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

QUALIFICATION WORK

on the topic: **«DESIGN OF MOLECULES WITH ANTI-INFLAMMATORY AND ANTIMICROBIAL PROPERTIES IN THE SERIES OF DERIVATIVES OF 3-BENZYLQUINAZOLIN-4(3*H*)-ONE»**

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ANNOTATION

Methods for the synthesis of 3-benzylquinazolin-4(3H)-one derivatives with oxo- and alkylthio substituents at position 2 based on synthetically available reagents have been developed. The structures of the obtained compounds were confirmed using instrumental methods. Among the synthesized target compounds, the compounds with the best binding parameters to the active sites of COX-2 and TRmD (*M. tuberculosis*) were selected by the molecular docking method, which made it possible to identify potential anti-inflammatory and anti-tuberculosis agents.

The work consists of an introduction, three chapters, general conclusions and a list of references, which consists of 82 sources. The content of the work is placed on 52 pages and contains 2 tables, 3 figure, 4 schemes.

Key words: quinazoline, benzyl, alkylation, molecular docking, antimicrobial activity.

АНОТАЦІЯ

Розроблено методики синтезу похідних 3-бензилхіназолін-4(3H)-ону із оксо- та алкілтіозамсниками у положенні 2 на основі синтетично доступних реагентів. Структури отриманих сполук підтверджено із застосіванням інструментальних методів. Серед синтезованих цільових сполук методом молекулярного докінгу відібрано сполуки із найкращими параметрами зв’язування з активним сайтами ЦОГ-2 та TRmD (*M. tuberculosis*), що дозволили виявити потенціальні протизапальні та протитуберкульозні агенти.

Робота складається із вступу, трьох розділів та загльних висновків, списка використаної літератури, який складає 82 джерело. Зміст роботи викладено на 52 сторінках і проілюстровано 2 таблицями, 3 рисунками, 4 схемами.

Ключові слова: хіназолін, бензил, алкілування, молекулярний докінг, протимікробна активність.

CONTENTS

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INTRODUCTION

Relevance of the topic. The qualification work is devoted to the study of biologically active compounds of synthetic origin among the derivatives of 3-benzylquinazolin-4(3H)-one. It is known that 3-benzylquinazolines with a hydrazone moiety at position 2 are known as the compounds with anti-inflammatory activity and 3-benzyl-2-alkylthioquinazolines were found to have anticancer properties as tyrosine kinase inhibitors. Inflammation accompanies most pathological processes in the human body, including those with bacterial etiology. Despite the fact that inflammatory process is a normal reaction of the organism to pathological stimuli, in many cases excessive inflammation is undesirable and its degree should be controlled. There are many effective drugs among NSAIDs, but all of them have initial side effects, so enlargement of the arsenal of drugs of this pharmacological group may always be appropriate. Therefore, the search for both compounds with anti-inflammatory and antimicrobial agents in a series of 3-benzyl-2-alkylthioquinazoline derivatives is expedient and can contribute both to solving the issues of creating new NSAIDs and does not preclude the possibility of obtaining antibacterial agents with fundamentally new mechanisms of action.

Purpose of the study. Development of effective methods of synthesis and study of the potential of anti-inflammatory and antimicrobial activity for 3-benzylquinazolin-4(3H)-one derivatives.

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

1. To carry out analysis of the market of modern anti-inflammatory drugs and suggest the innovative approaches to the development and creation of drugs of this group.

2. To develop effective methods for the synthesis of 3-benzylquinazolin-4(3H)-one derivatives with oxo- and alkylthio-substituents in position 2 of the heterocyclic system, to prove the structure of the obtained compounds using a number of instrumental methods of analysis.

3. To predict the pharmacological activity of the obtained compounds in relation to COX-2 and TrmD isolated from *P. aeruginosa*.

4. To make conclusions about the prospects of using 3-benzylquinazolin-4(3H)-one with oxo- and alkylthio-substituents at position 2 as promising NSAIDs or antimicrobial agents.

Object of the study. Synthetic derivatives of 3-benzylquinazolin-4(3H)-one with oxo- and alkylthio-substituents in position 2 of the heterocyclic system.

Subject of the study. Preparation methods, physicochemical characteristics and potential of anti-inflammatory and antimicrobial activity for 3-benzylquinazolin-4(3H)-one derivatives with oxo- and alkylthio-substituents at position 2 of the heterocyclic system.

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

Elements of scientific research. Obtained data on synthesis methods and pharmacological activity of 3-benzylquinazolin-4(3H)-one derivatives with oxo- and alkylthio-substituents at position 2 of the heterocyclic system will broaden the arsenal of drug candidates suitable for the development of new anti-inflammatory and antimicrobial agents.

Approbation of research results and publications. The results of the research were presented at XXX Scientific and Practical Conference of Young Scientists and Students “Topical issues of new medicines development”: (17-19 April 2024 p., Kharkiv). The abstract was published:

Battach Y., Vlasov S.V. Synthesis of 3-benzylquinazolin-4(3H)-one derivatives with predicted useful pharmacological properties. “Topical issues of new medicines development”: materials of XXX Scientific and Practical Conference of Young

Scientists and Students (17-19 April 2024 p., Kharkiv). – Kharkiv: NUPh, 2024. – P. 39-40.

Structure and scope of the qualification work. The work consists of an introduction, three chapters, general conclusions and a list of references, which consists of 82 sources. The content of the work is placed on 52 pages and contains 2 table, 3 figure, 4 schemes.

CHAPTER 1. MODERN ANTIINFAMMATORY DRUGS
(Literature review)

Inflammation and its consequences are the most serious threat to human health. The inflammatory response occurs in response to exposure to external factors such as infections, environmental exposures, or autoimmune reactions. The main purpose of this reaction is to protect tissues and improve the patient's well-being. However, if inflammatory signals are activated too intensively and inflammatory mediators are released for a long time, this can lead to the development of a persistent pro-inflammatory state, even if the initial process was moderate.

This can lead to the development of numerous degenerative diseases and chronic health problems such as arthritis, diabetes, obesity, cancer and cardiovascular disease. Although anti-inflammatory steroids and nonsteroidal drugs are widely used to treat various inflammatory conditions, long-term use can cause unwanted side effects and dangerous health consequences.

Inflammation is an abnormal reaction of the body to external stimuli, such as infections, chemicals, mechanical injuries. Depending on how the body reacts, inflammation can be classified as acute or chronic. Acute inflammation is characterized by an increased concentration of immune cells at the site of infection to fight the infecting agent, while chronic inflammation is characterized by high levels of inflammatory cells in tissues over a long period of time [1]. Molecular scientific studies confirm that chronic inflammation is a contributing factor in the development of various diseases such as diabetes, heart disease, cancer, stroke, arthritis and obesity [2].

It is important that chronic inflammation is a contributing factor in the development of various diseases such as diabetes, heart disease, cancer, stroke, arthritis and obesity to consider that inflammation is a natural self-healing process that goes

through three key stages that interact and occur sequentially: the first stage is swelling, the second is redness, immobility, pain, and the third is an increase in temperature [3]. The first stage begins with an increase in the permeability of blood vessels under the influence of mediators, which leads to the infiltration of immune cells. This process finally leads to granuloma formation and tissue repair [4]. An immunogenic response that stimulates the activity of mitogen-activated protein kinase (MAPK), Janus kinases/signal transducers (JAK-STAT), or JAK-STAT. activates the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL) 1β (IL-1β) and chemokines. Cytokines and chemokines play an important role in stimulating immune cells in the area of infection. Cytokines also increase the activity of cyclooxygenase-2 (COX-2), which promotes the synthesis of inflammatory prostaglandins [5, 6]. After the immune system ensures the removal of an immunogenic factor, it reprograms signaling pathways to terminate inflammation in a dynamic process controlled by multiple biological systems. Initially, effector cells that have been activated undergo apoptosis and return to normal levels after cessation of pro-inflammatory signals and agents. Uninvaded macrophages remove apoptotic vesicles of neutrophils and contribute to the restoration of tissue homeostasis. [7]. In certain conditions, the normal course of this inflammatory process may be disturbed in the body, as a result of an imbalance in the regulation of the inflammatory system. The results of this are the uncontrolled production of inflammatory mediators, and as a result, the formation of chronic inflammation and other degenerative diseases against its background. There is evidence of a link between inflammation and degenerative diseases, a known association between inflammation and obesity. [8].

1.1. Role of inflammation for different deaseses

Cardiovascular diseases

The progression of atherosclerosis, where inflammation is essential, increases the risk of cardiovascular disease (CVD). [9]. Atherogenic low-density lipoprotein (LDL) plays a key role in the development of atherosclerosis. Increased expression of IL-6 in atheromatous fat layers, endothelium, smooth muscle and adipose tissue promotes faster progression of atherosclerosis. TNF-α is critical in endothelial dysfunction, vascular dysregulation, monocyte adhesion to the endothelium, vascular oxidative stress, apoptosis, and the atherogenic response, which can lead to thrombosis [10].

Osteoarthritis

Arthritis is another chronic inflammatory disease that can lead to disability and pain, limiting a person's socioeconomic life. Osteoarthritis is a problem for about 250 million people worldwide, particularly among the elderly population. [11].

Osteoarthritis is characterized by cartilage loss, subchondral bone remodeling, osteophyte formation, and inflammation in the synovial membrane and joint capsule. [12]. Mediators such as cytokines or water-soluble prostaglandins can promote the activation of the synthesis of chondrocyte matrix metalloproteinases (MMPs), provoking inflammation. In the case of osteoarthritis, there is a disturbance in the balance between pro-inflammatory and anti-inflammatory cytokines in the synovial membrane. [13].

Diabetes

The number of diabetes cases is projected to increase to 578 million by 2030 and to 700 million by 2045. One of the main characteristics of diabetes is impaired glucose tolerance and hyperglycemia. This condition occurs against the background of insulin deficiency or resistance [14]. Type 1 diabetes develops due to an autoimmune disorder that causes β-cell death. At the time, type 2 diabetes (T2DM) was linked to genetic factors, ethnicity, age, obesity, diet and physical activity levels. There is increasing evidence from research that these factors interact through common inflammatory pathways that are key in the mechanism of diabetes development. [15]. The etiology of diabetes and its connection with obesity and the role of adipose tissue in the body are currently the subject of active scientific research. The number of inflammatory factors produced by macrophages in adipose tissue is of great importance in determining the degree of obesity. [16, 17].

When macrophages and immune cells invade adipose tissue, the development of low-level chronic inflammation is observed. This process activates the production of various cytokines and chemokines, such as TNF-α, IL-1, IL-6, IL-10, leptin, adiponectin, MCP, angiotensinogen, resistin, and others. There is a pathological relationship between obesity, insulin resistance and the development of diabetes. [18, 19].

Cancer

Lifestyle and environment, along with genetics, determine the development of most types of cancer in 90%-95% of cases. [20]. It can be concluded that inflammation plays a key role in the process of tumor development and progression based on its influence on the tumor microenvironment. In this microenvironment, inflammatory cells and mediators promote processes such as proliferation, signaling, migration, metastasis, and blood vessel growth [21].

An important factor in the death of patients from cancer is inflammation, which contributes to the acceleration of numerous stages of metastasis. [22, 23].

Obesity affects the release of adipokines and cytokines, which affects various systemic processes, including the tumor environment. Adiponectin, leptin, IL-6, TNF-α, YKL-40 (chitinase-3-like protein-1), osteopontin, and plasminogen activator inhibitor-1 (PAI-1), secreted by adipocytes, promote cancer growth, progression, and metastasis tumors [24].

Other pathologies

Alzheimer's, Parkinson's and Huntington's diseases are neurodegenerative diseases characterized by the destruction of neurons caused by inflammation. In this context, an increase in the concentration of cytokines and nitric oxide (NO) in inflammatory cells is noted [25].

1.2. Drugs for the treatment of inflammation

Corticosteroids

Glucocorticoid hormones, such as cortisol in humans and corticosterone in rodents, are steroid hormones produced and secreted by the adrenal glands in a circadian rhythm in response to physiological signals and stressors [26].

