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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: «**Design and study of novel tetrazol-5-thiole derivatives as potential anti-ulcer agents»**

Prepared by: higher education graduate of group
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specialty 226 Pharmacy, industrial pharmacy
educational program Pharmacy
Adnane ENNOUSSI
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

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ANNOTATION

A comprehensive study was conducted on the design of new acetamide derivatives of tetrazol-5-thiol as possible antiulcer agents. Based on the results of in silico molecular modeling, toxicity and docking prediction, synthesis and in vivo study on the model of ethanol-prednisolone ulcer in rats, the promising antiulcer agents – N-(2,6-dichlorophenyl)-2-(1-phenyltetrazol-5-yl) sulfonyl acetamide and N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl) sulfonyl acetamide were determined. The work consists of an introduction, 4 chapters, conclusions and a list of references consisting of 50 sources. The work is presented on 42 pages and contains 2 tables, 18 figures, 1 Scheme.

Key words: tetrazole, acetamide, molecular docking, toxicity, in silico, antiulcer activity

АНОТАЦІЯ

Проведене комплексне дослідження щодо дизайну нових ацетамідних похідних тетразол-5-тіолу як можливих противиразкових агентів. За результатами *in silico* молекулярного моделювання, прогнозу токсичності та докінгу, синтезу та *in vivo* дослідження на моделі етанол-преднізолонової виразки в щурів визначено перспективність противиразкові агенти – N-(2,6-дихлорофеніл)-2-(1-фенілтетразол-5-іл) сульфаніл-ацетамід та N-(2,5-диметоксифеніл)-2-(1-фенілтетразол-5-іл)сульфаніл-ацетамід. Робота складається зі вступу, 4 розділів, висновків і списку використаної літератури, який складається з 50 джерел. Робота викладена на 42 сторінках та містить 2 таблиці, 18 малюнків, 1 схему.

Ключові слова: тетразол, ацетамід, молекулярний докінг, токсичність, *in silico*, противиразкова активність

CONTENTS

LIST OF ABBREVIATIONS

CP	control pathology
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
GIT	gastrointestinal tract
IN SILICO	Research methods using mathematical calculation methods
IN VITRO	Research methods using cell cultures
IN VIVO	Methods of study in a living organism
MPGES1	microsomal prostaglandin synthase E-1
NSAIDs	nonsteroidal anti-inflammatory medicinal products
PPIs	proton pump inhibitors
PU	peptic ulcer
R	radical
SPU	State Pharmacopoeia of Ukraine (ed. 2)

INTRODUCTION

Actuality of the topic. The problem of peptic ulcer disease occupies a central place in gastroenterology. The rapid spread of this disease is associated with urbanization, emotional overstrain, violation of the rhythm of life, irrational nutrition, environmental pollution. Chronic recurrent course, polyetiology and polypathogenicity, polymorphism of clinical manifestations, severe complications that characterize peptic ulcer disease, as well as determining the role of *Helicobacter* infection in the development of the disease, determine the complexity of its successful treatment. Therefore, despite the availability of various methods of treatment of peptic ulcer disease and a significant arsenal of anti-ulcer medicinal products, the problem of treatment of this pathology remains unsolved. All the above-mentioned causes the necessity to create new, highly effective and safe medicinal products that can simultaneously affect the various links of ulcer pathogenesis, leading to the normalization of homeostasis systems.

Tetrazole derivatives are well known as the structural basis of effective agents with anti-inflammatory, anti-allergic, antihypertensive and other types of activity. Bioisosteriority of tetrazole cycle and imidazole cycle of histamine creates prerequisites for histamine-blocking activity of tetrazole derivatives as a key mechanism of anti-ulcer action. Therefore, the design of new derivatives of this heterocycle with potential anti-ulcer activity is an urgent and important issue.

The aim of the study was to model structures and investigate acetamide derivatives of tetrazol-5-thiols as potential antiulcer agents.

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

- To conduct a literature review on the aetiology and pathogenesis of peptic ulcer disease and modern antiulcer agents, in particular antisecretory agents;
- on the basis of logical-structural analysis of literature data, to design target substances as potential antiulcer agents;
- *in silico* studies to predict their toxicity;
- *in silico* studies to predict their activity towards anti-ulcer biotargets;

- synthesize target tetrazole derivatives and prove their structure using physicochemical methods of analysis;
- to carry out in vivo studies of antiulcer activity of synthesized substances.

The object of the study is the design and targeted synthesis of potential antiulcer agents.

Subject – acetamide derivatives of tetrazol-5-thiol: structure design; prediction of toxicity and antiulcer properties; methods of synthesis, investigation of physicochemical and pharmacological properties.

Research methods – organic synthesis, physical, physicochemical methods of analysis of organic compounds, docking studies – AutoDock Vina, BIOVIADraw 2021, Chem3D, HyperChem 7.5, Discovery Studio Visualizer 2021, ProTox, standard techniques for biological activity studies.

The practical value of the results. New organic compounds were synthesized, and their antiulcer activity was established. The library of 1H NMR spectra of organic biologically active substances was replenished. A new promising structure for further in-depth study of the antiulcer activity was revealed. Docking studies were carried out for promising compounds, possible mechanisms of effect realization were proposed; the ability of the compound to inhibit the enzyme microsomal prostaglandin synthase as a possible mechanism of antiulcer action was substantiated.

Elements of scientific research. The toxicity and activity to the enzyme microsomal prostaglandin synthase of tetrazol-5-thiol derivatives were predicted for the first time, their synthesis and in vivo studies were carried out.

Structure and scope of the qualification work.

The work consists of an introduction, four sections, conclusions, list of references. The work is outlined on 42 pages, illustrated with 1 scheme, 2 tables, 18 figures. The list of used literary sources contains 50 titles, 46 of which are by foreign authors.

CHAPTER 1

ETIOLOGY OF DEVELOPMENT AND PRINCIPLES OF ANTISECRETORY THERAPY OF PEPTIC ULCER DISEASE

(Literature review)

1.1 Etiology and pathogenesis of peptic ulcer disease

The problem of peptic ulcer disease occupies one of the central places in gastroenterology. This is due to both its prevalence and social and economic significance [1]. The data of world statistics show that peptic ulcer disease is one of the most frequent diseases of internal organs: 6-10% of the adult population suffers from peptic ulcer disease. In the countries of Eastern Europe in recent decades there has even been an increase in the incidence of the disease. In particular, in Ukraine the incidence of peptic ulcer disease is 7 cases per 1000 population, in Belarus – 5, in Moldova – 5.4, in Russia – 4.9. According to statistics, peptic ulcer disease in Ukraine ranks 2nd after chronic gastritis and duodenitis and in recent years accounts for 20-30% of all diseases of the digestive organs. The growth of morbidity is promoted by the pace and dynamism of modern human life, unbalanced diet and poor-quality products (alimentary factor), bad habits, stress, allergic diseases, irrational use of medicinal products, in particular non-steroidal anti-inflammatory medicinal products and glucocorticoids [2]. This is the main, but by no means complete list of etiologic factors in the development of peptic ulcer disease and other diseases of the gastrointestinal tract (GIT) such as gastritis, acute erosive lesions of the mucous membrane and peptic ulcer disease of the stomach and duodenum, inflammation of the intestine, cholelithiasis [3]. Psycho-emotional stress, heredity, disorders in the immune system, inflammatory and other mucosal changes, radiation lesions, the presence of Helicobacter infection, household (smoking and alcohol abuse) and other intoxications also play an important role in the development of peptic ulcer disease. Moreover, many of these etiologic factors combine with each other, causing the occurrence of peptic ulcer disease [4, 5].

Pharmacotherapy of peptic ulcer disease requires a comprehensive approach and includes the use of basic anti-ulcer medicinal products in combination with anti-

Helicobacter therapy, as well as auxiliary agents, which is due to the variety of etiologic factors in the development of the disease.

1.2 Classification of antiulcer agents

For the purpose of pharmacotherapy of peptic ulcer disease, various groups of medicinal products are traditionally used, including antacid, enveloping, adsorbing, antisecretory, cytoprotective, anti-Helicobacter agents, as well as medicinal products that accelerate gastric emptying and relax smooth muscles, means of central action [6].

According to the ATC classification system (Anatomical Therapeutic Chemical classification system), medicinal products used for the treatment of peptic ulcer disease belong to group A02B “Agents for the treatment of peptic ulcer and gastroesophageal reflux disease” [7].

I. Baseline remedies.

1. antacid medicinal products

2. Antisecretory medicinal products.

2.1. H2-histaminoblockers (H2-g/b)

2.2 Proton pump inhibitors (PPIs)

2.3 Selective M1-cholinoblockers (M-ch/b)

2.4. Medicinal products of different groups.

II. Complementary agents.

1. Gastroprotectors

2. Tissue-specific stimulators of regeneration.

III. Means of antihelicobacter therapy

1. antibiotics

2. synthetic antimicrobial agents

3. antisecretory medicinal products [8].

The process of ulcer formation in the stomach and duodenum is considered by most scientists as a result of disturbed interaction between the factors of acid-peptic aggression of gastric juice and elements of mucosal defines [9]. The main basic means are traditional antacid medicinal products of inorganic origin and antisecretory agents, the action of which is mainly aimed at reducing the acidity of gastric juice. Reduction of acidity can be achieved in two ways: chemical and physicochemical. In the first case, it is a matter of chemical neutralization of acid (acid-neutralizing action); in the second case – the binding of acid by its adsorption. Below we will consider antisecretory medicinal products, which are key in the treatment of peptic ulcer disease.

1.3 Antisecretory medicinal products

Antisecretory medicinal products reduce acidity by inhibiting the synthesis of hydrochloric acid. Hydrochloric acid production is controlled by three types of receptors located on the basal membrane of parietal cells – H_2-histamine, gastrin, M-cholinoreceptors. These include representatives of three important groups: H_2-histamine receptor blockers, proton pump inhibitors and M-choline blockers. Accordingly, it is possible to reduce hydrochloric acid production by blocking these receptors with the help of inhibitors [10].

1.3.1 H_2-histamine blockers

One of the most effective and widely prescribed in the clinic groups of anti-ulcer agents are H_2-histamine receptor blockers. They competitively inhibit the action of histamine (1.1) on H_2-histamine receptors of lining and principal cells, suppressing hydrochloric acid secretion. The first H_2-receptor antagonists were derived on the principle of similarity to the histamine molecule (Fig. 1.1.).

