intragastrically as a suspension in 3% starch glue one hour before the development of maximal edema. Group 5 animals were administered diclofenac sodium (0.1% solution) at a rate of up to 8 mg/kg of animal weight.

Edema was modeled by subplantar injection of 0.1 ml of 2% formalin solution into the hind paw. Paw volume was measured using digital plethysmography before drug injection and at the background of maximal edema 4 hours after modeling formalin injection.

Anti-exudative activity (AEA) was determined 4 hours after formalin injection by the degree of edema reduction in the studied groups compared to the control group and expressed in relative units calculated according to the formula:

$$\% \text{ AEA} = ,$$

where, Vc – means the difference between swollen and healthy paw volumes in the control group;

Vo – an average difference between swollen and healthy paw volumes in the studied group.

The results of studying the anti-exudative effect of compounds **AS111, AS112, AS113** are presented in Picture 4.1.

The screening results confirmed the predictive methods of the study and all synthesized compounds demonstrated some degree of anti-inflammatory activity. The highest activity was demonstrated by arylacetamide derivatives of 4-amino-3-thio-1,2,4-trizole with a methyl group in the phenyl radical of **AS111**. Its activity slightly exceeded that of the reference drug diclofenac sodium by 1.26 times. Compound **AS112** with 3-chloro- and **AS113** with 3,4-dichlorophenyl radicals were slightly inferior to diclofenac sodium radical by 7.4 and 20%, respectively.

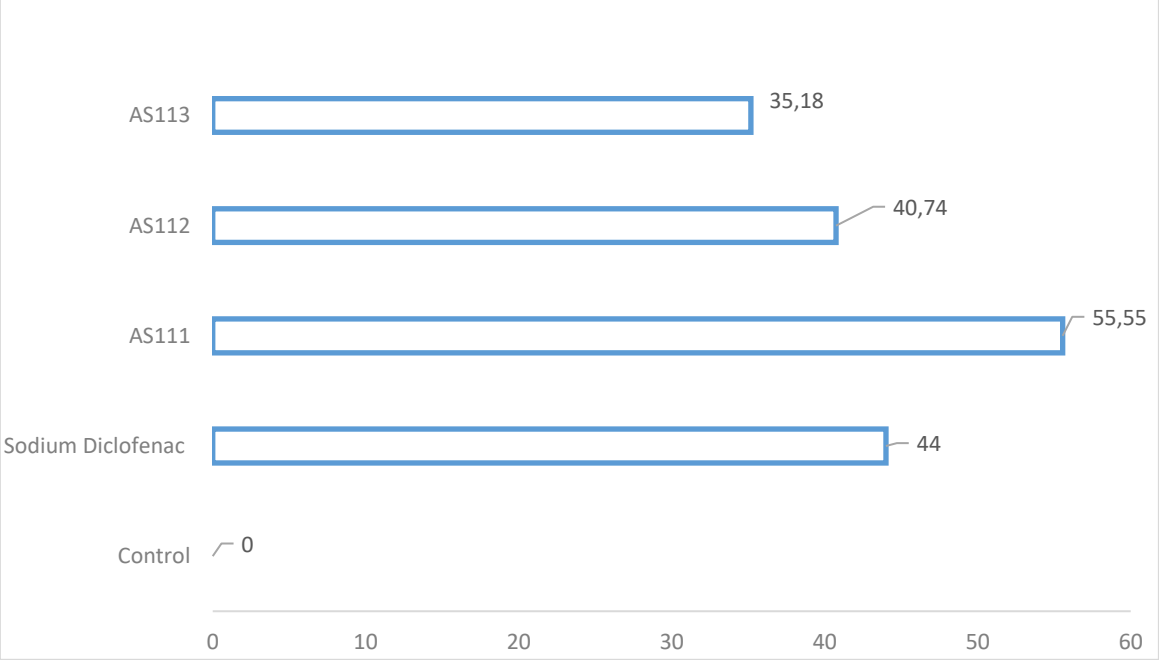


Figure 4.1. Anti-inflammatory activity of 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]thio]-N-arylacetamide compounds (**AS111, AS112, AS113**).

It can be stated that the anti-inflammatory activity decreases with the increase of chlorine atoms in the structure.

Conclusions to the Chapter 4

1. Conditions for the synthesis of 4-amino-5-(pyridin-2-yl)-1,2,4-triazole-3-ylthioacetamides have been studied, in particular, the direction of the alkylation reaction with N-aryl-substituted α-chloroacetamides in the presence of potassium hydroxide is selective for the sulfur atom.

2. The anti-inflammatory activity of the synthesized derivatives of 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]thio]-N-arylacetamides on the model of formalin edema in rats was studied.
3. It was found out that all the synthesized compounds exhibit anti-inflammatory activity at the level of reference drug – diclofenac.
4. The leading compound – 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]thio]-N-(3-methylphenyl)acetamide **AS111** was found to be 1.28 times more active than diclofenac sodium.

CONCLUSIONS

1. The scientific literature concerning the existing methods of preparation and biological activity of derivatives of 1,2,4-triazol-3-thione and the modern approach to the search for new substances with anti-inflammatory activity among its derivatives was analyzed.

2. Based on the principles of modern drug-design, the basic structure was determined and substances with potential anti-inflammatory effects were designed.

3. The generated database of compounds was analysed by ADMET parameters: bioavailability radar, physicochemical parameters, lipophilicity, water solubility, pharmacokinetic parameters and drug-like parameters: satisfactory drug-like parameters were determined, except for poor water solubility.

4. The toxicity of the generated compounds was predicted by the ProTox program and it was determined that all 1,2,4-triazole-3-thione derivatives belong to the 4th class of toxicity with LD50 of 500 and 1000 mg/kg.

5. Molecular docking was performed and predicted high affinity of the target derivatives to the cyclooxygenase-2 inhibitor site and the feasibility of *in vivo* studies was confirmed.

6. The alkylation conditions of 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-thione with N-aryl-substituted α-chloroacetamides in the presence of potassium hydroxide were studied and 4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-ylthioacetamides were obtained.

7. According to the results of screening for anti-inflammatory activity of 2-[[4-amino-5-(2-pyridinyl)-1,2,4-triazol-3-yl]thio]-N-arylacetamides derivatives on the model of formalin edema in rats, it was found that all compounds exhibit anti-inflammatory activity at the level of reference drug – diclofenac.

8. The leader compound – 2-[[4-amino-5-(2-pyridyl)-1,2,4-triazol-3-yl]thio]-N-(3-methylphenyl)acetamide was found to be 1.28 times more active than diclofenac sodium. Comparability of the results of *in vivo* and *in silico* experiment and the probability of realization of anti-inflammatory activity through inhibition of cyclooxygenase-2 enzyme were established.

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