The release of glucocorticoids from the adrenal glands is regulated by the hypothalamic-pituitary-adrenal axis. Signals from the suprachiasmatic nucleus stimulate the paraventricular nucleus of the hypothalamus to release corticotropin-releasing hormone and vasopressin. These hormones affect the anterior lobe of the pituitary gland, where they activate corticotrophic cells to secrete adrenocorticotropic hormone into the blood. It in turn, acts on the cortex of the adrenal glands, promoting

the synthesis and release of glucocorticoids [27]. After being released from the adrenal glands, glucocorticoids enter the bloodstream and reach target tissues, where they regulate many physiological processes, such as metabolism, immune function, skeletal growth, cardiovascular function, reproductive function, and cognitive processes. The synthesis of glucocorticoids in these tissues occurs rapidly under the influence of ACTH stimulation, since they cannot be synthesized and accumulated in the adrenal glands in advance. This mechanism of direct communication in the HPA system (hypothalamus-pituitary-adrenal gland) is regulated by the negative feedback of glucocorticoids, which affect the anterior lobe of the pituitary gland and the hypothalamus. This leads to suppression of further release of ACTH and corticotropin-releasing hormone, respectively.[28].

The process of synthesis of biologically active glucocorticoids from cholesterol using a multienzyme process is known as steroidogenesis [29, 30]. The action of ACTH consists in increasing the activity of the adrenal glands by activating protein kinase A (PKA), which in turn leads to non-genomic regulation of steroidogenic proteins. This process involves the phosphorylation of hormone-sensitive lipase (HSL), which increases intracellular cholesterol, and the phosphorylation of the steroidogenic regulatory protein (StAR). These proteins facilitate the transport of cholesterol into the mitochondria, where cholesterol is converted to pregnenolone by the enzyme cytochrome P450 (P450scc). This process involves sequential enzymatic reactions occurring in the mitochondria and endoplasmic reticulum of the cell. The final result of these reactions is the synthesis of glucocorticoids, which are released into the bloodstream [30].

It was found that the hypothalamic-pituitary-adrenal axis (HPA) exhibits circadian oscillations that link the synthesis of glucocorticoids with daily rhythms. Therefore, the concentration of cortisol in blood serum reaches its maximum level in the morning and has its lowest level at night. The hypothalamic-pituitary-adrenal axis

(HPA) is the central stress response system, which is responsible for the adaptive component of the stress response and is aimed at restoring homeostasis [31].

Dysregulation of the stress response can lead to various pathologies, such as autoimmune diseases, hypertension, affective disorders and major depression. Serum glucocorticoid levels are maintained by adrenal synthesis, but glucocorticoid availability is regulated at the tissue or cellular level. In humans, approximately 80-90% of circulating glucocorticoids are bound to corticosteroid-binding globulin (CBG), only 5% of systemic glucocorticoids are free and biologically active. [32]. Cellular availability of glucocorticoids is ensured by tissue-specific metabolic enzymes such as 11β-hydroxysteroid dehydrogenases (11β-HSDs). [33].

NSAIDs

Cyclooxygenase (COX) enzymes consist of COX-1 and COX-2 isoforms, which are responsible for the synthesis of prostaglandins. Prostaglandins play a key role in inflammation, and their activity should be regulated with selective nonsteroidal anti-inflammatory drugs (NSAIDs).

During the coronavirus pandemic, selective cyclooxygenase-2 (COX-2) inhibitors became one of the most popular nonsteroidal anti-inflammatory drugs (NSAIDs). This is mainly due to their ability to reduce pain and protect against inflammatory diseases.

Cyclooxygenase-2 (COX-2) is an inducible enzyme and is a target for drugs aimed at reducing pain through selective inhibition. However, due to serious side effects, such as cardiovascular disease, risk of stroke, and cardiac arrest, this approach requires careful consideration [34] First-generation COX-2 inhibitors, such as rofecoxib (Vioxx™) and valdecoxib (Bextra™), were withdrawn from the market due to serious side effects that included cardiovascular complications [35]. Drugs approved by the US Food and Drug Administration, such as celecoxib (Celebrex™), have high

selectivity for cyclooxygenase-2 and include warnings about possible side effects on the packaging, but are still available in the US. In contrast, nonselective NSAIDs such as ibuprofen, naproxen, nimesulide, diclofenac, and sulindac can cause serious side effects [36] The most common side effects of pain management include gastrointestinal bleeding, peptic ulcer, duodenal ulcer, hypertension, dyspepsia, and stroke [37]

Cyclooxygenase-1 (COX-1) is mainly expressed on platelets, kidneys, gastric mucosa and lungs. On the other hand, cyclooxygenase-2 (COX-2) shows a low level of constitutive expression in the brain, kidney, gastrointestinal tract, and thymus, but its level can increase upon exposure to inflammatory stimuli. [38] An increase in the expression of cyclooxygenase-2 (COX-2) leads to a decrease in the pain threshold due to the production of prostaglandins. This can pave the way for the development of diseases related to inflammation in the long term. [38]

The regulation of inflammation is a complex process in which both major forms of cyclooxygenase play a modulatory role that can sometimes be contradictory. When blocking the formation of prostaglandins PGG2 and PGH2 with the help of non-selective non-steroidal anti-inflammatory drugs and further limiting the function of the mucous membrane, there may be a risk of damage to the gastrointestinal tract [39] Instead, cyclooxygenase-2 (COX-2) plays a key role in regulating kidney function. Thus, in patients with an increased risk of renal ischemia, liver cirrhosis, renal failure, cardiovascular disorders, and congestive heart failure, the use of cyclooxygenase-2 (COX-2) inhibitors may have serious side effects and therefore requires special attention [36]. When prostaglandin production is stopped by blocking cyclooxygenase (COX), arachidonic acid (AC) switches to an alternative metabolic pathway via lipoxygenase (LOX). The leukotrienes formed are associated with diseases such as asthma and allergic reactions and should be considered when analyzing the safety of nonsteroidal anti-inflammatory drugs (NSAIDs) [40]. Due to the high prevalence of inflammatory diseases in many patients, selective cyclooxygenase-2 (COX-2) inhibitors have become one of the most widely used drugs during the COVID-19

pandemic. This highlights the importance of having safe and effective medications and points to the need for further research and development of new medications. Recent scientific research is aimed at studying new compounds or drugs that inhibit the activity of cyclooxygenase-2 (COX-2), considering them from a structural and mechanistic point of view. These studies will contribute to the understanding of the relationship between the structure and activity of compounds, and will also determine future research directions for medicinal chemists and biologists.

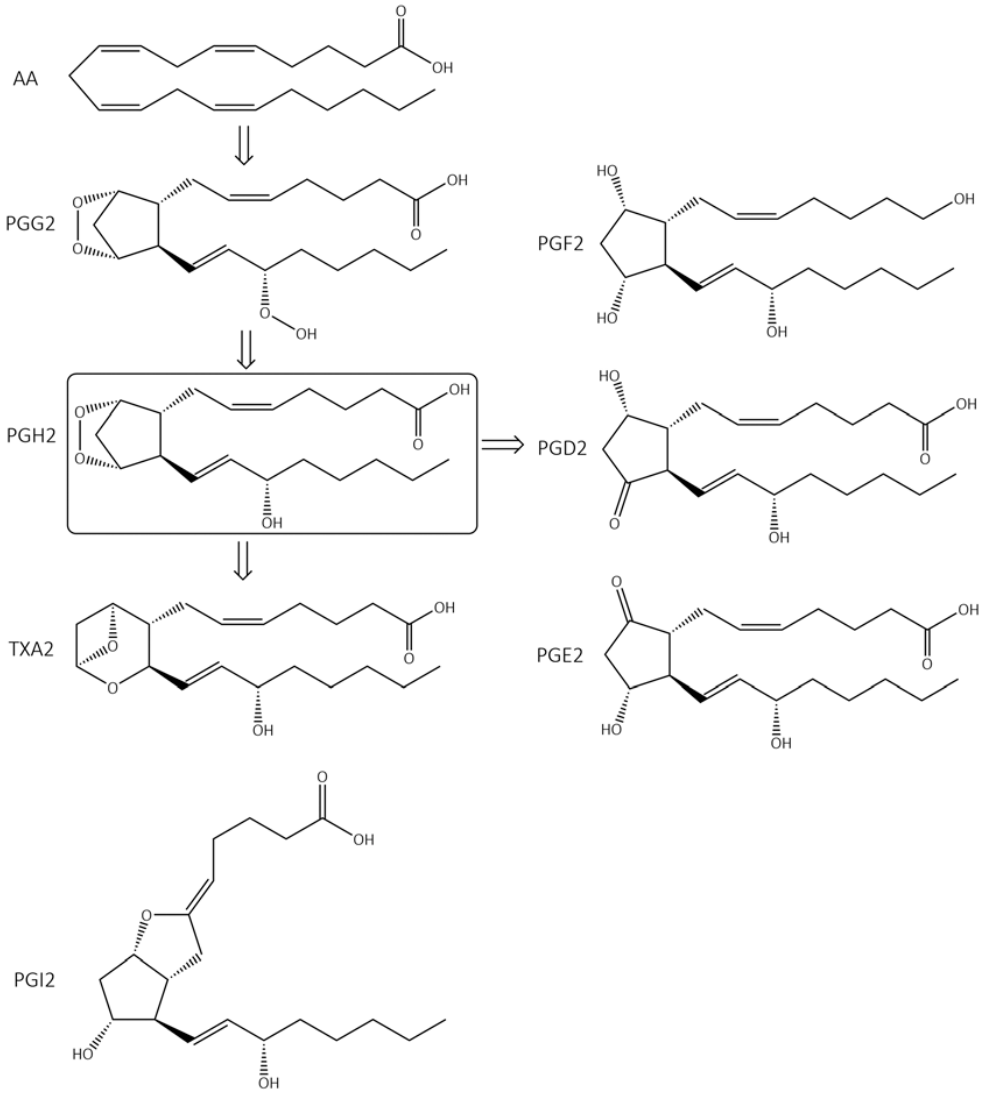


Fig 1.1. Inflammation pathways (Arachidonic acid (AA) cascade. PG = prostaglandin, TX =thromboxane).

Phospholipase A2 promotes the cleavage of arachidonic acid (AA) from phospholipids, which activates three key inflammatory pathways: cytochrome P450 monooxygenase, lipoxygenase (LOX), and cyclooxygenase (COX). These pathways are major components of the inflammatory response in mammals. [40] Cyclooxygenases are bifunctional enzymes involved in the conversion of long-chain (C20-C24) polyunsaturated monocarboxylic acids to oxidized cyclic products, mainly prostaglandins (PGs) and thromboxanes (TXs). [38]

Cyclooxygenases act on arachidonic acid (AA) by introducing two oxygen atoms into the C–H bonds, forming a bicyclic peroxide intermediate, PGG2. Rapidly reduced to PGH2, this peroxide is further converted to PG E2, D2, I2, F2, and TXA2 in response to a variety of stimuli. [38]

Interleukins, TNF-α, lipopolysaccharides, transforming growth factor-α, interferon-γ, platelet-activating factor, endothelin-1, retinoic acid, as well as arachidonic acid itself are various signaling molecules that contribute to the activation of cyclooxygenase-2. [41, 42] The functions of leukotrienes (Leu), epoxyeicosatrienoic acids (EET) and hydroxyeicosatetraenoic acids (HETE) in signaling pathways have not yet been fully elucidated. These compounds are formed as a result of the metabolism of arachidonic acid (AC) with the participation of specific enzymatic pathways, such as lipoxygenases (LOX) and soluble epoxide hydrolase (sEH). These enzymes play an important role in signaling networks associated with inflammation and allergic diseases. For example, some studies have shown that cyclooxygenase-2 selective inhibitors can reduce antithrombotic PGD2 levels during cardiovascular events [38].

Proinflammatory cytokines such as TNF-α and IL-6 contribute to the perpetuation of inflammation by activating the production of cyclooxygenase-2 (COX-2), which in turn causes the production of prostaglandins and IL-6 [43].