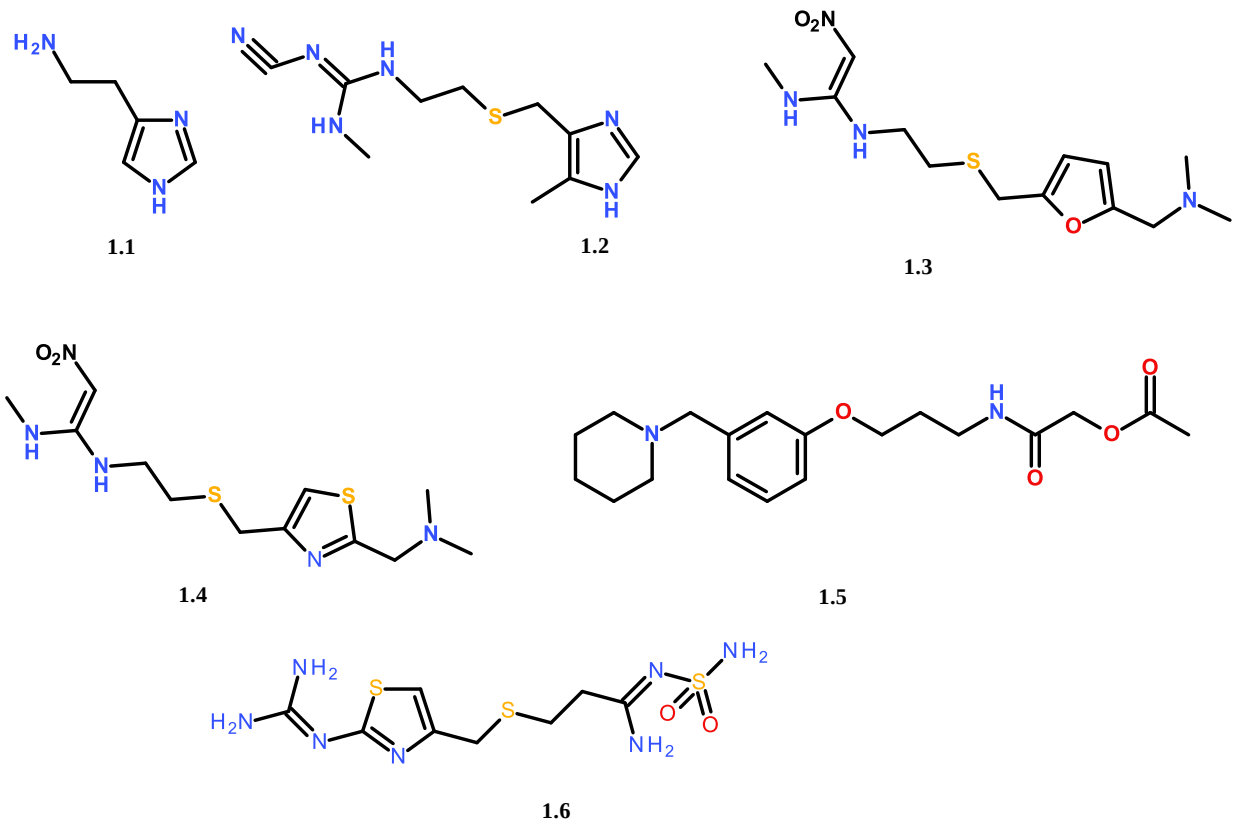


Figure 1.1. Representatives of H_2-histamine-blockers

As a result of successful targeted search for histamine receptor antagonists based on histamine molecule in the 70s, the first H_2-blocker of histamine receptors acceptable in terms of safety – cimetidine (1.2) was synthesized, and its inventor English scientist J. Black was awarded the Nobel Prize [11]. Cimetidine, a representative of the first generation, is no longer used because it has many serious side effects: 10-20% of patients experienced side effects such as diarrhea, nausea, vomiting, psychomotor agitation, headache, speech disorders, seizures, increased prolactin synthesis, impotence, drug-induced hepatitis, nephritis, hematological disorders and others [12].

Further, the medicinal products of II generation – ranitidine (1.2), roxatidine (1.3), nizatidine (1.4) and III - famotidine (1.5) were synthesized and introduced into medical practice (Fig. 1.1.). They all differ from each other in their pharmacokinetic characteristics and safety profile due to differences in their chemical structure. For example, cimetidine and oxmethidine molecules are based on imidazole heterocycle, ranitidine – furan, famotidine, nizatidine, thiotidine – thiazole [12]. All modern H_2-

histamine blockers contain guanidinethiazole groups, which are the backbone groups for binding to histamine receptors.

1.3.2 Proton pump inhibitors

Another effective group of frequently prescribed antisecretory medicinal products are proton pump blockers [13, 14]. The process of hydrochloric acid secretion is based on transmembrane proton transfer and is directly carried out by a specific proton pump – H^+, K^+-dependent ATPase. Binding of substances to the active sites of this enzyme leads to its blocking and cessation of hydrochloric acid secretion. The first medicinal product among proton pump inhibitors – omeprazole (1.7) – was synthesized in 1979 in Sweden by a group of scientists led by Ivan Estholm (Fig. 1.2) [15].

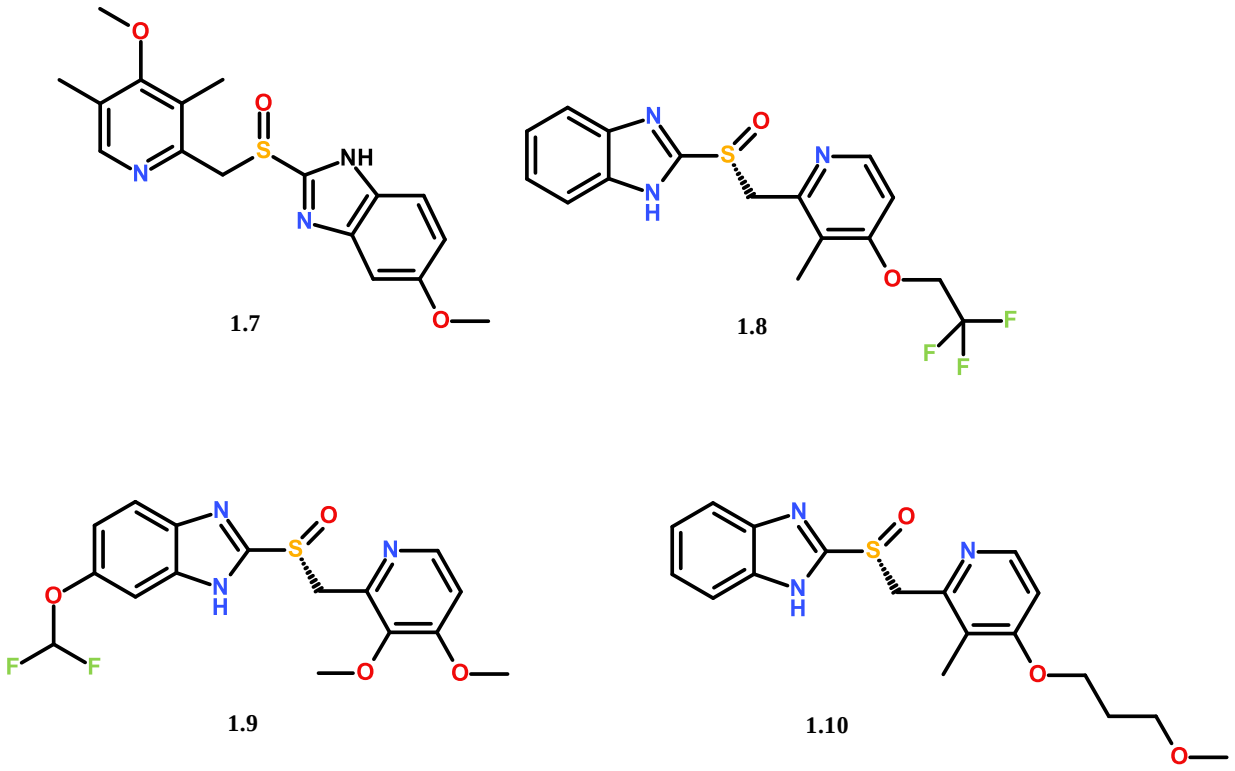


Рис. 1.2. Representatives of proton pump blockers

The next representatives of this class of compounds were lansoprazole (1.8), pantoprazole (1.9) and rabeprazole (1.10) (Fig. 1.2) [16,17]. The most recent development was esomeprazole – proton pump inhibitors, which is a product of

stereoselective synthesis technology and is an optical S-monoisomer of omeprazole [18].

Proton pump inhibitor medicinal products in comparison with H_2-histamine receptor blockers more strongly inhibit the production of hydrochloric acid and, therefore, more effectively promote the healing of ulcer defects. By chemical structure proton pump inhibitors are benzimidazole derivatives, which differ from each other by the structure of radicals on pyridine and benzimidazole rings [19]. Inhibition of H+,K+-ATPase by substituted benzimidazoles is irreversible [20]. In order for the cell to start acid secretion again, synthesis of new proton pumps free from binding to blockers is required. The duration of medicinal product effect is determined by the rate of proton pump renewal. It is known that half of H+,K+-ATPase molecules are renewed in humans in 30-48 h, which causes prolonged suppression of acid production.

Adverse reactions when taking proton pump inhibitors in the form of dyspepsia, headaches, dizziness, feeling of fatigue, visual disturbances, microcirculation disorders, ricochet acid hypersecretion after medicinal product withdrawal due to secondary hypergastrinemia are observed in 10-20% of patients [21,22]. A point of view expressed about the need for careful study of the effects of long-term use of “proton pump” blockers, because in parallel with the positive therapeutic effect may occur a decrease in the production of protective hexosamine-containing gastric mucin [22].

1.3.1 M-choline blockers

M-choline blockers are among the most long-used medicinal products for the treatment of peptic ulcer disease. The antisecretory action of medicinal products of this group is based on their ability to block M-cholinoreceptors. Non-selective M-cholinoblockers bind to M1-cholinoreceptors in intramural ganglia of the stomach and M3-cholinoreceptors on the lining and gastrin-producing cells of the gastric mucosa. This binding results in a reduction in hydrochloric acid secretion by up to 50%. In addition, blockade of M3-cholinoreceptors markedly affects the motility of the GI tract – the tone, amplitude and frequency of peristaltic contractions decreases,

sphincters relax. These medicinal products can be divided into selective and non-selective. Non-selective M-choline blockers have been known for a long time. They include methacin (1.11), atropine (1.12), chlorosil, and platifylline (Fig. 1.3) [23].

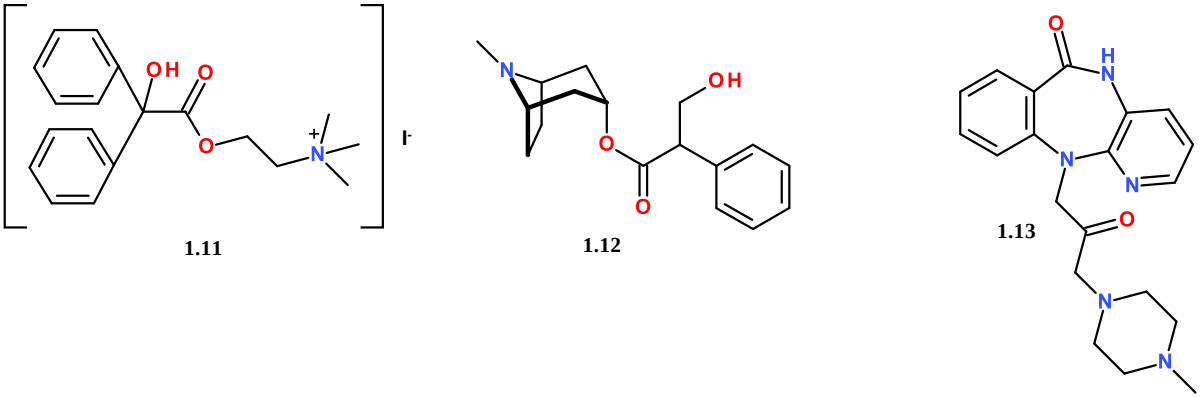


Figure 1.3 Representatives of m-choline blockers

Platifylline has only weak antisecretory properties. Metacin exhibits antisecretory properties only when administered parenterally, which significantly limits the possibility of its effective clinical use [24]. Metacin poorly penetrates the blood-brain barrier and therefore is a selectively acting peripheral cholinolytic. Thus, atropine has been the main representative of this group of medicinal products for several decades [25].

According to the results of chemical and pharmacological studies of preparations of atropine group, it was found that the carrier of cholinolytic activity is an ester of tropic acid with a free hydroxyl group, and the effect practically does not change when tropic acid is replaced by almond acid. The advantages of atropine include rapid and complete absorption from the digestive tract, pronounced antispasmodic and antisecretory effects [26]. However, the latter is characterized by relative short duration, and the next activation of secretion sometimes begins to exceed the initial level. Currently, atropine and other derivatives of krasavka are used in gastroenterology mainly as antispasmodic, but not as antisecretory agents. This is due to the impossibility of achieving persistent suppression of gastric secretion, as well as the overly broad spectrum of action and toxicity of atropine, which serve as a source of its adverse reactions.

The discovery of two subtypes of cholinoreceptors - M1- and M2 - and especially their different localization in the digestive tract forced to reconsider the traditional ideas about cholinolytics as a homogeneous pharmacological group. This discovery prompted scientists to search for selective blockers of M-cholinoreceptors and, as a result, a medicinal product selectively affecting M1-cholinoreceptors – pirenzepine (gastrocepin) – was synthesized (1.13) [27]. By chemical structure, pirenzepine is a tertiary amine and a benzodiazepine derivative. By blocking M1-muscarinic receptors, the medicinal product leads to a decrease in the production of hydrochloric acid and pepsin, the consequence of which is a decrease in gastric acidity. It has quite pronounced antisecretory activity, decreasing basal secretion by 50-60% (with intravenous injection up to 80-90%) and surpassing in clinical effect both antacids and non-selective choline blockers, but inferior to H_2-histaminoblockers and proton pump inhibitors. When using pirenzepine, side effects are rarely observed, and the probability of their occurrence increases with increasing dosage of the medicinal product. Pirenzepine is currently used for the treatment of moderate to mild peptic ulcer disease.

Once widely represented alkaloids of the atropine group and synthetic choline blockers, they have now given way in the therapy of peptic ulcer disease much more effective and safe means.

1.3.4 Prostaglandins

It is a well-known fact [28] that without the participation of prostaglandins of E series (E1 and E2) it is impossible to produce gastroduodenal mucus, secrete hydrocarbonates into the gastric lumen, maintain sufficient volume blood flow, and ensure mucosal repair. Deficiency of prostaglandins E1 and E2 decisively reduces the protective properties of the gastroduodenal mucosa. Taking NSAIDs is quite often the cause of gastroduodenal ulceration, including the most common cause of gastric and duodenal ulcers for those uninfected with Helicobacter pylori. Often, when NSAID therapy is necessary, medicinal products - chemical analogs of natural prostaglandins - are used to compensate for NSAID-inhibited prostaglandins. They have a selective effect and do not cause several serious side

effects, and, in addition, are not inactivated as quickly as natural ones [29]. In addition, prostaglandins can potentiate the antisecretory effect of H_2-histamine blockers. The group of anti-ulcer medicinal products “A02BB Prostaglandins” includes misoprostol (1.14) and enprostil (1.15) – synthetic analogs of prostaglandins, E1 and E2, respectively (Fig. 1.4).

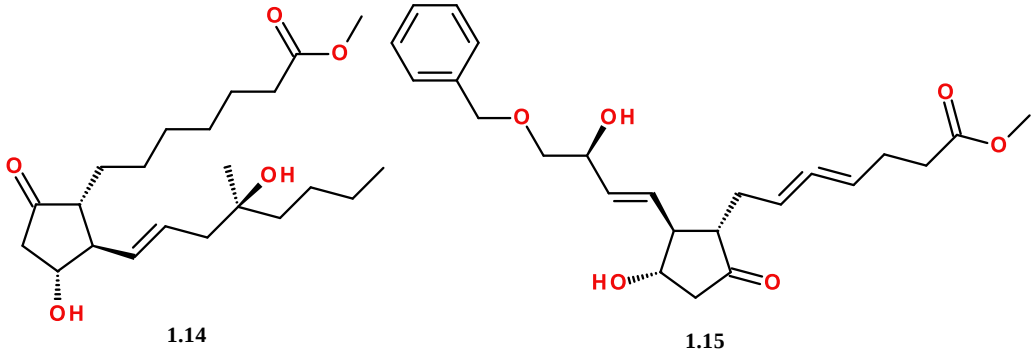


Figure 1.4 Representatives of prostaglandins

Misoprostol and enprostil have antisecretory and cytoprotective properties. Binding to receptors of gastric parietal cells, they inhibit basal, stimulate nocturnal secretion of gastric juice and hydrochloric acid, increase the formation of bicarbonate and mucus, improve blood flow. Increasing the resistance of the gastric mucosa and preventing the development of erosive and ulcerative lesions promotes healing of peptic ulcers. In patients taking NSAIDs, prostaglandins reduce the incidence of gastric and duodenal ulcers and the risk of ulcer bleeding [29].

Given the polyetiology and polypathogenicity of the disease, the treatment of peptic ulcer disease should be complex, i.e., it should include dietary nutrition, use of anti-ulcer agents, cessation of smoking, alcohol consumption and intake of ulcerogenic medicinal products, normalization of work and rest regimen of patients, sanatorium and resort treatment. However, such a variety of medicinal products does not completely solve the problem of successful treatment of peptic ulcer disease, since the problem of relapses remains relevant, the frequency of which within 1.5-2 years after the withdrawal of medicinal products reaches almost 100%.

One of the most important problems of modern medicine is the creation of new highly effective medicinal products that have a more pronounced therapeutic effect and a lower degree of side effects than the medicinal products currently used.

Conclusions

The data of literature concerning etiology and epidemiology of peptic ulcer development were summarized, aspects of peptic ulcer therapy were considered. The mechanisms of action of the key antisecretory medicinal products in the treatment of peptic ulcer disease have been studied in detail. The relevance of the search for new antiulcer agents has been determined.

CHAPTER 2

DESIGN OF POTENTIAL ANTIULCER AGENTS AND PREDICTION OF TOXICITY

The search for new highly effective and low-toxic medicinal products always remains a priority task for pharmaceutical science. One of the main ways to search for promising biologically active compounds is targeted synthesis based on modern ideas about the regularities of the structure-activity relationship. The fundamental step at the stage of synthesis planning is the choice of the basic structure - a fragment of the molecule on the basis of which further chemical modification is planned to be carried out.

2.1 Realization of logical-structural approach in the design of new tetrazole derivatives

Over the past 30 years, the pharmaceutical industry has been actively searching for new medicinal products based on compounds containing a tetrazole fragment in their structure. Analysis of scientific publications, patent documents in the field of chemistry, biochemistry, and medicinal chemistry of tetrazoles revealed intensive growth in the number of bibliographic sources. The growth rate of the number of publications in the field of tetrazole medicinal chemistry is higher than for other representatives of the azole series. This fact indicates the interest to tetrazoles as potential objects of the modern pharmaceutical market.

Currently, the pharmaceutical market offers dozens of highly effective medicinal products whose active pharmaceutical ingredients contain the tetrazole cycle [30]. Along with long-known medicinal products, such as antibiotics of the cephalosporin series – Cefazolin, Ceftezol and Cefpiramide (Fig. 2.1) [31, 32], new generation tetrazole-containing medicinal products with high efficacy and selectivity of action have found application.

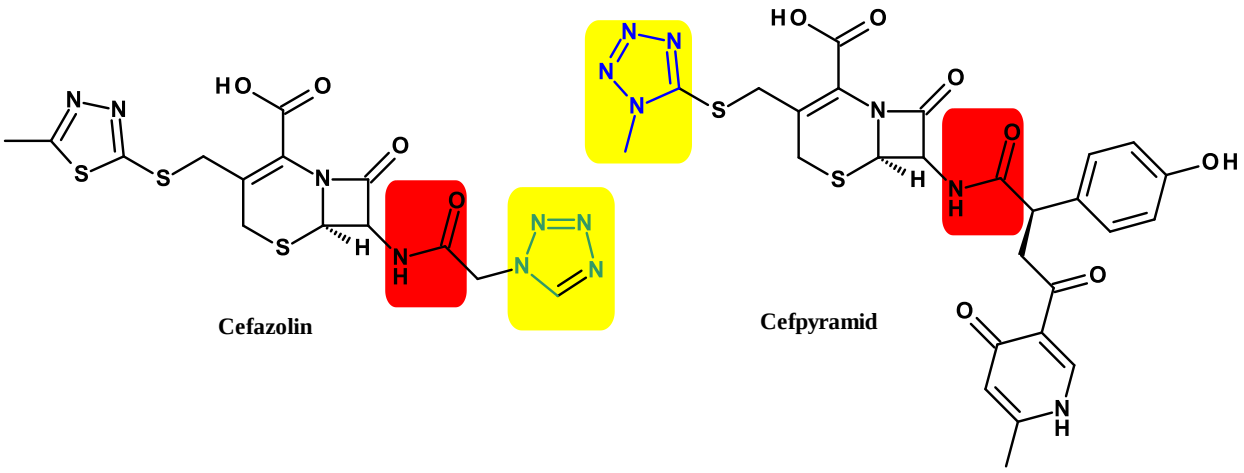


Figure 2.1 Stucture of antibiotics that contain a tetrazole fragment

Among them, a large group of substances with hypotensive action – Losartan [33], Valsartan, Candesartan, Irbesartan – selectively blocking angiotensin II receptors [34] (Fig. 2.2).

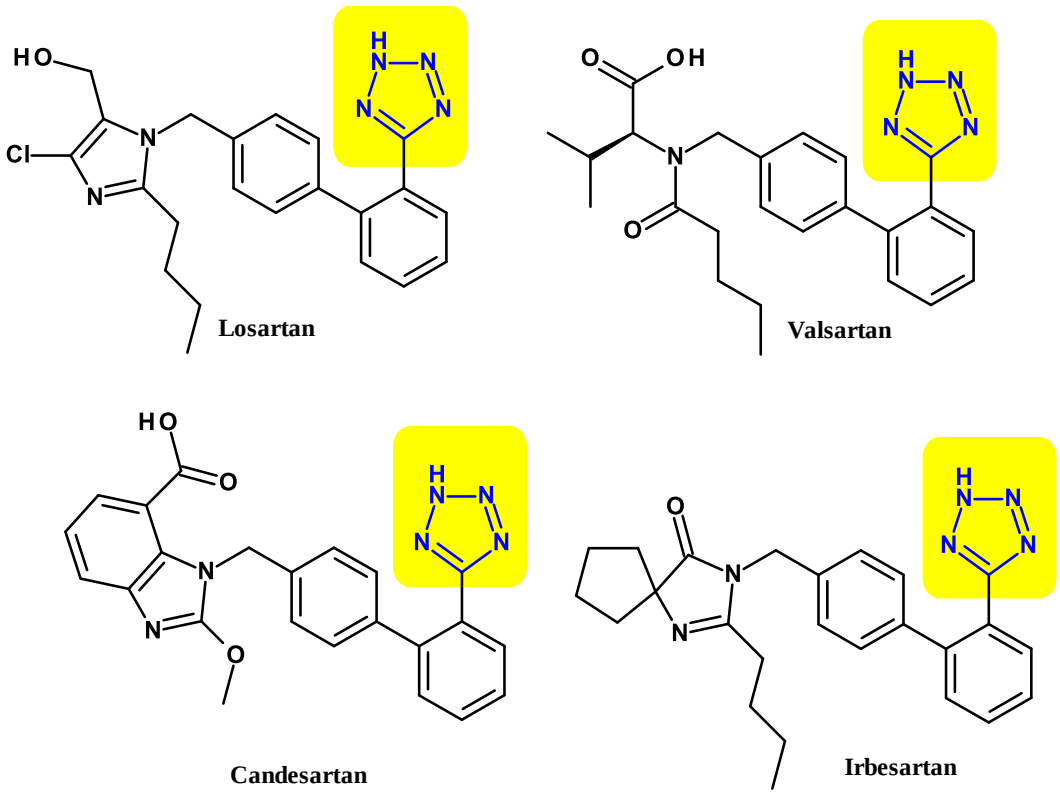


Figure 2.2 Antihypertensive medicinal products that contain a tetrazole fragment

Substances with an unsubstituted NH-group of the tetrazole cycle – Pranlucast and Pemiroplast – have also emerged as a new class of anti-allergic medicinal

products that act on both H_1 and H_2-histamine receptors of mast cells, and pranlucast is a leukotriene receptor-1 antagonist (Figure 2.3) [35].