Arachidonic acid (AA) as a natural substrate enters the active center (ASC), where the catalytic process of its transformation into PGG2 begins. Tyrosine (Tyr)385 is located next to the hemecofactor and is important for splitting off a hydrogen (H)

atom according to the redox states of the iron center. The formation of PGH2 is controlled by peroxygenase. Tyr385 is also important for the separation of the second hydrogen atom. The formation of prostaglandins in different human tissues is regulated by different mechanisms of biological activity, which is determined by the types of PG receptors that interact [38] Prostaglandins (PGs) are crucial in the regulation of inflammatory processes. The participation of PGI2 and PGE2 in the change of vascular permeability, the formation of tissue edema and the secretion of gastric mucus reflects the characteristic manifestations of inflammation [38] Prostaglandin D2 (PGD2) regulates bronchoconstriction and influences the immune response to SARS-CoV-2, including innate and adaptive immunity. Prostaglandin F2 (PGF2) is associated with various aspects of the reproductive system [44].

Synthesis of thromboxane A2 (TXA2) by cyclooxygenase-1 (COX-1) promotes activation and aggregation of platelets through their own receptors [45] In addition, prostaglandins play important roles in a variety of regulatory processes, such as body thermoregulation, renal blood supply (renal perfusion), gastrointestinal integrity, and immune modulation [46].

Oxidoreductase enzymes, which belong to the EC 1 category, play a role in redox reactions by moving hydrogen atoms, oxygen atoms, or electrons from one compound to another [47].

Cyclooxygenases are enzymes of the oxidoreductase type that function as bifunctional catalysts, contributing to the deoxygenation of arachidonic acid. In this group, there are two main isoforms of cyclooxygenases - cyclooxygenase-1 and cyclooxygenase-2. In addition, a splice variant of cyclooxygenase-1, called cyclooxygenase-3, was discovered [48].

COX-1 and COX-2 enzymes are homodimers consisting of 72 kDa subunits. Each subunit includes three domains: an epidermal growth factor domain, a membrane-binding domain, and an active center (ASC), which is responsible for catalytic reactions. [49] COX-1 and COX-2 have almost identical amino acid sequences in the

active site. They include four amphipathic helices with hydrophobic interactions near the active site cavity (ASC). The hydrophobic "pocket" of COX-2 is located at the deep end of ASC, while the entrance of ASC is surrounded by amino acid residues arginine (Arg)120, Tyr355 and glutamic acid (Glu)524. [50]

The substrate binding site extends from Arg120 to Tyr385. COX-2 has a wider binding cavity (~17%) containing the amino acids valine (Val)523, Val434, leucine (Leu)503 and Arg513. Compared to COX-1, where the amino acids isoleucine (Ile)523, Ile434, L-phenylalanine (Phe)503 and histidine (His)513 are found. Replacing the amino acid Val at position 523 with another sterically challenging residue, such as Ile, creates an additional sub-pocket that may allow bulkier inhibitors to occupy the active site without inhibiting COX-1 activity. Arg513 can interact with polar parts in the active site of COX-2. At the same time, deletion of Val434 may cause a further increase in access to the active site compared to COX-1, which has the Ile434 residue. For example, the interaction of celecoxib with the active site of COX-1 and COX-2 at the molecular level is studied using the X-ray crystal structure of COX-1 (Protein Database (PDB) entry 3KK6, resolution 2.75 Å) [51] and COX-2 (PDB 3LN1, resolution 2,40 Å) [52].

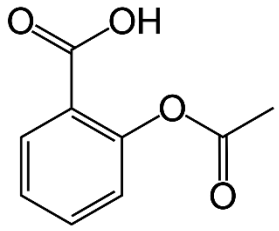
Celecoxib interacts with different regions of the active site of both forms of cyclooxygenase. The pyrazole ring is located in the middle of the hydrophobic "pocket", where it interacts with amino acids Val116, Val349, Leu359 and Ala527 in COX-1, as well as with amino acids Arg106, Leu517, Val335 and Ala513 within the active site of COX-2. The sulfonamide moiety interacts with amino acids glutamine (Gln)192, Leu352, His90, and Ser516 in COX-1, while COX-2 interacts with amino acids Arg499, Leu338, Gln178, and Ser339 through hydrogen bonds.

These interactions indicate small structural differences in the active site between the isoforms affecting the selective profile of the compounds. Overall, the study of key COX-2 amino acids that correlate with the chemical structure of the inhibitor suggests the possibility of creating potent, selective, and safe COX-2 inhibitors with an improved safety profile.

Cyclooxygenase-1 (COX-1) is distributed in all tissues, while cyclooxygenase-2 (COX-2) is present in various cell types such as brain, kidney, endothelial cells, as well as in reproductive tissues, inflammatory tissues, and tumor cells [53]. Selectivity to cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) for various NSAIDs is evaluated in vitro by whole blood analysis and is expressed as a ratio of half-maximal inhibitory concentration (IC_{50}) values [54].

The ratio between cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which depends on the concentration, helps to estimate the relative selectivity and the IC_{50} ratio, which is expressed as the selectivity index (SI = IC_{50} COX-1/IC_{50} COX-2). Most often, NSAIDs are divided into three categories depending on the kinetics of their interaction with COX-1 and COX-2: (i) irreversible inhibitors, such as aspirin; (ii) time-dependent and slowly reversible inhibitors, such as diclofenac and celecoxib; (iii) freely circulating, such as ibuprofen and piroxicam.

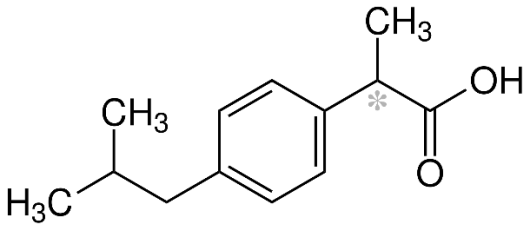
Acetylsalicylic acid (aspirin) is a cyclooxygenase-1 (COX-1) inhibitor, and it inhibits COX-1 irreversibly. This occurs due to the formation of a salt bridge with Arg120 and interaction with Ser530 through its acetyl group in the region of the active site [50].



Aspirin

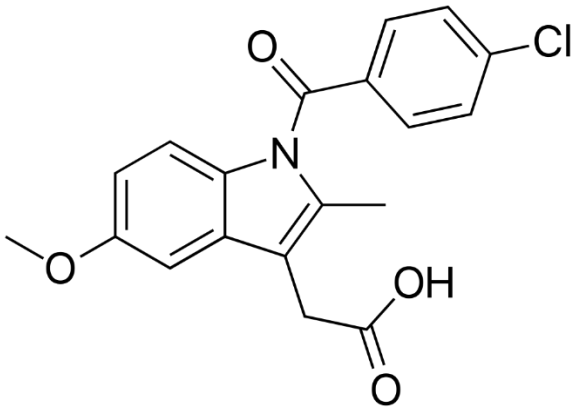
Ser530 is located near the active site of cyclooxygenase-1 (COX-1), and the acetyl group of aspirin blocks the entrance to the active site, preventing the access of arachidonic acid to Tyr385 through steric hindrance. Instead, acylation of cyclooxygenase-2 (COX-2) converts its function from cyclooxygenase to lipoxygenase, resulting in the formation of monooxygenated 15R-hydroxy-

eicosatetraenoic acid (15R-NETE) [55, 56, 57]. One of the important characteristics of aspirin is that even the minimum effective dose can cause long-term inhibition of cyclooxygenase-1 (COX-1), which leads to inhibition of prostaglandin (PG) and thromboxane A2 (TXA2) production. It plays an important role in reducing platelet aggregation [58, 59] Traditional representatives of the most well-known nonsteroidal anti-inflammatory drugs (NSAIDs) include ibuprofen, ketoprofen, flurbiprofen, indomethacin, sulindac, and diclofenac. These drugs are characterized by slow reversibility in their inhibition of cyclooxygenase and are widely used for the treatment of inflammation. [50]



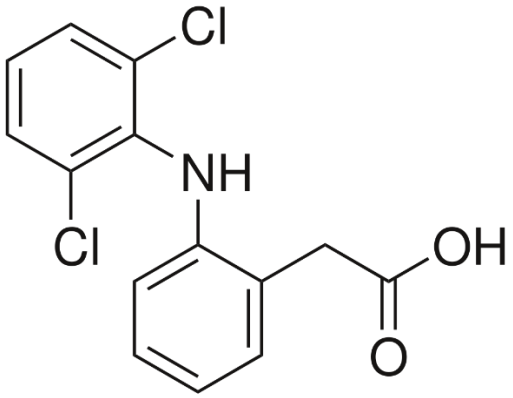
Ibuprofen

Traditional representatives of the most well-known nonsteroidal anti-inflammatory drugs (NSAIDs) include ibuprofen, ketoprofen, flurbiprofen, indomethacin, sulindac, and diclofenac. These drugs are characterized by slow reversibility in their inhibition of cyclooxygenase and are widely used for the treatment of inflammation.



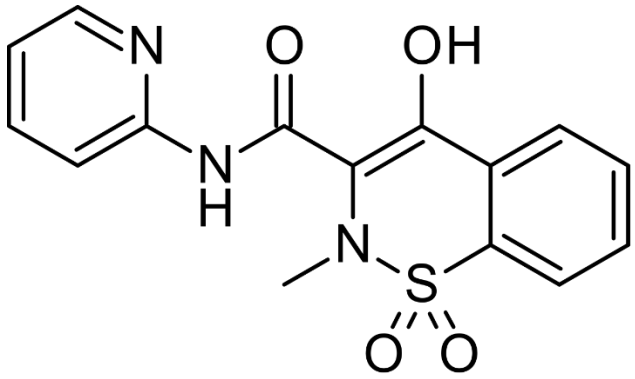
Indometacin

Indomethacin is known as a time-dependent COX inhibitor that interacts with the hydrophobic "pocket" using the amino acids Ser530, Ala527, Val349 and Leu531.



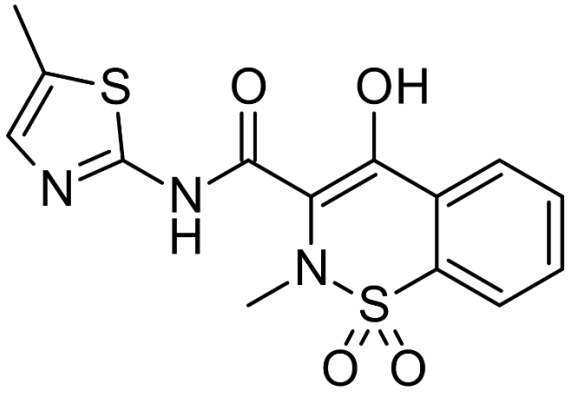
Diclofenac

Interestingly, diclofenac has a carbonyl group and two chlorine atoms that form several hydrogen bonds with amino acids Ser530 and Tyr385. This may lead to a certain increase in its selectivity compared to other traditional anti-inflammatory drugs [60].



Piroxicam

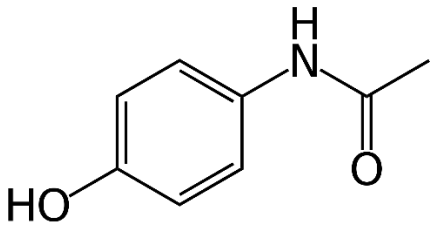
Pyrazolidine-3,5-dione derivatives such as oxicams, including piroxicam, are known for their nonselective cyclooxygenase-2 (COX-2) inhibitory activity, with the exception of meloxicam, which exhibits only minor COX-2 inhibitory activity [61].



Meloxicam

Physicochemical characteristics of oxicams, such as keto-enol tautomerism, are mainly related to their ability to inhibit enzymes. Several H-bonds between thiazine and carboxamide fragments and residues Ser530, Tyr385, Arg120 and Tyr355 were detected. Binding of oxicam in the active site causes changes in protein conformation, which is a difference compared to classic NSAIDs. The latter can usually rotate the Leu531 residue and have access to the side pocket for binding [62].