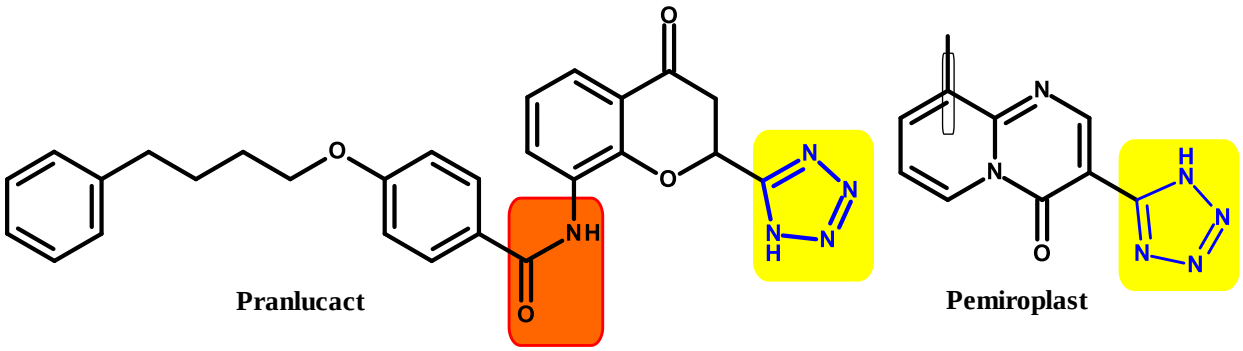


Figure 2.3. Anti-allergic medicinal products that contain a tetrazole fragment

The long-acting loop diuretic Azosemid is widely used in the treatment of heart failure and is not inferior to furosemide [36]. Cilostazol, a selective inhibitor of phosphodiesterase-III, is widely used as an inhibitor of platelet aggregation [37].

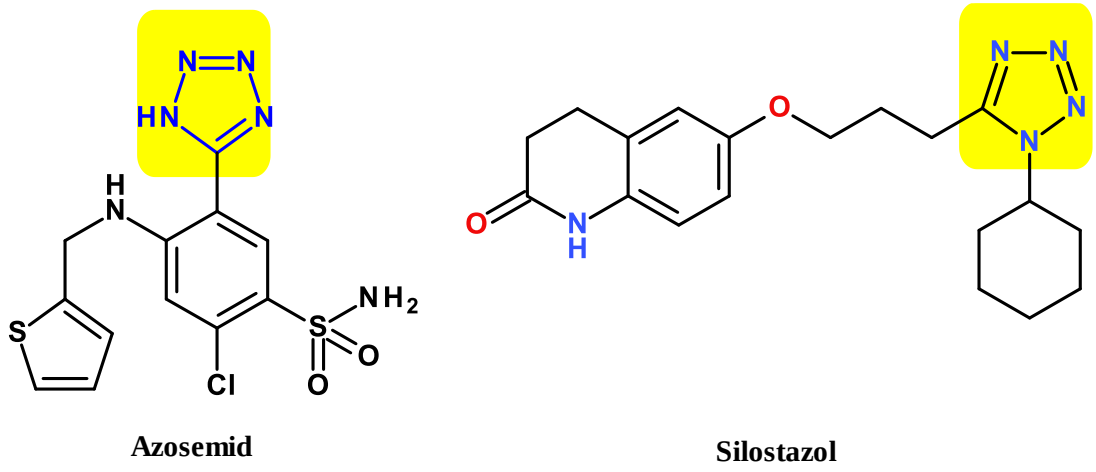


Figure 2.4. Medicinal products that contain tetrazole fragment of different pharmacological groups

The nature of biological activity of this heterocyclic pharmacophore group has been debated. The biological activity of tetrazoles is attributed to the fact that they are bioisosters of functional groups key to biomolecules, namely the peptide bond -C(O)-NH- and the carboxyl group -C(O)OH. Presumably, it is the N1-tetrazolyl fragment that determines the metabolic activity of tetrazole-containing substances,

and endocyclic nitrogen atoms form hydrogen bonds that reliably fix the enzyme-substrate complex [38].

2.2 Design of tetrazole derivatives as anti-ulcer agents

However, it is obvious that the pharmacological potential of this class of compounds at the present stage is not exhausted and remains promising for the search of new biologically active compounds. That is why we have chosen tetrazole derivatives in order to search for the structure of a promising substance of anti-ulcer action. In addition, tetrazole derivatives have some structural similarity to the amino acid histamine, so they can compete with it by blocking histamine receptors [39].

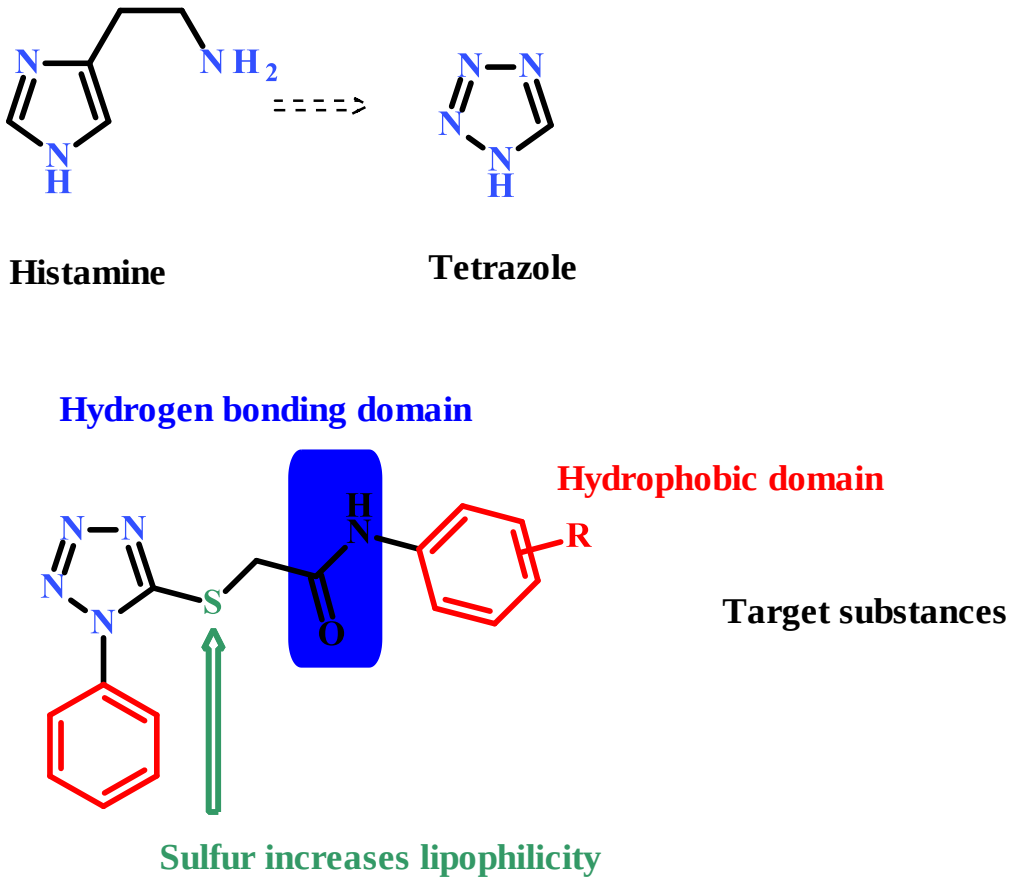
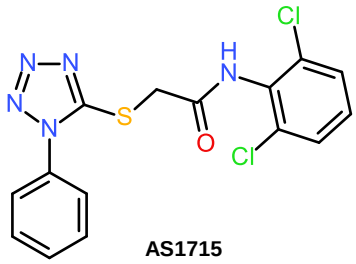


Figure 2.5. Design direction of new tetrazal derivatives

In order to increase the lipophilicity of the molecule, it was decided to introduce hydrophobic fragments – phenyl radicals with different substituents and sulfur atom, and for the purpose of possible additional fixation in the active site of

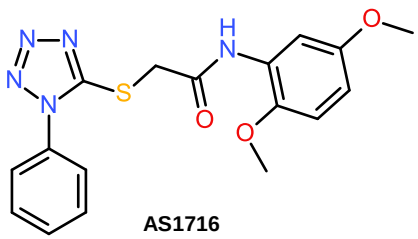
the receptor, a residue of acetamide fragment was introduced as a hydrogen bonding domain. Thus, N-(R-aryl)-2-(1-phenyltetrazol-5-yl)sulfanyl-acetamide were designed for study of anti-ulcer activity:



AS1715

N-(2,6-dichlorophenyl)-2-(1-phenyltetrazol-5-yl)sulfanyl-acetamide

Clc1cccc(Cl)c1NC(=O)CSc2nnnn2c3ccccc3



AS1716

N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl)sulfanyl-acetamide

COc1ccc(O)c(NC(=O)CSc2nnnn2c3ccccc3)c1

2.3 Toxicity prediction of engineered derivatives by the ProTox program

In silico toxicity predictions are a fast and inexpensive alternative to animal experiments. They rely on known toxicity data that are used to develop a model capable of predicting the toxicity of new compounds.

The novelty of the ProTox web server is that the prediction scheme is categorized by different levels of toxicity, such as oral toxicity (acute rodent toxicity), organ toxicity (hepatotoxicity), toxicological endpoints (such as mutagenicity, carcinotoxicity, cytotoxicity and immunotoxicity (B cell growth)), molecular initiating events, toxicological pathways and targets of toxicity, thus providing insight into the possible molecular mechanism behind such a toxic reaction.

According to the results of the prediction, the compound AS1715 was assigned to the 4th class of toxicity with an LD50 of 500 mg/kg, while the dimethoxy

derivative AS1716 was assigned to the 5th class of toxicity with an LD50 of 1000 mg/kg (Fig. 2.6).

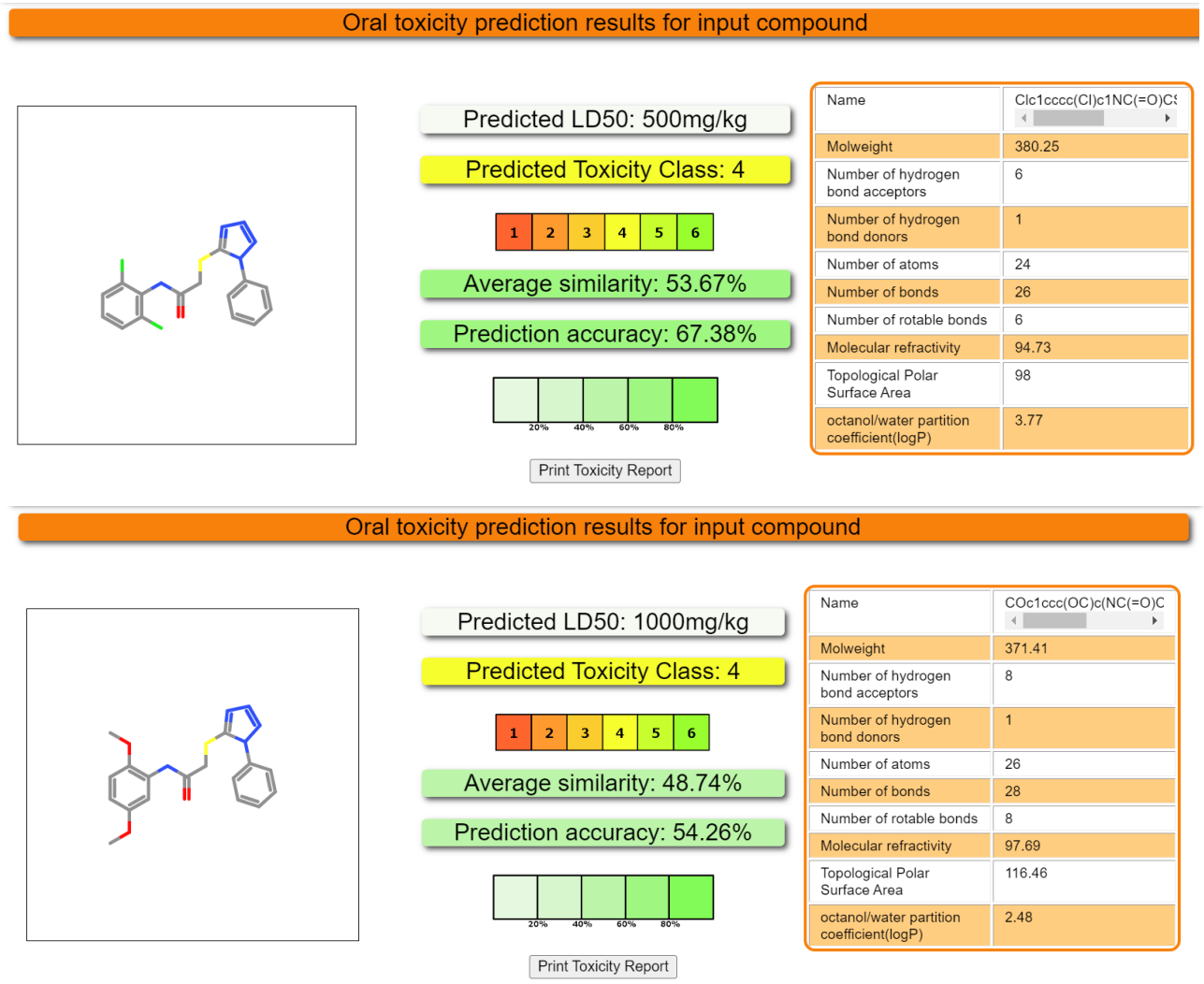


Figure 2.6 Toxicity results of compounds AS1715 and AS1716

As can be seen from the generalized toxicity radar for compound AS1716 (Fig. 2.7), possible Ecotoxicity (0.75) and Neurotoxicity (0.74), as well as the ability to penetrate the BBB-barrier are predicted for compound AS1715.

The generalized toxicity radar for compound AS1716 (Fig. 2.8) and for compound AS1715 demonstrates only the ability of the substance to penetrate the blood-brain barrier (0.71).

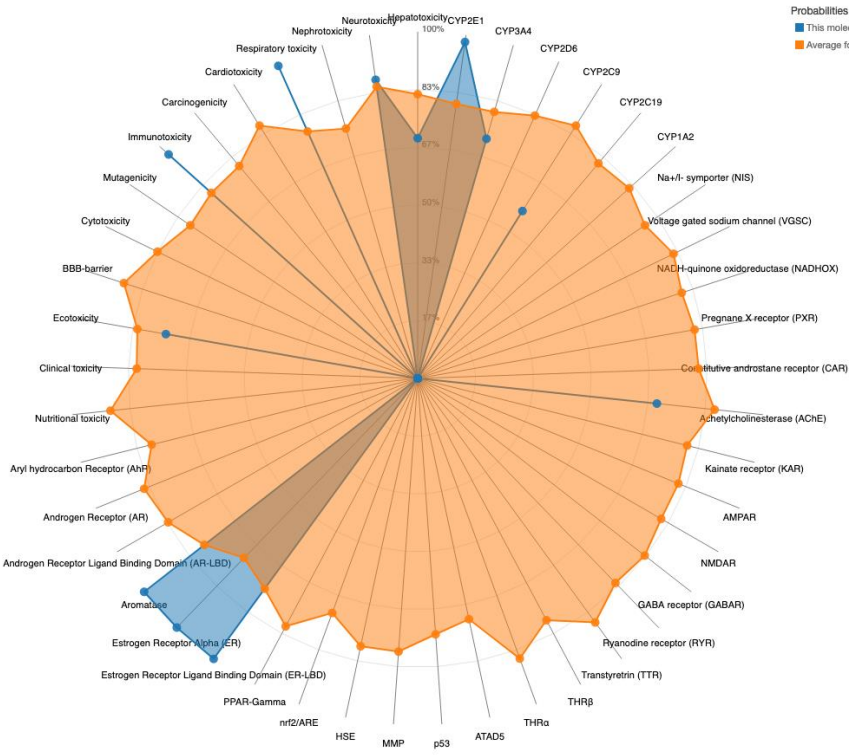


Figure 2.7 The toxicity radar of compound AS1715

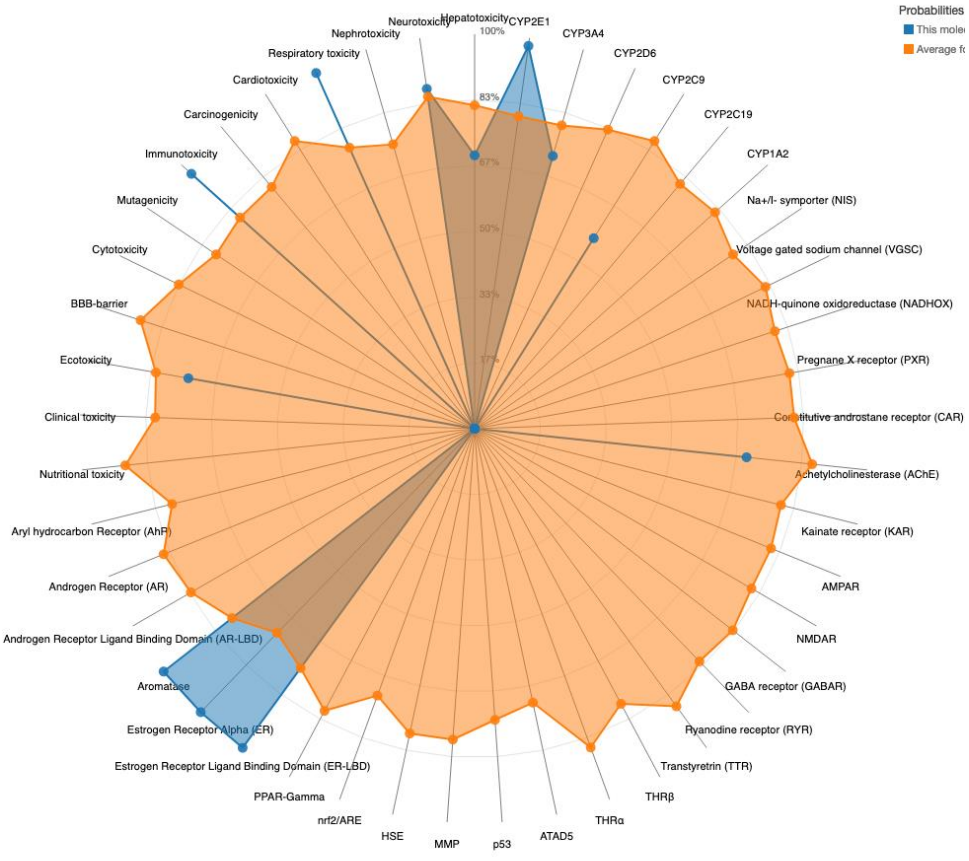


Figure 2.8 The toxicity radar of compound AS1716

Conclusions to chapter 2

1. A logical-structural analysis of the pattern of structure-anti-ulcer activity relationship as well as the fragmented functional effect on binding to the active site of the receptor was performed.

2. Acetamide tetrazole derivatives AS1716 and AS1716 were designed as potential anti-ulcer agents.

3. The toxicity of the designed compounds was predicted using ProTox program: compound AS1716 belongs to toxicity class 4 (LD50 500 mg/kg) and AS1716 belongs to toxicity class 5 (LD50 1000 mg/kg), both compounds are able to penetrate the blood-brain barrier.

CHAPTER 3
IN SILICO STUDY OF CONSTRUCTED CANDIDATE STRUCTURES AS ANTIULCER AGENTS

3.1 Rationale for the selection of macromolecules for *in silico* study

The use of computer technologies, with the help of which it becomes possible to consider the contribution of individual structural fragments to biological activity and provide for the interaction of potential medicinal products with biotarget molecules, makes it possible to reduce the number of experiments on laboratory animals, which significantly increases the economic efficiency of creating new medicinal products. One of the most promising methods of virtual screening is molecular docking, which makes it possible to assess the affinity of a substance to a specific biological target - enzyme or receptor.

Various agents such as serotonin, histamine, kinin system and prostaglandins are involved in different stages of ulcer inflammation. In our research we planned to study the anti-ulcer effect of the designed ligands on ethanol-prednisolone pharmacological model of gastric mucosal injury. This model is one of the rapidly reproducible experimental models of “acute” ulcer, the key role in the formation of which is played by inhibition of prostaglandins biosynthesis in the gastric mucosa. Prostaglandins have an antisecretory effect on gastric hydrochloric acid and protect the mucosa from the damaging effect of this acid. Consequently, ligands that can prevent the rapid metabolic conversion of prostaglandins to inactive products can be used to treat ulcers caused by NSAID intake.

The enzyme microsomal prostaglandin synthetase was chosen to predict the activity of compounds on ethanol-prednisolone ulcer.

In addition, we decided to investigate the ability of tetrazole derivatives to inhibit carbonic anhydrase, which is a target for famotidine, an anti-ulcer medicinal product containing a sulfamide moiety that acts as a nanomolar inhibitor of several hCA II, VI, VII and *Helicobacter pylori*.

3.2 Determination of affinity of the investigated ligands in microsomal prostaglandin E synthase 1

A three-dimensional crystallographic model of the enzyme macromolecule was obtained from Protein Data Bank - PDB ID 3DWW (Figure 3.1) [39].

Figure 3.1 3D visualization of microsomal prostaglandin synthase E-1 (MPGES1)

Microsomal prostaglandin synthase E-1 (MPGES1) is an inducible glutathione-dependent integral membrane protein that catalyzes the oxidoreduction of prostaglandin H2 to prostaglandin E2.

Docking studies for the investigated tetrazole derivatives and the comparison medicinal product were performed using the AutoDockVina and AutoDockTools 1.5.6 program [40].

The structures of the substances were obtained using BIOVIADraw 2021 R2 software and saved in .mol format. After that, they were optimized by Chem3D

software using MM2 molecular mechanical algorithm and saved as .pdb files. The molecule was converted to .pdbqt format using AutoDockTools-1.5.6 program.

The macromolecule of the enzyme was prepared by removing water molecules and native ligands, glutathione molecules, in the first step of the study. Polar protons and partial charges were added using the program AutoDockTools-1.5.6, and the number of torsional angles was set as default. Next, the protein molecule was converted into .pdbqt format.

Validation of the docking methodology was performed by docking the native glutathione ligand into the binding site. According to the experimentally determined data, the glutathione binding site is represented by the following amino acid residues: Arg38, Arg70, Arg73, Arg126, Arg126, Arg110, Tyr28, Tyr130, His113. The two arginine/tyrosine pairs (Arg70, 126) are in close contact with the glycine and cysteine parts of glutathione, respectively.

The ability of the docking algorithm used to reproduce the experimental data in the case of the MPGES1 enzyme is demonstrated in Figure 3.2. The location of the ligand and all interactions are consistent with the experimental data. The success of the technique is also confirmed by the low value of the binding energy of the native ligand -6.4 kcal/mol, which is a quantitative characteristic of the degree of affinity of the ligand to the receptor – affinity.