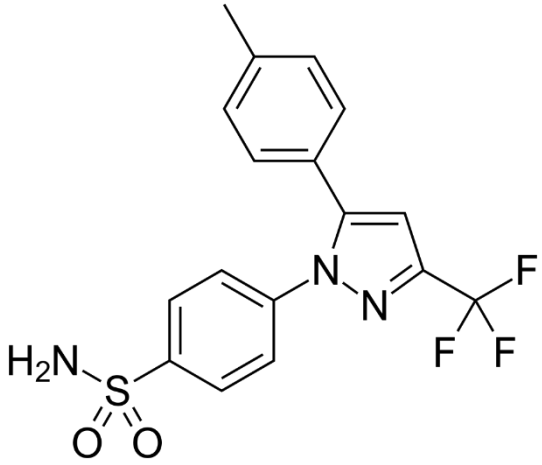
The main difference of the new generation of COX-2 inhibitors is that they do not have a carboxylic acid, which usually leads to inhibition of the enzyme due to interaction with the amino acid Ser530 and no interaction with the salt bridge with the amino acid Arg120.



Paracetamol

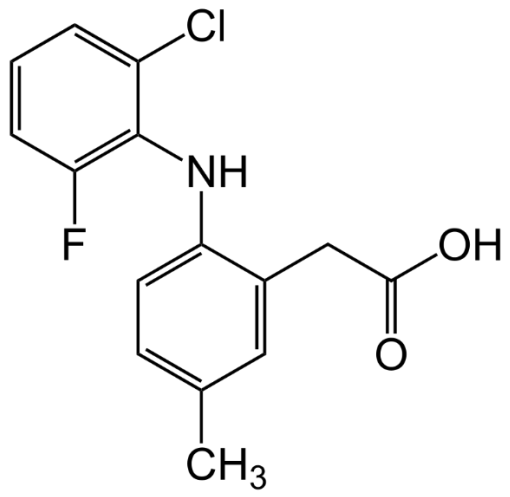
Paracetamol is used to reduce pain and reduce fever when elevated. It does not contain carboxylic acid and is characterized by non-selective inhibition of cyclooxygenase. [63, 64] The mechanism of action of paracetamol remains unspecified. The unpredictable pharmacological nature of this drug, which includes its high efficiency in reducing fever, is not accompanied by pronounced anti-inflammatory activity, which may indicate a possible interaction with COX-1/2. Some evidence supports the idea that paracetamol may interact with the cyclooxygenase 1 (COX-3) splice variant, which was once thought to be active in the human pituitary and hypothalamus, but the COX-3 concept was later rejected [48, 65, 66] The modern approach involves the use of drugs with lipophilic properties for mixed inhibition of COX-1/2 in the central nervous system. In addition, diarylheterocyclic coxibs, such as celecoxib, valdecoxib, rofecoxib, etoricoxib, have been developed as selective COX-2 inhibitors [63, 67, 68] This category of cyclooxygenase (COX) inhibitors contains substituted diaryl structures in the central cores of five- and six-membered heterocycles. Among them, it is worth noting pyrazole (as in celecoxib), furanone (found in rofecoxib), isoxazole (used in valdecoxib) and bipyridine (characteristic of etoricoxib).

Sulfonamide and methanesulfonyl groups play an important role in selective blocking of COX-2.



Celecoxib

Thus, for the selective COX-2 inhibitor celecoxib, the binding of the $–SO_2NH_2$ group in the side pocket with amino acids His90 and Arg513 is observed, which occurs with the participation of sulfonyl oxygen atoms, as well as with amino acid Phe518 through the oxygen atom of the carbonyl group. These atoms may contribute to additional substitution interactions with residues Val434 and Arg513. Even small structural changes in COX-2 inhibitors such as celecoxib highlight the importance of similarity in interaction with COX-2 to maximize efficacy and selectivity by introducing steric hindrance between the sulfonamide oxygen and the Ile523 residue in COX-1.



Lumiracoxib

Lumiracoxib is an analogue of diclofenac in its structure, where one of the chlorine-containing groups is replaced by a fluorine atom and methyl is a substitute in the meta-position of phenylacetic acid. This creates an inhibitory structure based on a salt bridge [50, 69] Diaryl-substituted heterocycles, such as thiazole, imidazole, triazole, oxazole, oxadiazole, pyrrole, thiophene, have undergone a wide range of studies as potential selective cyclooxygenase-2 (COX-2) inhibitors [69].

Cyclooxygenase-2 (COX-2) inhibitors, which are based on pyrimidine and fused bicyclic structures, also show selectivity in COX-2 inhibition [63, 70] Acyclic nondiaryl heterocyclic molecules represent a new class of cyclooxygenase-2 (COX-2) inhibitors, characterized by the absence of a central heterocyclic core [63, 71]

Acyclic inhibitors of cyclooxygenase-2 (COX-2) include a variety of chemical structures, such as olefin, acetylene, azo, imine, unsaturated ketones, which can be modified with di- or triaryl (or alkyl) groups. In addition, a wide range of natural compounds such as xanthines, alkaloids, stilbenes, flavonoids, terpenoids and quinones are known for their ability to inhibit COX-2 [72, 73].

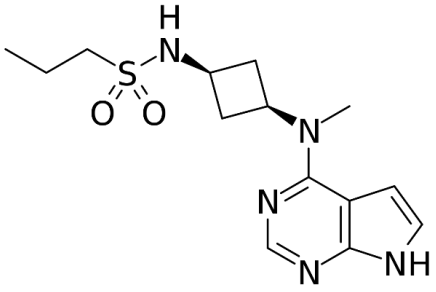
1.3. Janus kinase (JAK) inhibitors

Protein tyrosine kinases includes the Janus kinase family (JAK) which consist of four members: JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2) [74]. This type of kinases is found in mammals. Fish, birds and insects also have Janus kinases.

Cytokine signal transduction is regulated through interaction with the membrane-proximal intracellular domains of cytokine receptors, which is controlled by Janus kinases (JAKs) [74, 75, 76]. When cytokines are binding with target receptors, JAKs are activated and phosphorylate the receptors. Then JAKs create docking sites for signaling molecules.

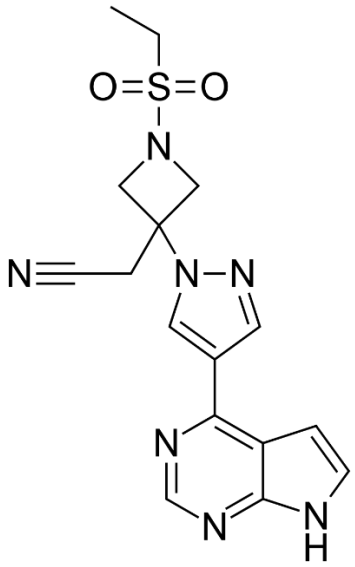
Janus kinase inhibitors are very promising because they used to treat different disorders associated with chronic inflammation. Many scientists all over the world try to create molecules that will block Janus kinase receptors and help doctors to treat different diseases with minimal side effects.

Some molecules which are used in medical practice as inhibitors of Janus kinase present below.



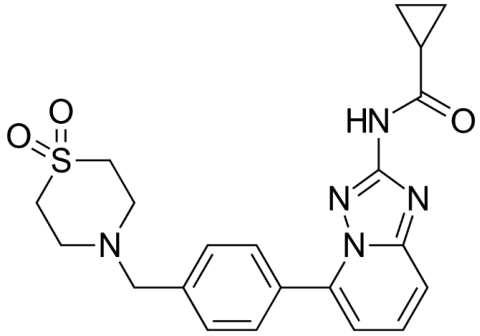
Abrocitinib

The first medicine that used for treatment of moderate to severe atopic dermatitis (AD) in adults and adolescents is Abrocitinib (Cibinqo®). It is a small molecule of selectively Janus kinase 1 (JAK1) inhibitor developed by pharmaceutical company Pfizer and approved for treatment in 2021 [77].



Baricitinib

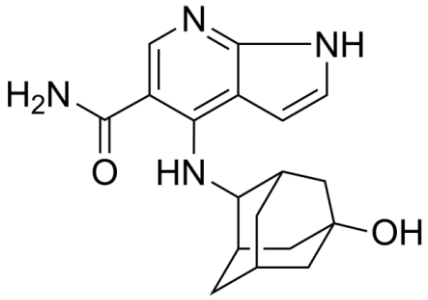
Next medicine that helps in treatment is Baricitinib. The trade name of this molecule is Olumiant®. This drug blocks JAK1 and JAK2 and may be used in treatment of some diseases. Baricitinib is a perspective medicine that treats severe alopecia, atopic dermititis and idiopathic arthritis in adult and paediatric patients 2 years of age and older. Olumiant may be used in combination with methotrexate or as monotherapy [78].



Filgotinib

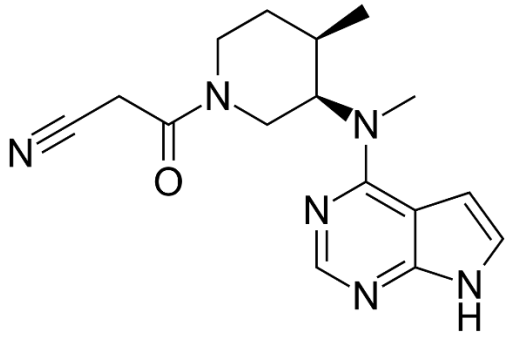
Filgotinib (also known by the trade name Jyseleca®) is preferential JAK1 inhibitor. The JAK-STAT pathway plays a key role in the pathogenesis of inflammatory and autoimmune diseases, and filgotinib modulates this pathway by preventing STAT

phosphorylation and activation. Jyseleca is used not only in treatment rheumatoid arthritis, it can be used for treating severely active ulcerative colitis [79].



Peficitinib

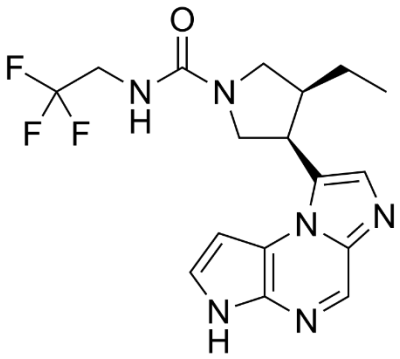
Peficitinib is the active substance of the drug with trade name Smyraf®. Introduced into medical practice by a pharmaceutical company Astellas Pharma. Peficitinib is a small molecule with unique feature to inhibit all types of JAK receptors. This medicine approved in Japan for the treatment of rheumatoid arthritis. Peficitinib has properties to inhibit activation of cytokine signaling pathways and considerably improve measures severity of disease [80].



Tofacitinib

Tofacitinib (trade name is XELJANZ®) developed by pharmaceutical company Pfizer and approved into medical practice by the FDA in 2018. This molecule inhibits mainly Janus kinase 1 and Janus kinase 3. However, it is also have abilities to inhibit other

types of Janus kinases. Tofacitinib used for treatment patients with rheumatoid or active psoriatic arthritis, ankylosing spondylitis and ulcerative colitis [81].



Upadacitinib (Rinvoq)

Upadacitinib is the active substance of the drug with trade name Rinvoq®. Molecule inhibits Janus kinase 1 and Janus kinase 3. Upadacitinib can used to treat different diseases as rheumatoid and psoriatic arthritis, ankylosing spondylitis, ulcerative colitis, Crohn's disease, eczema (atopic dermatitis) [82].

Conclusions to the Chapter 1

1. The analysis of approaches to the treatment of inflammatory processes showed that despite the large arsenal of anti-inflammatory drugs, the variety of inflammatory processes leaves the need for the creation of new anti-inflammatory agents that would be more effective for a specific pathology.
2. Development of new anti-inflammatory agents based on rational target-oriented design is an urgent task of modern pharmaceutical science.

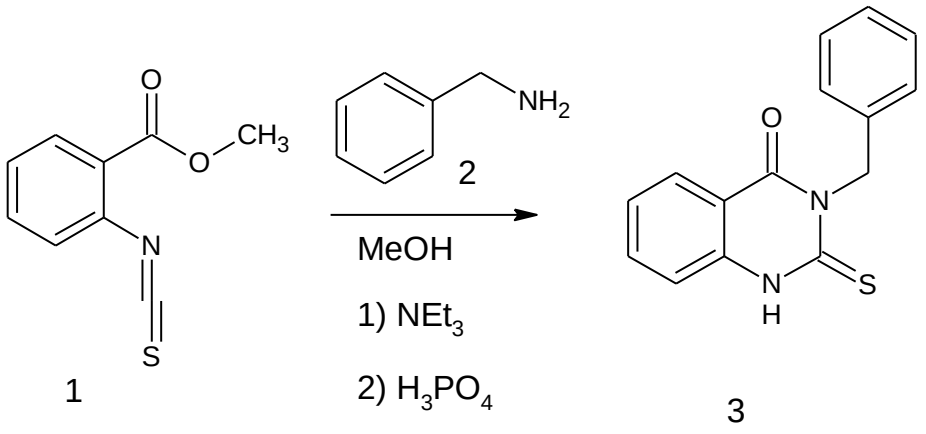
CHAPTER 2. SYNTHESIS OF 2-[(3-BENZYL-4-OXO-3,4-DIHYDROQUINAZOLIN-2-YL)THIO]ACETAMIDE AND 2-(3-BENZYL-2,4-DIOXO-3,4-DIHYDROQUINAZOLIN-1(2*H*)-YL)ACETAMIDE DERIVATIVES

The synthesis of 3-benzylquinazolin-4(3H)-one derivatives presented in the work was carried out in two directions. The first is the synthesis and modification by alkylation of 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 3. The second is the synthesis of 3-benzylquinazoline-2,4(1H,3H)-dione and its modification by alkylation of position 1.

2.1. Synthesis and modification of 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one

The synthesis of the key intermediate 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 3 was carried out on the basis of the interaction of commercially available methyl 2-isothiocyanatobenzoate with benzylamine in a methanol environment (Scheme 2.1).

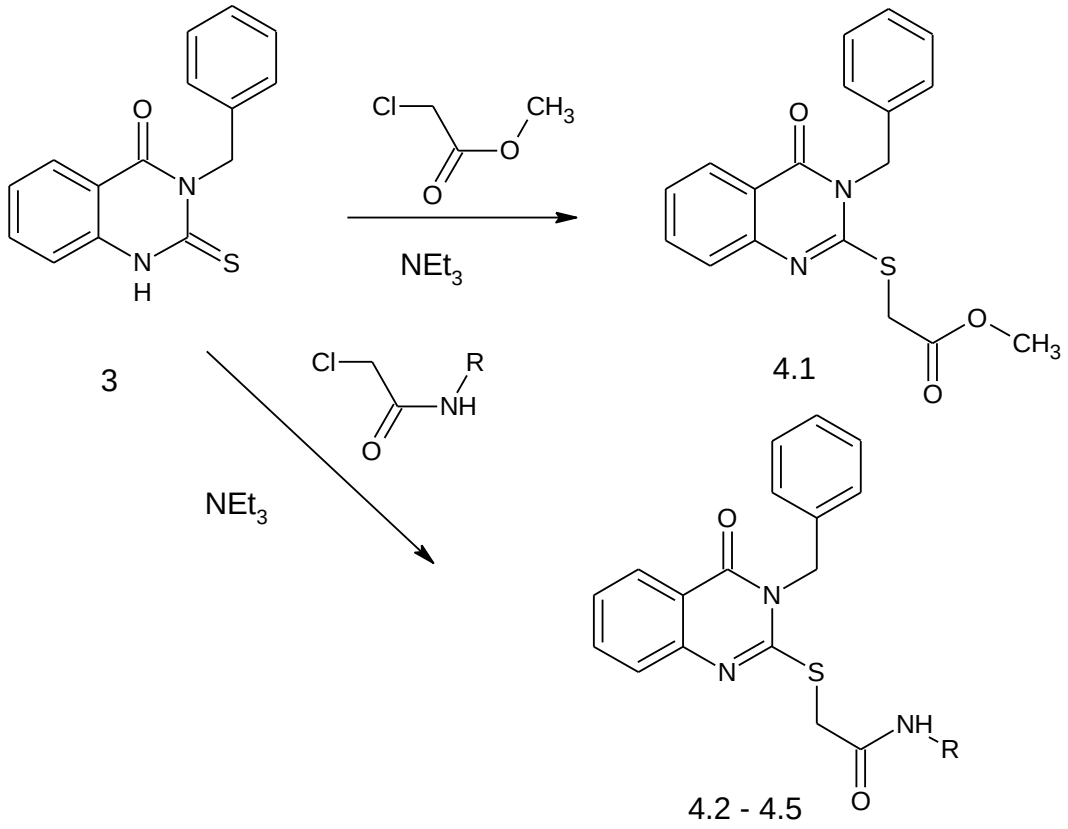
Scheme 2.1



Moreover, the stage of formation of the intermediate thiourea and its cyclization was carried out in a single reactor in the presence of triethylamine. The action of this weak base was sufficient for efficient cyclization to the corresponding triethylammonium salt, which was destroyed by the addition of stronger phosphoric acid before the isolation of the target thione **3**.

Further compound **3** was alkylated with methyl chloroacetate and chloroacetic acid amides, as a result of which te series of **4** was obtained (Scheme 2.2)

Scheme 2.2



The structure of the obtained compounds **4** was confirmed using ^{1}H NMR spectroscopy data. In the spectra of compounds **4**, the signals of the benzylic protons of the substituent in position **4** of the quinazoline system at 5.32–5.36 ppm and also the signals of the methylene protons of the S-alkyl fragment in the range of 4.04–4.22 ppm

are observed. For amides **4.2** - **4.5**, the position of the NH signal of the amide fragment is in the range of 8.72 - 10.38 ppm.

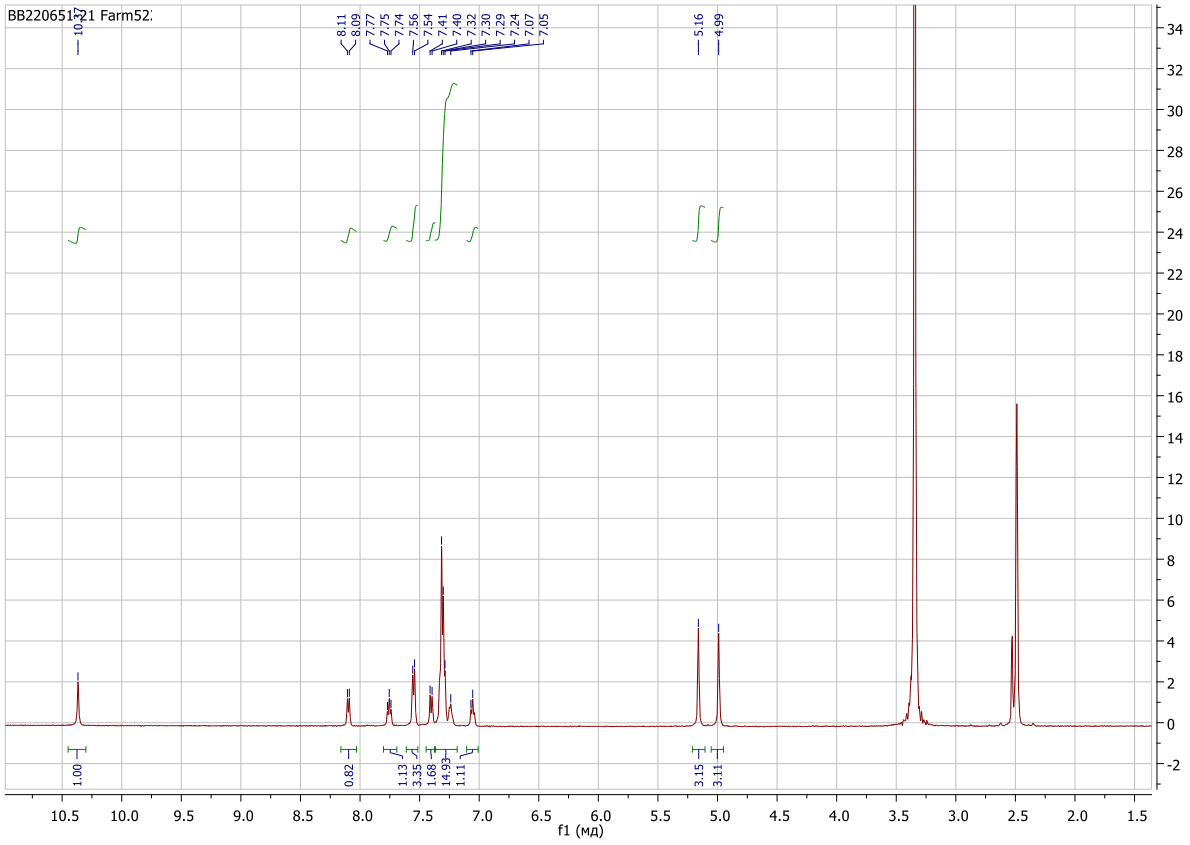


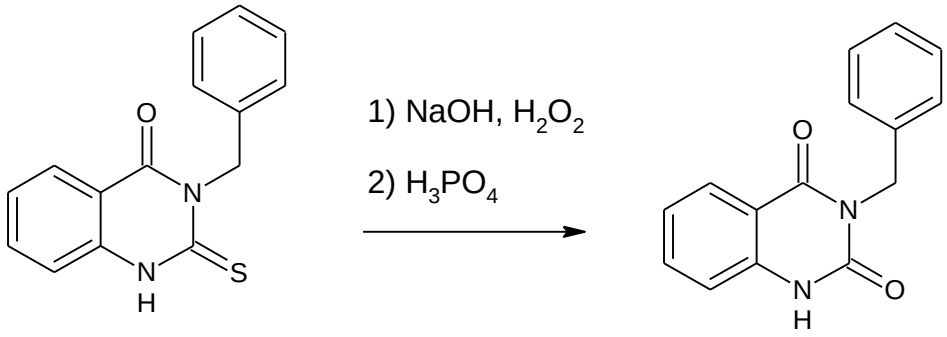
Fig. 2.1. ^{1}H NMR spectrum of 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)sulfanyl]-N-phenylacetamide **4.3**

2.2. Synthesis and alkylation of 3-benzylquinazoline-2,4(1H,3H)-dione

Another way of modifying 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one **3** was its oxidation with hydrogen peroxide in an alkaline medium, which allowed to obtain 3-benzylquinazoline-2,4(1H,3H)- dione **5** (Scheme 2.3). The reaction takes place during cooling in order to prevent the destruction of the molecule and increase

the yield of the target product. In order to isolate the oxo-form of compound **5**, before isolation, the reaction mixture was acidified with orthophosphoric acid to an acidic reaction of the medium, and the precipitate formed was filtered and washed with a large amount of water.

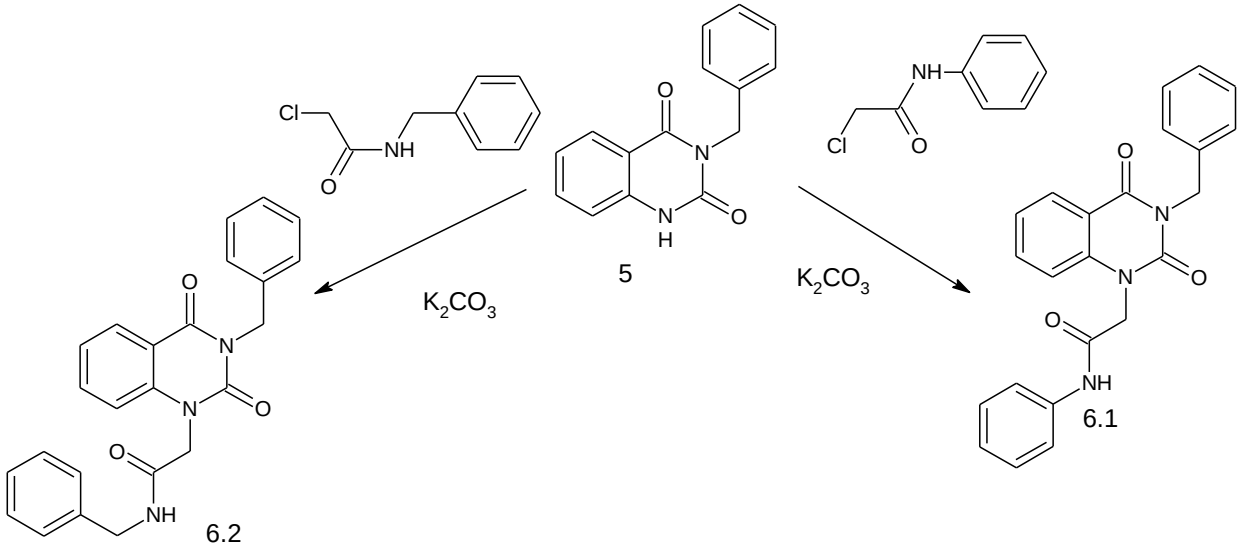
Scheme 2.3



3 5

Further alkylation of 3-benzylquinazoline-2,4(1H,3H)-dione **5** was carried out by reaction with phenyl or benzyl chloroacetamides in the medium of dimethylformamide and in the presence of potassium carbonate, which was added to bind chloride ions, which are by-products of the reaction (scheme 2.4).