Further flexible docking procedure using the conformational search algorithm made it possible to search for the most suitable positions and orientations of the ligand in the ligand-binding center of the microsomal prostaglandin synthetase enzyme and to identify factors whose change can lead to an improvement of the ligand-receptor interaction. The result of modeling was the ligand conformation that best interacts with the protein binding site.

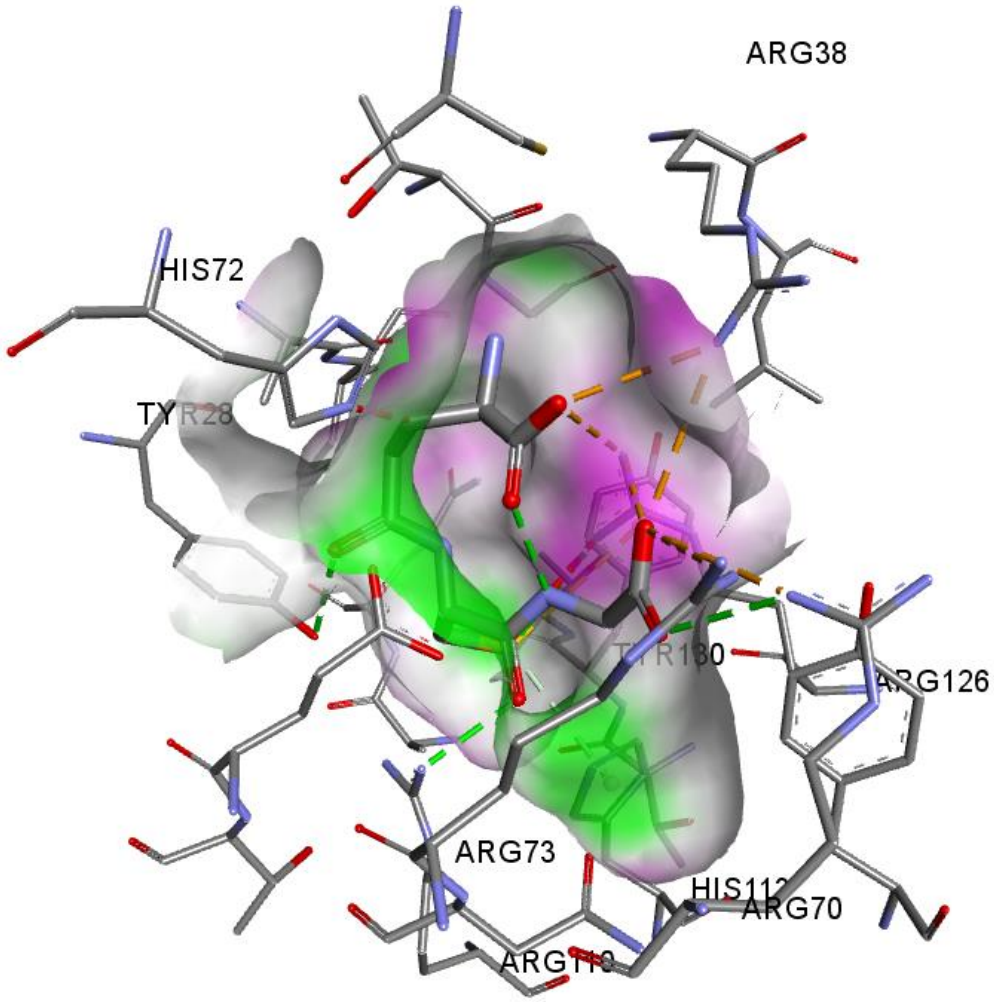


Figure 3.2. 3D interaction of native reference ligand with MPGES1 enzyme

The tetrazole derivatives studied showed a high degree of affinity to the glutathione active site of microsomal prostaglandin synthase E-1 (Table 3.1). The binding energy for N-(2,6-dichlorophenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1715 was -7.6 kcal/mol and for N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1716 was -7.4 kcal/mol. The affinity of the investigated ligands exceeded the calculated affinity of the reference ligand, which indicates the possibility of competitive inhibition of this enzyme.

The detailed placement and fixation of the AS1715 ligand in the active site of MPGES1 in the active site is demonstrated in Figure 3.3. The possibility of forming 10 bonds with active site amino acids is predicted. Strong 4 hydrophobic interactions are formed between the phenyl radical in the first position of the tetrazole cycle and

the benzene ring of the 4-hydroxyphenyl fragment of tyrosine, the alkyl radical of Arg73 and the methyl groups of leucine Leu69.

Table 3.1.

Results of docking of tetrazol-3-thiol derivatives AS1715, AS1716 and native ligand into the active site of microsomal prostaglandin synthase E-1

Ligand	Binding energy kcal/mol	Hydrophobic interaction	Hydrogen bonds	Other interactions
	-6.4	–	Arg70, Arg110, Arg126, Tyr28, Tyr130, His113	Arg38(2), Arg70, Arg73, Arg126(2), Tyr130 – Electrostatic
AS1715	-7.6	Arg73(2), Val65, Cys68, Leu69(2)	Arg70, Arg38, Arg73	Arg73, Arg38, Met76 Electrostatic, Pi-Cation, Pi-Sulfur
AS1716	-7.4	Arg73, Leu69 Arg73, Tyr117	Arg70	Arg70, Arg73, Glu77, Met76, His72 Pi-Cation, Pi-Anion, Pi-Sulfur

Simple hydrogen bonding is possible between the tetrazole cycle and the guanidine moiety of arginine Arg73. The thioacetamide fragment forms a Pi-Sulfur bond with the imidazole cycle of histidine His72, and the aromatic ring of the amide residue forms a Pi-Sulfur bond with the thiograppe of methionine Met76. In addition, electrostatic interaction is predicted to form a Pi-Anion bond with the carboxyl group of glutamic acid Glu77 and a Pi-Cation bond with the guanidine moiety of two arginine residues Arg70, 73.

Analyzing the conformation of N-(2,6-dichlorophenyl)-2-(1-phenyltetrazol-5-yl)sulfanyl-acetamide AS 1715 when co-imaged with the native reference ligand at the active site of the MPGES1 domain, it can be seen (Figure 3.4) that both molecules are arranged in space in the same plane and actually overlap.

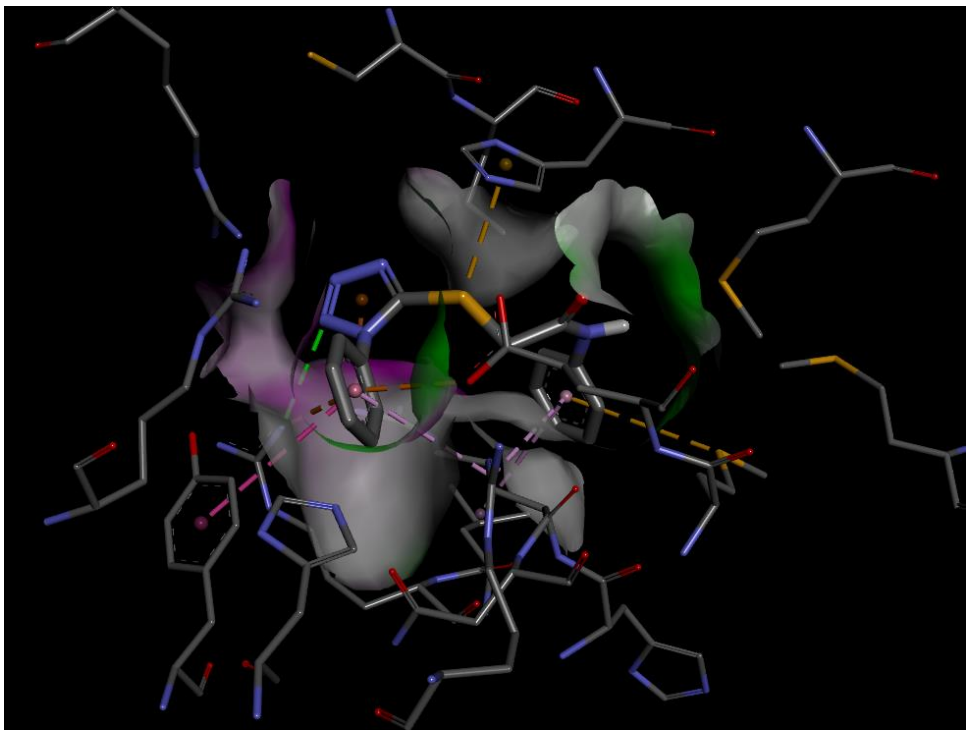


Figure 3.3. Interaction of N-(2,6-dichlorophenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1715 with amino acid residues of MPGES1.

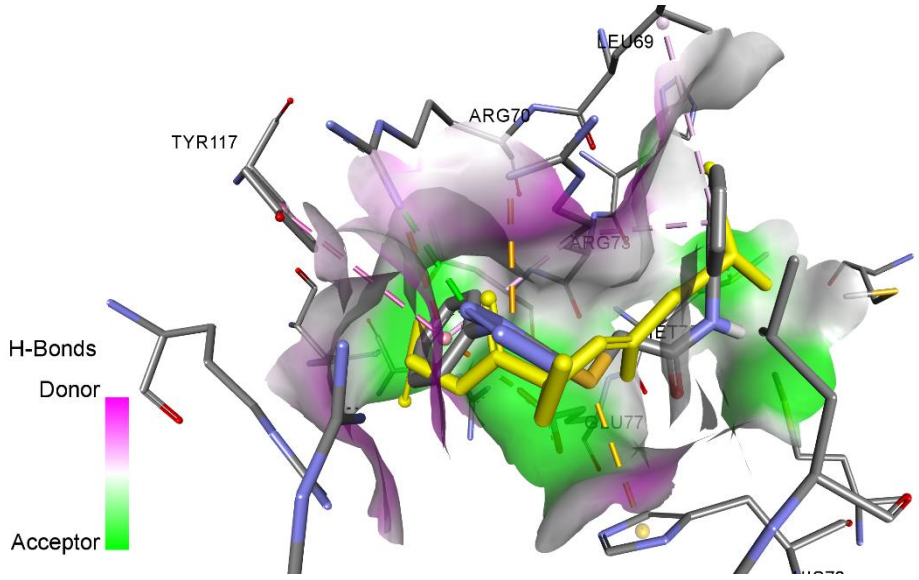


Figure 3.4. Conformation of N-(2,6-dichlorophenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1715 (gray molecule) and glutathione (yellow molecule) in the active site of MPGES1.

A detailed analysis of the interaction with the amino acid residues of the active site for N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1716 predicted the formation of 14 bonds (Fig. 3.5). Hydrophobic bonds are formed between the phenyl radical at the first position of the tetrazole cycle and the alkyl

residues of valine Val65, cysteine Cys68 and leucine Leu69; a bidentate bond is formed between the tetrazole cycle and arginine Arg73(2); the phenylacetamide moiety interacts with leucine Leu69 and arginine Arg73. Electrostatic Pi-cationic interactions are predicted between the tetrazole cycle and the guanidine moiety of arginine Arg73; the phenyl ring of the acetamide moiety and the sulfo-group of methiognin Met76. The formation of a simple hydrogen bond between the guanidine residue of arginine Arg70 and the tetrazole cycle is also predicted.

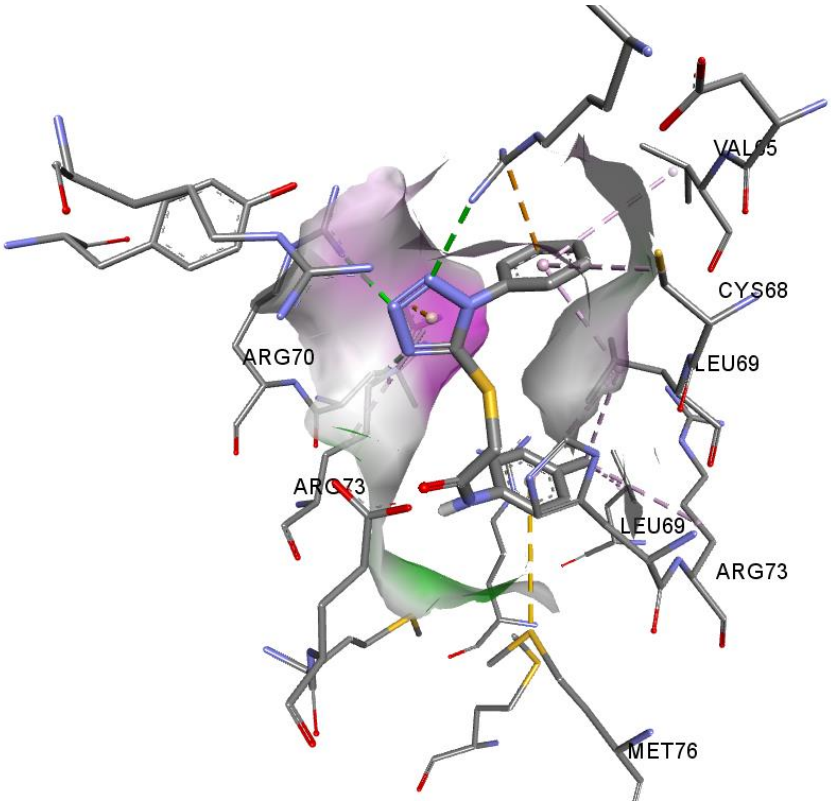


Figure 3.5. Interaction of N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1716 with amino acid residues of MPGES1.

The conformation of N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1716 compared to the conformation of native glutathione in the active site are visualized in Fig. 3.6. Despite the much larger size of the tetrazol derivative molecule, it perfectly fits into the pocket in the same plane as the reference ligand.

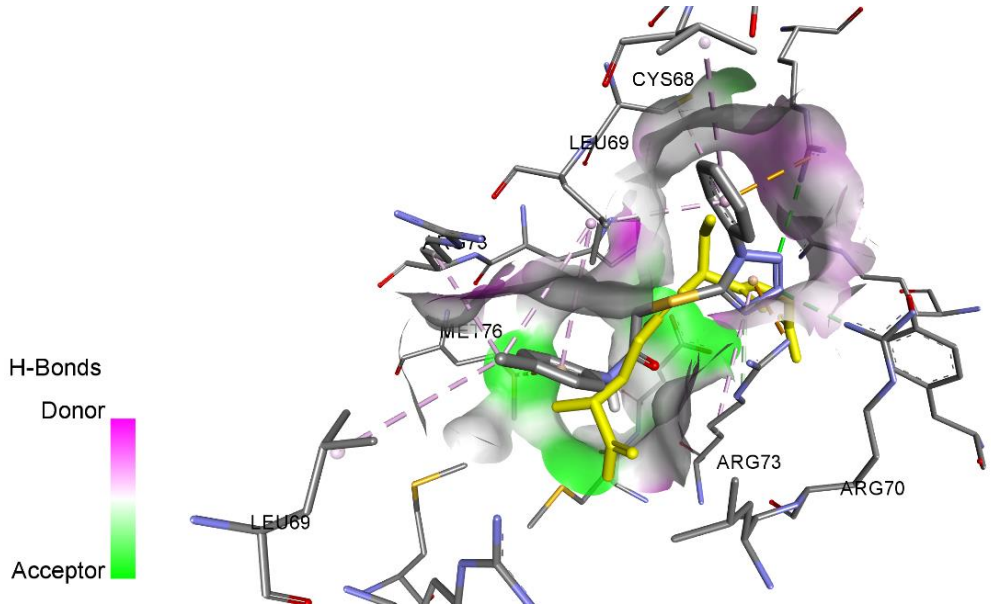


Figure 3.6 Conformation of N-(2,5-dimethoxyphenyl)-2-(1-phenyltetrazol-5-yl) sulfanyl-acetamide AS 1716 (grey molecule) and glutathione (yellow molecule) in the active site of MPGES1.

The obtained results indicate that the investigated structures can exert anti-ulcer effect due to the influence on the inflammatory process by inhibiting the enzyme of microsomal prostaglandin synthase E-1. Accordingly, the obtained prediction determines the expediency of their synthesis and further in vivo study on the model of ethanol-prednisolone ulcer in mice.

Conclusions to Chapter 3

1. Docking of the target tetrazoles was carried out and a high degree of affinity of the target tetrazoles to the active site of microsomal prostaglandin synthetase E-1 was established.
2. The obtained prediction confirmed the feasibility of synthesizing and further in vivo study of tetrazole derivatives in the ethanol-prednisolone ulcer model in mice.

CHAPTER 4

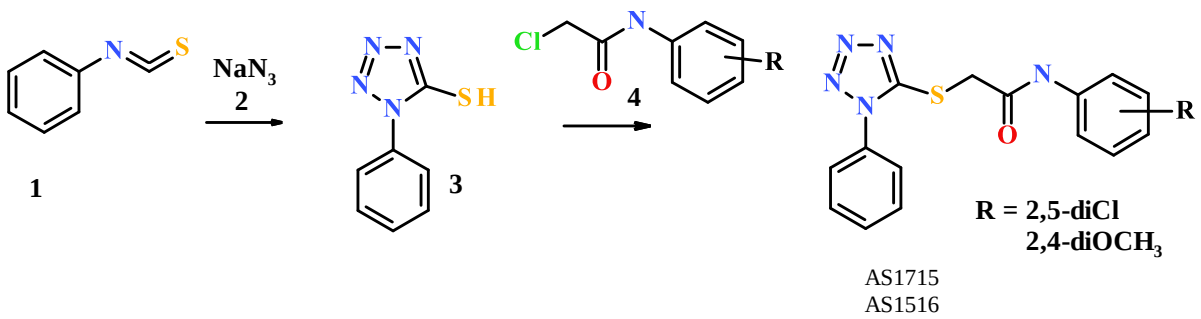
DISCUSSION ON THE SYNTHESIS AND ANTI-ULCER ACTIVITY OF TETRAZOLE DERIVATIVES

4.1 Synthesis of the of target derivatives of N-aryl-2-(1-phenyl-tetrazol-5-ylthio) acetamides

Synthesis of potential antiulcer agents was carried out in two steps. At the first stage it was necessary to obtain the starting tetrazol-5-thiol (3). For a long time, the only way to synthesize 1-alkyl(aryl)-tetrazol-5-thiols was the addition of nitrogen-hydrogen salts to the corresponding thiocyanates in DMFA or aqueous dioxane [41]. Currently, the most common method for the preparation of tetrazole-5-thiols is the interaction of isothiocyanate derivatives with sodium azide in aqueous medium under heating [42]. Tetrazol-5-thiols can also be obtained in several steps from alkyl(aryl)-thiosemicarbazide, benzyl chloride, and sodium nitrite with strict observance of temperature conditions [43]. The latter method is rather labor-intensive and requires the use of a large number of auxiliary reagents and solvents

In our case, we used the method of obtaining the target tetrazolthiol from isothiocyanates [44] as the most accessible and easy to perform. The synthesis of initial tetrazol-5-thiol (3) was carried out by boiling phenylisothiocyanate (1) and sodium azide (2) in aqueous solution under stirring (Scheme 4.1).

Scheme 4.1



The obtained reaction product is a pale-yellow powder with a melting point of 147-8 °C, which fully corresponds to the literature data and is a confirmation of the structure of the obtained 1-phenyl-tetrazol-5-thiole [45].

The next stage of our research was the introduction of pharmacophore fragments into the starting 1-phenyl-tetrazol-5-thiol (3). It is known that 1-aryl-tetrazol-3-thiol can exist in thione-thiol tautomeric forms and its alkylation can proceed ambiguously, with the formation of individual substances or a mixture of isomeric products depending on the reaction conditions with electrophiles both on SH- and NH-groups of the heterocycle. To clarify the matter with the reaction directionality, we have carried out quantum mechanical calculations of the molecule of 1-phenyl-tetrazol-5-thiole (3) using the HyperChem 8 program. As follows from these calculations, the highest electron density is observed at the sulfur atom (-0,476), the nitrogen atom in the fourth position has a charge of -0.154, in the third position the nitrogen atom has a charge of -0.161, in the second position -0.116. The charge of the nitrogen atom that has the phenyl substituent is -0.013. Based on these data, we can conclude that the alkylation of 1-phenyl-tetrazol-5-thiole (3) should proceed with more electronegative effect.

Alkylation of 1-phenyl-tetrazol-5-thiole (3) with arylamides of chloroacetic acid (4) was carried out under conditions of homogeneous basic catalysis (Scheme 4.1), in the presence of potassium hydroxide in ethyl alcohol medium. The alkylation reaction yielded the target products of N-aryl-2-(1-phenyl-tetrazol-5-ylthio) acetamides (SA1715, SA1716). Compounds are insoluble in water, soluble in ethanol, dioxane, DMF and DMSO [46,47].

After crystallization from ethyl alcohol or propanol-2, they are white with a yellowish tinge crystalline substances with distinct melting points.

4.2 Results of studying the antiulcer activity of tetrazol-5-thioly derivatives on the model of acute alcohol-prednisolone ulcer in rats

Screening study of anti-ulcer activity of the investigated compounds N-aryl-2-(1-phenyl-tetrazol-5-ylthio) acetamides SA1715, SA1716 was carried out on the

model of alcohol-prednisolone ulcer in rats - acute ulcerative lesion of the stomach. The experimental animals were mongrel white rats of both sexes weighing 180-280 g, which were pre-treated for 24 hours on starvation diet without restriction of water drinking. Acute ethanol-prednisolone gastric ulcer in rats was induced by intragastric single injection of ethanol-prednisolone mixture: prednisolone at a dose of 20 mg/kg and 80% ethyl alcohol at a rate of 0.6 ml per 100 g of rat weight [48]. The studied substances were administered intragastrically at a dose of 20 mg/kg, the comparison medicinal product ranitidine – at a dose of 40 mg/kg in prophylactic mode 1 hour before administration of alcohol-prednisolone mixture. Thus, 8 groups of animals with 6 animals in each group participated in the experiment:

- 1 group - intact control;
- 2 group - control pathology;
- 3 group - animals injected with reference medicinal priduct ranitidine at a dose of 40 mg/kg (comparison group);
- 4 group - animals treated with substance **SA1715** at a dose of 20 mg/kg;
- 5 group - animals treated with substance **SA1716** at a dose of 20 mg/kg.

At the end of the experiment, animals were removed from the examination 24 hours after the administration of the ulcerogenic agent under euthanasia conditions. Stomachs were removed, cut open, washed with physiological solution and macroscopic examination of the gastric mucosa was performed.

The intensity of ulcerative lesions and anti-ulcer activity of the preparations were assessed by macroscopic indicators of the state of the gastric and gastrointestinal mucosa and the intensity of ulcer defects formation:

The results of studies on the anti-ulcer activity of tetrazole derivatives SA1715 and SA1716 are presented in Table 4.1.

As a result of these studies, it was found that all the studied substances have one or another antiulcer activity: substance SA1716 – weakly expressed 23.02%, substance SA1715 – high antiulcer activity 64.29%.

In the course of the experiment, it was found that no defects of the gastric mucosa were observed when examining the stomachs of rats from the intact control group. The animals had good appetite, mobility and reaction to external stimuli was normal.