Scheme 2.4



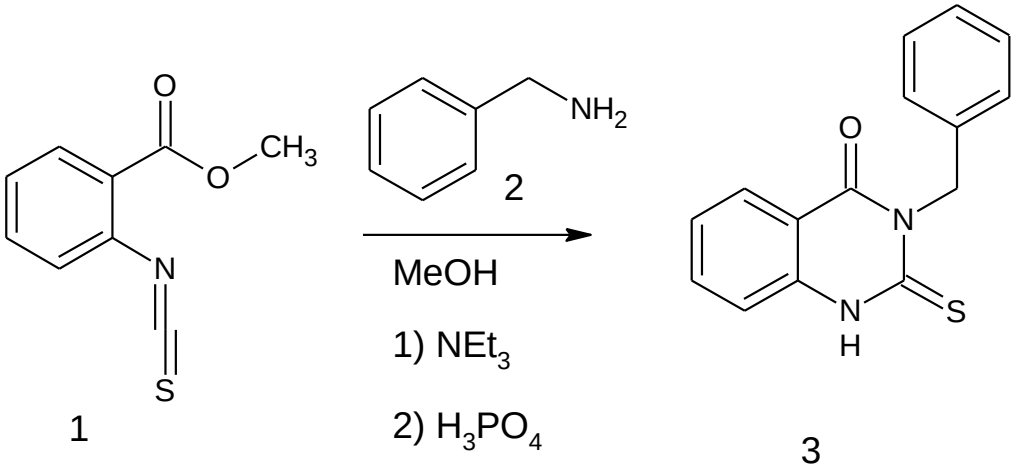
The structure of the obtained compounds **6** was confirmed using ^{1}H NMR spectroscopy, and the spectral data for these compounds indicate their correspondence to the suggested structures. In the spectra, the signals of methylene protons of the benzylic fragment are reflected in the range of 5.11–5.16 ppm, at the same time, signals of protons of methylene groups of acetamide fragments appear in the range of 4.83 - 4.99 ppm. For 2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)-N-benzylacetamide **6.2**, the signal of benzyl protons appears at 4.29 ppm. The set and nature of the signals and the resonance range of the aromatic photons correspond to the proposed structures for both compounds **6**.

2.3. Experimental part

All solvents and reagents were obtained from commercial sources. The melting points were determined in a capillary using an electrothermal IA9100X1 (Bibby Scientific Limited, Staffordshire, UK) digital melting point apparatus. The elemental analyses were performed on a Euro Vector EA-3000 (Eurovector SPA, Redavalle, Italy) microanalyzer and were within 0.4% of the theoretical values. ^{1}H NMR spectra for the compounds were recorded on Bruker Avance DRX 500 500 MHz and Varian-400 at 400 MHz spectrometers, respectively; solvents - DMSO-d_6, internal standard TMS. LC/MS spectra were recorded on Agilent 1100 HPLC instrument equipped with diode matrix and mass detectors (Agilent LC- MSD SL), column Zorbax SB-C18 (4.6 mm × 15 mm). Atmospheric Pressure Chemical Ionization (APCI) was used in the experiment.

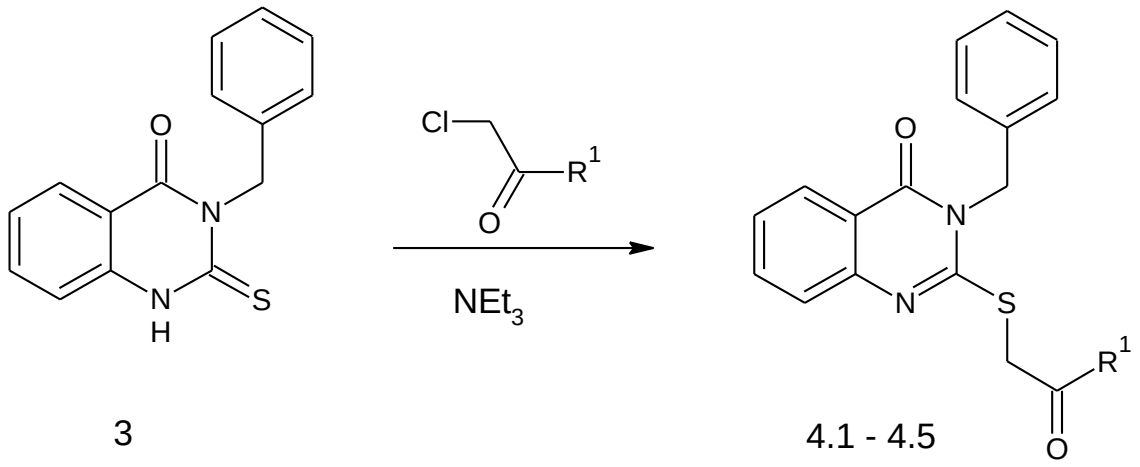
Synthesis of 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetic acid derivatives

Synthesis of 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 3



Benzylamine **2** (2.8 ml) was added to 5 g (0.026 mol) of methyl 2-isothiocyanatobenzoate **1** in 40 ml of methanol, and the mixture was heated with stirring at 60ºC for 30 minutes, and then 3.6 ml of triethylamine was added and heating was continued. After cooling, the reaction mixture was diluted with water and acidified with 3 ml of orthophosphoric acid. The precipitate 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one **3** that formed was filtered and washed with a large amount of water. The precipitate of compound **3** was dried and used in further transformations without further purification.

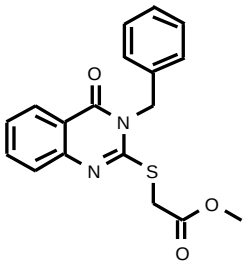
General technique of alkylation of 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 3 with chloroacetic acid methyl ester and chloroacetamides



3 4.1 - 4.5

Triethylamine (0.14 ml) and 3 ml of dimethylformamide were added to 0.267 g (0.001 mol) of 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 3, and then 0.001 mol of the corresponding chloro derivative. The reaction mixture was stirred at 70°C for 5 hours. Then the reaction mixture was cooled and diluted with water, the resulting precipitate was filtered off and recrystallized from ethanol.

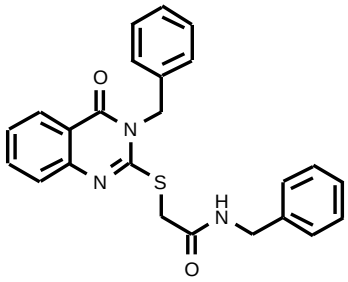
4.1 Methyl [(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetate



Yield – 76 %, white crystals. M. p. 139-140 °C. Anal. Calcd. for $C_{18}H_{16}N_2O_3S$, % (340.40): C, 63.51; H, 4.74; N, 8.23. Found, C, 63.56; H, 4.79; % N, 8.31. ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.10 (d, *J* = 6.8 Hz, 1H), 7.80 (s, 1H), 7.47 (s, 2H),

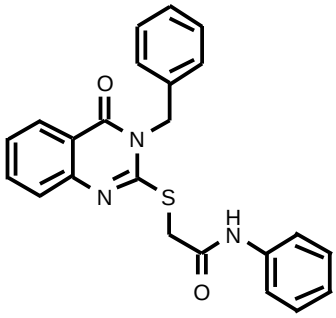
7.30 (d, *J* = 14.5 Hz, 5H), 5.32 (s, 2H), 4.10 (s, 2H), 3.67 (s, 3H). LC-MS m/z (ES+) 341.0 (MH^+).

4.2 *N*-Benzyl-2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetamide



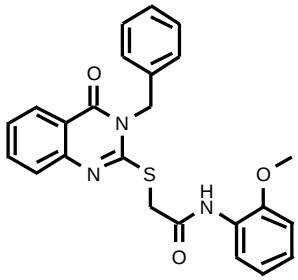
Yield – 85 %, white crystals. M. p. 198-200 °C. Anal. Calcd. for $C_{24}H_{21}N_3O_2S$, % (415.13): C, 69.42; H, 5.13; N, 10.12. Found, C, 69.46; H, 5.22; % N, 10.27. ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.72 (s, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 2H), 7.40 – 7.06 (m, 10H), 5.35 (s, 2H), 4.30 (d, *J* = 5.9 Hz, 2H), 4.04 (s, 2H). LC-MS m/z (ES+) 416.0 (MH^+).

4.3 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]-N-phenylacetamide



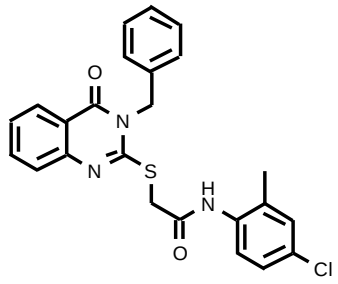
Yield – 76 %, white crystals. M. p. >250 °C. Anal. Calcd. for $C_{23}H_{19}N_3O_2S$, % (401.49): C, 68.81; H, 4.77; N, 10.47. Found, C, 68.94; H, 4.85; N, 10.57. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.18 – 8.04 (m, 1H), 7.84 – 7.71 (m, 1H), 7.59 (d, *J* = 7.9 Hz, 2H), 7.53 – 7.40 (m, 2H), 7.38 – 7.21 (m, 8H), 7.04 (t, *J* = 7.5 Hz, 1H), 5.36 (s, 2H), 4.19 (d, *J* = 2.9 Hz, 2H). LC-MS m/z (ES+) 402.0 (MH^+).

4.4 *N*-(2-methoxyphenyl)-2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetamide



Yield – 83 %, white crystals. M. p. 167-169 °C. Anal. Calcd. for $C_{24}H_{21}N_3O_3S$, % (431.52): C, 66.80; H, 4.91; N, 9.74. Found, C, 66.80; H, 4.94; % N, 9.77. ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.58 (s, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.84 (t, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.39 – 7.21 (m, 5H), 7.03 (dt, *J* = 15.9, 7.8 Hz, 2H), 6.88 (t, *J* = 7.5 Hz, 1H), 5.36 (s, 2H), 4.22 (s, 2H), 3.66 (s, 3H). LC-MS m/z (ES+) 432.0 (MH^+).

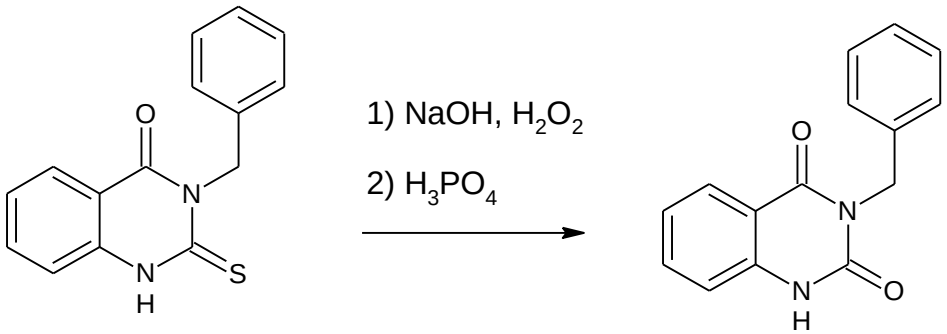
4.5 *N*-(4-chloro-2-methylphenyl)-2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetamide



Yield – 88 %, white crystals. M. p. 224-226 °C. Anal. Calcd. for $C_{24}H_{20}ClN_3O_2S$, % (449.96): C, 64.06; H, 4.48; N, 9.34. Found, C, 64.08; H, 4.49; % N, 9.36. ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.74 (s, 1H), 8.11 (d, *J* = 7.7 Hz, 1H), 7.81 (t, *J* = 7.3 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.3 Hz, 1H), 7.42 – 7.24 (m, 7H), 7.19 (d, *J* = 7.8 Hz, 1H), 5.36 (s, 2H), 4.22 (s, 2H), 2.16 (s, 3H). LC-MS m/z (ES+) 450.0 (MH^+).