Administration of alcohol-prednisolone mixture in the control pathology group caused deterioration of the general condition of the animals compared to that in the intact control group. Lack of appetite decreased mobility, weak reaction to external stimuli were observed in this group of animals [49,50].

At macroscopic examination of stomachs (Table 4.1) in the control pathology group, the animals had bloating of stomach and intestines, disturbance of folding; edema and hyperemia of mucous membrane were observed in the majority of rats, and pallor with areas of necrosis was observed in the rest. In addition, point deep hemorrhages, various ulcerative defects of the gastric mucosa were observed.

It should also be noted that the general condition of animals and appearance (behaviour, reflexes in particular, "food", coat condition, etc.) were the best for all groups receiving the studied substances, both in relation to the KP group and to the group receiving the comparison medicinal product – ranitidine.

The best condition of mucous membranes according to the indicators: bloating, hemorrhagia, hyperemia, edema and folding, was observed in the group of animals receiving substance SA1715, and in most cases the values of all indicators were statistically reliable with respect to the control group and the group receiving the comparison medicinal product – ranitidine.

Table 5.1.

Screening of anti-ulcer activity of compounds SA1715 and SA1716 in a model of acute alcohol-prednisolone ulcer in rats

Experimental groups, (n=6)	Number of animals with ulcers in the group, %	Average area of ulcers, mm^2	Anti-ulcer index	Anti-binding activity, %
1	2	3	4	5
Intact control	_	_	_	_
Control	100	59,5±2,12	59,5	_

pathology				
Ranitidine	100	48,5±2,48*	48,5	18,49
SA1715	100	21,25±2,42*/**	21,25	64,29
SA1716	100	45,8±1,13*	45,8	23,02

Notes:

n – number of animals in the group

* – Differences are statistically significant with respect to the control pathology group at the significance level of $p < 0.05$ (by Student's t-criterion)

**– Differences are statistically significant with respect to the group receiving ranitidine at the significance level of $p < 0.05$ (by Student's t-criterion)

As can be seen from Table 4.1, the number of animals with ulcers was equal to 100% in all groups of experimental animals receiving alcohol-prednisolone mixture, i.e. the reproducibility of the model was absolute.

The average area of ulcers was minimal in the group of animals receiving substance SA1715 – 21.25±2.42 mm2, i.e. by 27.25 mm^2 less for the reference preparation, respectively. This index had a value of 48.5±2.42 mm^2 in the reference group and 45.8±1.13 mm^2 in the SA1716 group. Ulcer index was minimal for SA1715 animal groups – 21.25.

Anti-ulcer activity was maximal in substance SA1715 – 64,29%, i.e. by 45,8% more than the comparison preparation, and these indicators are statistically reliable both in relation to the group of control pathology, and in relation to the group of animals receiving the comparison preparation. Antiulcer activity at the level of the comparison preparation was demonstrated by substance SA1716 – 23,02%.

Thus, we can summarize that the study design was successful and both synthesized compounds have anti-ulcer activity on alcohol-prednisolone model of gastric ulcer. Furthermore, the results of in vivo and in silico experiment are comparable to each other. Therefore, it can be assumed that the mechanism of realization of anti-ulcer activity occurs by inhibiting the enzyme microsomal prostaglandin synthase E-1.

Experimental part

The study of the physicochemical properties of the synthesized substances was carried out according to the methods given in the State Pharmacopoeia of Ukraine (SFU, ed. 2) [46]. Reagents produced by Sigma-Aldrich, USA were used in this work. Melting points (°C) were determined in a capillary on an electrothermal digital instrument IA9100X1 (Bibby Scientific Limited, Staffordshire, UK).

Method for the preparation of N-aryl-2-(1-phenyl-1H-tetrazol-5-ylthio)-acetamides (SA1715, SA1716).

A mixture of 0.49 g (0.005 mol) of phenylisothiocyanate (1) and 0.39 (0.006 mol) of sodium azide (2) in 10 mL of water was boiled with a reflux condenser under stirring until complete dissolution of the reagents. The reaction mixture was cooled to room temperature and acidified with HCl to acidic reaction of the medium (pH=3-4). The formed precipitate was filtered off, washed with water, dried.

To a solution of 0.002 mol (0.28 g) of 1-phenyl-1H-tetrazol-5-thiole (3) in 20 mL of ethanol was added 20 mL of an aqueous solution of 0.002 mol KOH. A solution of 0.002 mol of the corresponding arylchloroacetamide (4) in ethyl alcohol was added to the obtained reaction mixture under stirring. The resulting solution was boiled with a reflux condenser for 3 h, cooled, poured into 200 ml of water. The resulting precipitate of the target product 5 was filtered off, dried. It was crystallized from propanol-2 or ethanol.

Conclusions to the Chapter 4

1. A methodology for the synthesis of the target antiulcer agents N-aryl-2-(1-phenyl-tetrazol-5-ylthio)-acetamides by alkylation of 1-phenyl-1H-tetrazol-5-thiole with the corresponding chloroacetamides under alkaline catalysis conditions is presented.
2. The target products of N-aryl-2-(1-phenyl-tetrazol-5-ylthio)-acetamides were obtained in good yields and high purity.
3. Screening of the synthesized compounds on ethanol-prednisolone model of ulcer in rats was presented and high anti-ulcer activity was determined for

dichlorophenyl substituted derivative **SA1715**, which was 3.4 times higher than the reference medicinal product ranitidine. Dimethoxyphenyl substituted derivative **SA1716** showed activity at the level of the reference medicinal product.

4. Comparability of the results of in vivo and in silico experiment was established and it is likely that the mechanism of realization of anti-ulcer activity occurs by inhibition of the enzyme microsomal prostaglandin synthase E-1.

CONCLUSIONS

1. Literature sources were analyzed and data on epidemiology, etiology and aspects of peptic ulcer therapy were summarized. Mechanisms of action of antisecretory antiulcer agents were studied.

2. Based on logical and structural analysis, the target acetamide derivatives of tetrazoles **AS1716** and **AS1716** were designed as potential antiulcer agents.

3. To optimize the design of potential biologically active substances, a prediction of the toxicity of the designed compounds was carried out. The toxicity of the designed compounds was predicted using ProTox program: compound **AS1715** belongs to toxicity class 4 (LD50 500 mg/kg) and **AS1716** belongs to toxicity class 5 (LD50 1000 mg/kg), both compounds can penetrate the blood-brain barrier.

4. Molecular docking was carried out and a high degree of affinity of the target tetrazoles to the active site of microsomal prostaglandin synthase E-1 was revealed.

5. The synthesis of the target derivatives N-aryl-2-(1-phenyl-tetrazol-5-ylthio)-acetamides AS1716 and AS1716 is presented. The compounds were obtained in good yields.

6. Based on the results of screening in an ethanol-prednisolone model of ulcer in rats, high anti-ulcer activity was determined for N-(2,6-dichlorophenyl-2-(1-phenyl-tetrazol-5-ylthio) acetamide SA1715, which is 3.4 times higher than the activity of the reference medicinal product ranitidine. N-(3,6-dimethoxyphenyl)-acetamide derivative SA1716 showed activity at the level of the reference medicinal product.

7. Comparability of results of in vivo and in silico experiment and probability of realization of antiulcer activity through inhibition of microsomal prostaglandin sintase E-1 enzyme were established.

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APPLICATION

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ГРАМОТА

за участь

отримав(ла)

Adnane Ennoussi

у секційному засіданні студентського наукового товариства кафедри фармацевтичної хімії

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Національного фармацевтичного університету

Алла КОТВІЦЬКА

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DETERMINATION OF AFFINITY OF TETRAZOLE DERIVATIVES TO MICROSOMAL PROSTAGLANDIN SYNTHASE

Ennoussi A., Severina H.I.
National University of Pharmacy, Kharkiv, Ukraine
severina.ai@ukr.net

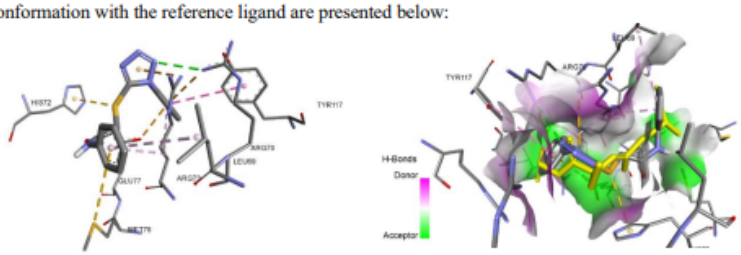
Introduction. The problem of peptic ulcer disease occupies a central place in gastroenterology. Chronic recurrent course, polyetiological and pathogenicity, polymorphism of clinical manifestations, severe complications that characterize peptic ulcer disease, as well as determining the role of *Helicobacter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

treatment. Therefore, despite the availability of various methods of treatment of peptic ulcer disease and a significant arsenal of anti-ulcer drugs, the problem of treatment of this pathology remains unsolved. All the above-mentioned causes the necessity to create new, highly effective, and safe drugs that can simultaneously affect the various links of ulcer pathogenesis, leading to the normalization of homeostasis systems. Tetrazole derivatives are well known as the structural basis of effective agents with anti-inflammatory, anti-allergic, antihypertensive, and other types of activity. The similarity of tetrazole cycle and imidazole cycle of histamine creates prerequisites for histamine-blocking activity of tetrazole derivatives as a key mechanism of anti-ulcer action. Therefore, the design of new derivatives of this heterocycle with potential anti-ulcer activity is an urgent and important issue.

Aim. The aim of the presented study is molecular docking of tetrazole derivatives as potential antiulcer agents.

Materials and methods. The program BIOVIA Draw 2017R2 was used for compound construction. Docking studies – AutoDock Vina, Chem3D, HyperChem 7.5, Discovery Studio Visualizer 2017/R2 for interpretation of results. Enzyme was obtained from Protein Data Bank – PDB ID 3DWW.

Results and discussion. The study of anti-ulcer effect of tetrazole derivatives is planned to be carried out on ethanol-prednisolone pharmacological model of gastric mucosal injury. This model is one of the rapidly reproducible experimental models of "acute" ulcer, the key role in the formation of which is played by inhibition of prostaglandins biosynthesis in the gastric mucosa. Prostaglandins have an antisecretory effect on gastric hydrochloric acid and protect the mucosa from the damaging effect of this acid. Consequently, ligands that can prevent the rapid metabolic conversion of prostaglandins to inactive products can be used to treat ulcers caused by NSAID ingestion. The enzyme microsomal prostaglandin synthetase was selected to predict the activity of the compounds on ethanol-prednisolone-induced ulcer. The investigated tetrazole derivatives showed a high degree of affinity to the glutathione active site of microsomal prostaglandin synthase E-1, exceeding the reference drug: the binding energy for N-phenyl-2-(1-phenyltetrazol-5-yl)thioacetamide was -7.6 kcal/mol, and for its 4-methylphenyl substituted analogue -7.4 kcal/mol. Details of the placement and fixation in the MPGES1 active site of the ligand exemplified by N-phenyl-2-(1-phenyltetrazol-5-yl)thio-acetamide in the active site and co-conformation with the reference ligand are presented below:



The possibility of forming 10 bonds with active site amino acids was predicted. Analysing the conformation of N-phenyl-2-(1-phenyltetrazol-5-yl)thioacetamide when co-imaged with the native reference ligand at the active site of the MPGES1 domain, it can be seen (Fig.) that both molecules are arranged in space in the same plane and actually overlap each other.

Conclusions. The obtained results indicate that the investigated structures can exert anti-ulcer effect due to the influence on the inflammatory process by inhibiting the enzyme of microsomal prostaglandin synthase E-1. Accordingly, the obtained prediction determines the expediency of their synthesis and further in vivo study on the model of ethanol-prednisolone ulcer in mice.