General method of synthesis of 2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetamides

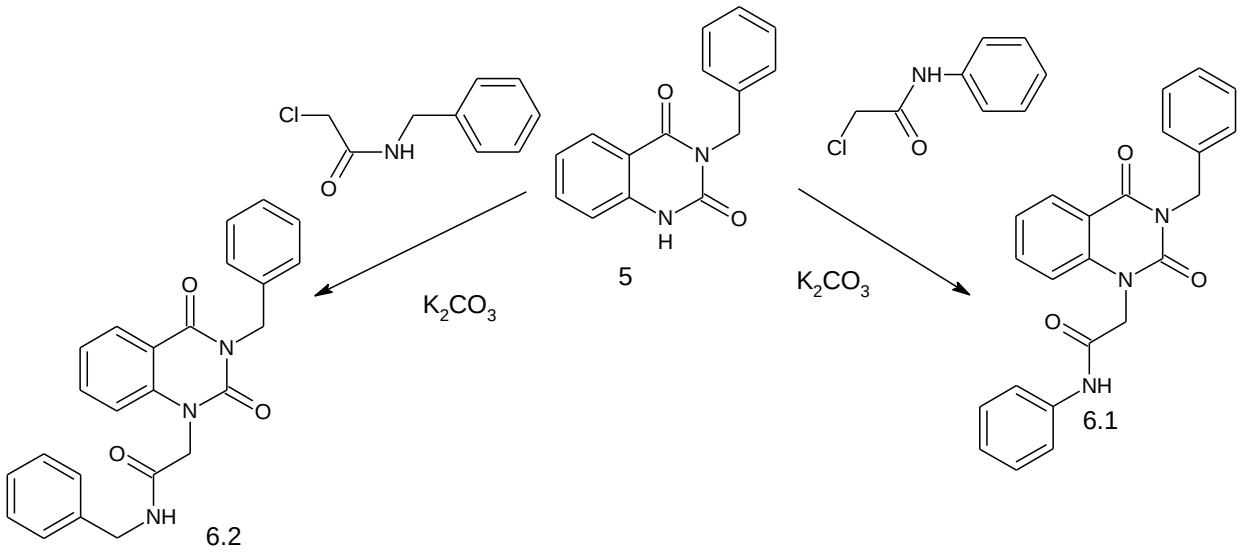
Synthesis of 3-benzylquinazoline-2,4(1H,3H)-dione 5



3 5

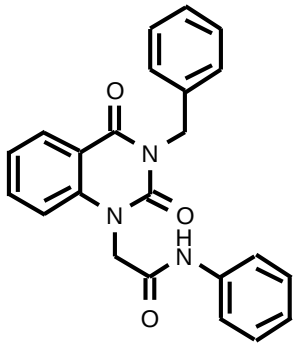
To 3 g (0.011 mol) of 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 3 was added 1.35 g (0.033 mol) of sodium hydroxide and the amount of water sufficient to dissolve 3 at a temperature of 50ºC. Next, the reaction mixture was cooled in an ice bath and 7 ml of hydrogen peroxide solution (30%) was added. The reaction mixture was stirred for 15 hours and the medium reaction was made acidic by adding orthophosphoric acid. The precipitate formed was filtered and washed with a large amount of water. Further, compound 5 was used without further purification.

General method of alkylation of 3-benzylquinazoline-2,4(1H,3H)-dione with 5 chloroacetamides



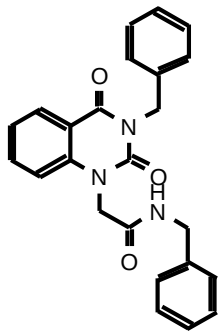
Potassium carbonate (0.14 g) 0.001 mol of the corresponding chloroacetamide and 3 ml of dimethylformamide were added to 0.25 g (0.001 mol) of 3-benzylquinazoline-2,4(1H,3H)-dione 5. The reaction mixture was stirred under heating at 70 C for 5 hours. After cooling, the reaction mixture was diluted with water and the resulting precipitate was filtered off and crystallized from ethanol.

6.1 2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)-*N*-phenylacetamide



Yield – 58 %, white crystals. M. p. 239-241 °C. Anal. Calcd. for $C_{23}H_{19}N_3O_3$, % (385.43): C, 71.68; H, 4.97; N, 10.90. Found, C, 71.77; H, 4.99; % N, 10.95. ^{1}H NMR (500 MHz, DMSO-D_6) δ 10.37 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.75 (t, *J* = 7.4 Hz, 1H), 7.55 (d, *J* = 7.7 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.29 (dd, *J* = 22.9, 15.7 Hz, 8H), 7.06 (d, *J* = 7.4 Hz, 1H), 5.16 (s, 2H), 4.99 (s, 2H). LC-MS m/z (ES+) 386.0 (MH^+).

6.2 2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)-*N*-benzylacetamide



Yield – 55 %, white crystals. M. p. 235-237 °C. Anal. Calcd. for $C_{24}H_{21}N_3O_3$, % (399.45): C, 72.17; H, 5.30; N, 10.52. Found, C, 22.302; H, 5.47; % N, 10.55. ^{1}H NMR (500 MHz, DMSO-D_6) δ 11.51 (s, 1H), 8.74 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.66 (s, 1H), 7.42 – 7.15 (m, 9H), 5.11 (d, *J* = 33.8 Hz, 2H), 4.83 (s, 2H), 4.29 (d, *J* = 5.3 Hz, 2H). LC-MS m/z (ES+) 400.0 (MH^+).

Conclusions to the Chapter 2

1. An effective procedure of obtaining 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetic acid derivatives by alkylation of synthetically available 3-benzyl-2-thioxo-2,3-dihydroquinazolin- 4(1H)-one with chloroacetic acid methyl ester and chloroacetamides.
2. A method for obtaining 3-benzylquinazoline-2,4(1H,3H)-dione with acetamide substituents in position 1 of the heterocyclic system was developed
3. The structures of the obtained compounds were confirmed by modern instrumental methods.

CHAPTER 3. PREDICTION OF THE ANTI-INFLAMMATORY AND ANTIBACTERIAL EFFECT OF 3-BENZYLQUINAZOLIN-4(3H)-ONE DERIVATIVES BY MOLECULAR DOCKING STUDY

3.1. Prediction of anti-inflammatory effect of 3-benzylquinazolin-4(3H)-ones

3-Benzylquinazolines with a hydrazone moiety in position 2 are known for their anti-inflammatory activity, while 3-benzyl-2-alkylthioquinazolines are better known as anticancer agents with a tyrosine kinase mechanism of action. Taking into account the prospects of the quinazoline scaffold for modern medical chemistry, we conducted a study of a number of 3-benzylquinazolin-4(3H)-one derivatives for the possibility of being COX-2 inhibitors, and therefore acting as anti-inflammatory agents with a reduced number of side effects characteristic of non-selective COX inhibitors.

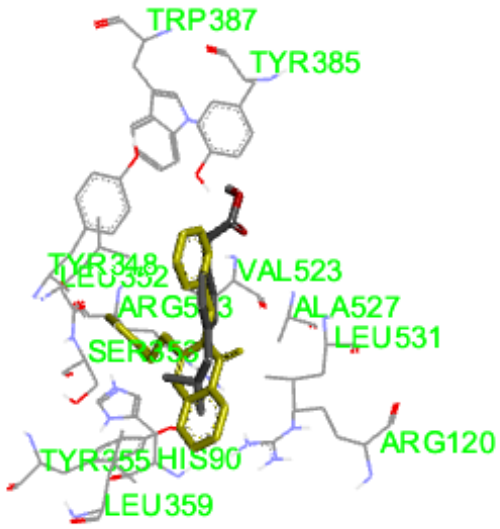


Fig. 3.1. Interaction of methyl [(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)sulfanyl]acetate 4.1 (yellow) with theactive site of COX-2 in coparison with Ibuprofen

Docking analysis of derivatives of 3-benzylquinazolinylquinazolin-4(3*H*)-one in order to establish the structures of molecules that can have anti-inflammatory activity and revealed their effect by binding to the active site of COX-2. The results are presented in table 3.1.

Table 3.1

The predicted COX-2 inhibitory activity for the derivatives of 3-benzylquinazolin-4(3H)-one

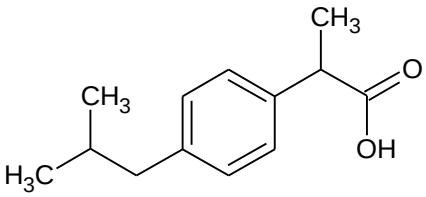
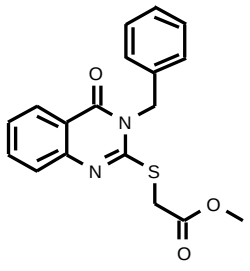
Code/Stucructure	**Interaction with the active site***	**Interacting amino acids**
1	2	3
Ibuprofen 	-	ARG120 VAL349 SER353 TYR355 LEU384 TYR385 TRP387 PHE518 GLY526 ALA527
4.1 	+	HIS90 ARG120 TYR348 LEU352 SER353 TYR355 LEU359 TYR385 TRP387 ARG513 VAL523 ALA527 LEU531

Table 3.1 (continued)

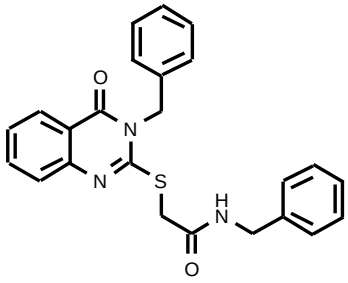
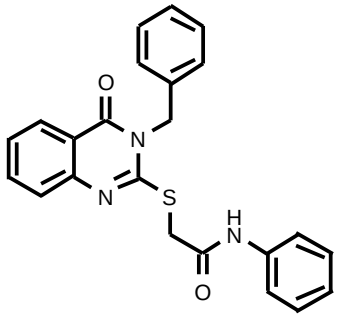
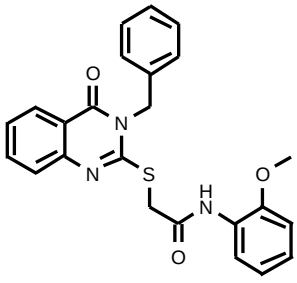
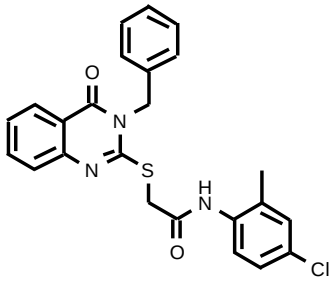
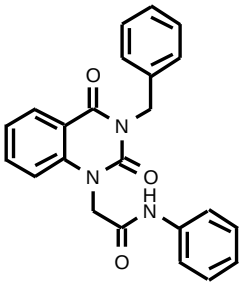
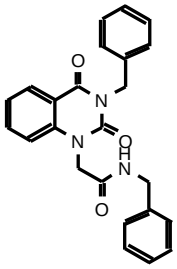
1	2	3
4.2 	+ (additional linking)	HIS90 ARG120 GLN192 VAL349 LEU352 SER353 TYR355 ARG513 ASP515 ALA516 PHE518 VAL523
4.3 	- (no interaction with active site)	-
4.4 	- (no interaction with active site)	-

Table 3.1 (continued)

1	2	3
4.5 	- (no interaction with active site)	-
6.1 	- (no interaction with active site)	-
6.2 	- (no interaction with active site)	-

* Interaction with the active site: + - ligand fits the active site cavity; +/- - ligand partially fits the active site cavity; - - ligand does not interact with the active site

Based on the molecular docking results, it was possible to identify methyl [(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)sulfanyl]acetate **4.1** as a potential COX-2 inhibitor that can be used in the therapy of inflammatory diseases and is probably devoid of side effects typical for compounds capable of binding to the active site of COX-1 only. Form the docking studies it is obvious that the substitution of the exocyclic sulfur atom with the small derivatives of acetic acid is the preferable way of modification of the core structure of 3-benzylquinazolinylquinazolin-4(3*H*)-one for preparation of the effective COX-2 inhibitors.

3.2. Prediction of antimicrobial activity for the derivatives of 3-benzylquinazolinylquinazolin-4(3*H*)-one molecular docking

Tuberculosis is a disease that is difficult to treat, which is due to the peculiarities of the structure of the cell wall of mycobacteria and their ability to form antibiotic resistance. Currently, there is a therapeutic scheme for the treatment of tuberculosis, which is effective in most cases. Patients who have suppressed immunity as a result of additional diseases usually deserve special attention in therapy. Therefore, the problem of development of new drugs to overcoming the problems in therapy of tuberculosis is urgent. Recent studies have allowed the isolation of TRmD from *M. tuberculosis*. This enzyme is an important target for the search for innovative antibiotics because its blocking disrupts protein synthesis in the bacterial cell. Docking analysis of derivatives of (3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetic acid in order to establish the structures of molecules that can have anti-tuberculosis activity and realize their effect by the innovative mechanism of bacterial TRmD inhibition (table 3.2).

According to the results of docking studies of the series of amides in the active site of TRmD isolated from *M. tuberculosis*, it was established that phenyl and benzyl amides of (3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetic acid were proved to be the effective ligands.

Table 3.2

The predicted interaction with TRmD from *M. tuberculosis* inhibitors active site for 3-benzylquinazolin-4(3H)-one

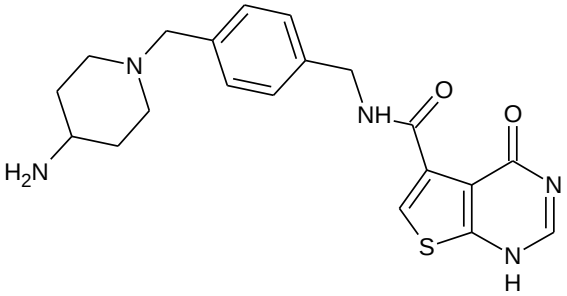
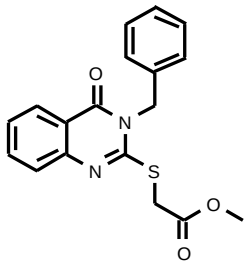
Code/Stucructure	**Interaction with the active site***	**Interacting amino acids**
1	2	3
Native inhibitor 		PRO83 THR84 PRO85 GLU112 SER132 ILE133 GLY134 TYR136 VAL137 LEU138 GLY140 GLY141 ALA144
4.1 	-	-

Table 3.2 (continued)

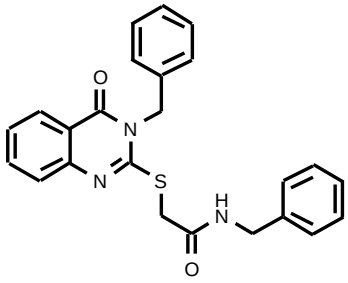
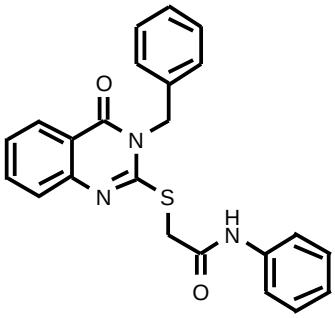
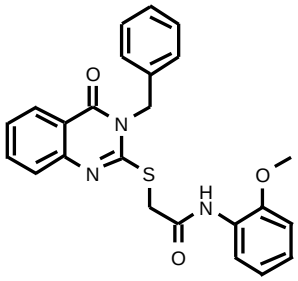
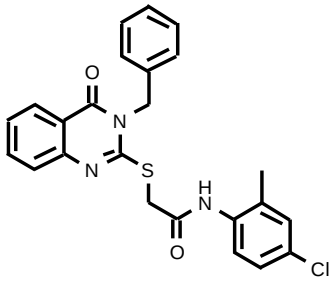
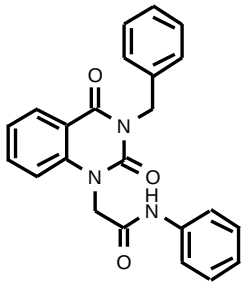
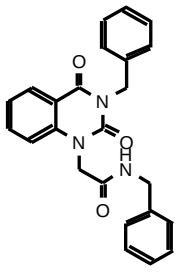
1	2	3
4.2 	-	-
4.3 	+ (good interaction)	PRO83 THR84 PRO85 GLY109 GLU112 GLY113 ILE114 ILE133 TYR136 LEU138
4.4 	-	-

Table 3.2 (continued)

1	2	3
4.5 	-	-
6.1 	+	VAL60 VAL82 PRO83 THR84 PRO85 GLU112 GLY113 ILE114 LEU138 GLY140
6.2 	+ (good inhibitor)	PRO83 THR84 PRO85 ALA86 GLY109 GLU112 ILE114 LEU138

* Interaction with the active site: + - ligand fits the active site cavity; +/- - ligand partially fits the active site cavity; - - ligand does not interact with the active site

Based on the molecular docking results, it was possible to identify 2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)-N-phenylacetamide and N-benzyl-2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)-acetamide as potential *M. tuberculosis* TRmD inhibitors that can be used in the therapy of tuberculosis. 2-[(3-Benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]-N-phenylacetamide **4.3** showed the best binding result in the series of 2-alkylthio-sustituted derivatives.

Eperimental part

The structures of the compounds were drawn using ACD/ChemSketch (freeware) and saved in .pdb format using Discovery Studio Visualizer 2021. AutoDockTools-1.5.7 was used to convert .pdb files to .pdbqt, the number of active rotatory bonds was set by default. AutoDock Vina was used to calculate molecular docking. Discovery Studio 2021 was used for visualization. Biotarget macromolecules was selected from Protein Data Bank (Protein Data Bank): COX-2 PDB ID – 1CX2; *M.tuberculosis* Trmd PDB ID – 6JOF.

Conclusions to the Chapter 3

1. Based on the molecular docking results, it was possible to identify methyl [(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetate **4.1** as a potential COX-2 inhibitor.
2. According to the results of docking of the synthesiszed compounds in the active site of TRmD isolated from *M. tuberculosis*, it was established that phenyl and benzyl amides of (3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetic acid were effective ligands; 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]-N-phenylacetamide showed the best binding result in the series of 2-alkylthio-sustituted derivatives

GENERAL CONCLUSION

1. The analysis of approaches to the treatment of inflammatory processes showed that despite the large arsenal of anti-inflammatory drugs, the variety of inflammatory processes leaves the need for the creation of new anti-inflammatory agents that would be more effective for a specific pathology. On the other hand tuberculosis is great problem for moderm methods of therapy.
2. The effective procedure of obtaining 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetic acid derivatives by alkylation of synthetically available 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one with chloroacetic acid methyl ester and chloroacetamides and the method for obtaining 3-benzylquinazoline-2,4(1H,3H)-dione with acetamide substituents in position 1 of the heterocyclic system were developed.
3. Based on the molecular docking results, it was possible to identify methyl [(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]acetate as a potential effective COX-2 inhibitor.
4. According to the results of docking of the synthesiszed compounds in the active site of TrmD isolated from *M. tuberculosis*, it was established that phenyl and benzyl amides of (3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetic acid were effective ligands; 2-[(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio]-N-phenylacetamide showed the best binding result in the series of 2-alkylthio-sustituted derivatives

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Appendices

Appendix A

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ГРАМОТА

за участь

отримав(ла)

Baltach Yassine

у секційному засіданні студентського наукового товариства кафедри фармацевтичної хімії

XXX Міжнародна науково-практична конференція молодих вчених та студентів **"Актуальні питання створення нових лікарських засобів"**

В.о. ректора
Національного фармацевтичного університету

Алла КОТВІЦЬКА

17-19 квітня 2024 р.
м. Харків

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

АКТУАЛЬНІ ПИТАННЯ СТВОРЕННЯ НОВИХ ЛІКАРСЬКИХ ЗАСОБІВ

МАТЕРІАЛИ
XXX МІЖНАРОДНОЇ НАУКОВО-ПРАКТИЧНОЇ
КОНФЕРЕНЦІЇ МОЛОДИХ ВЧЕНИХ ТА СТУДЕНТІВ

17-19 квітня 2024 року
м. Харків

Харків
НФаУ
2024

УДК 615.1

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

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

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

УДК 615.1

глутаміну і глутамату. Також він здатний підвищує рівень дофаміну, ГАМК і гліцину в різних областях мозку. Ось ця зміна балансу медіаторів забезпечує ноотропну властивість теаніну.

На ринку знаходиться 4 виробники дієтичних добавок з теаніном. Сюди входить: Now Food, Stark Pham, Solgar, Віола.

Більшість дієтичних добавок представлена компанією Now Food, її частка становить 40%. А найменшу частину займає виробник Віола, її частка становить 20%

Наступним етапом наших маркетингових досліджень було вивчити цінову вартість дієтичних добавок. Для цього ми склали критерій за ціною дієтичної добавки: висока вартість – від 1000 грн., середня вартість – від 500 грн., а низька – від 150 грн.

За результатами було встановлено, що 5 дієтичних добавок має низьку вартість, 8 препаратів має середню вартість та 3 дієтичних добавки мають високу вартість.

Висновки. Теанін є важливою амінокислотою, основним джерелом якого є лист зеленого чаю. Основний механізм когнитивноъ дії є інгібування зворотного захоплення глутаміну. Лідером серед виробників є NOW Food, найбільша кількість дієтичних добавок з теанином має ціну від 500 грн.

SYNTHESIS OF 3-BENZYLQUINAZOLIN-4(3H)-ONE DERIVATIVES WITH PREDICTED USEFUL PHARMACOLOGICAL PROPERTIES

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Introduction. Among the most dangerous pathological conditions, inflammatory processes, as well as diseases of bacterial etiology, occupy an important place in terms of prevalence and danger. At the same time, recent studies show that 3-benzylquinazolines with a hydrazone moiety at position 2 are known for their anti-inflammatory activity, while 3-benzyl-2-alkylthioquinazolines are better as tyrosine kinase mechanism inhibitors. Considering the literature, the development of new approaches to the synthesis of compounds with the 3-benzylquinazolin-4(3H)-one fragment may provide new objects for further pharmacological research.

Aim. Testing of synthesis methods and development of an effective approach to the creation of 3-benzylquinazolin-4(3H)-one derivatives with oxo- and thio-substituents in position 3, which may be of interest as anti-inflammatory and anti-tuberculosis agents.

Materials and methods. Methods of organic synthesis and instrumental methods of elucidation of the structure (^{1}H NMR, mass-spectroscopy, etc.); the methods of virtual screening of compounds (molecular docking).

Results and discussion. The synthesis of the key intermediate 3-benzyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one was carried out on the basis of the interaction of commercially available methyl 2-isothiocyanatobenzoate with benzylamine in a methanol medium. Moreover, the stage of formation of the intermediate thiourea and its cyclization was carried out in a single reactor in the presence of triethylamine. Subsequently, thione was alkylated with methyl chloroacetate and chloroacetic acid amides, as a result of which 3-benzyl-2-(alkylthio)quinazolin-4(3H)-one derivatives were obtained. The key thione was also oxidized with hydrogen peroxide in an alkaline medium, and 3-benzylquinazoline-2,4(1H,3H)-dione was obtained, the further modification of position 1 of which

was carried out by reaction with phenyl or benzyl chloroacetamides in the medium of dimethylformamide and in the presence of potassium carbonate.

Conclusions. An effective technique was developed, which allowed to effectively obtain a number of potential anti-inflammatory and anti-tuberculosis agents from 3-benzylquinazolin-4(3H)-one series with oxo- and thio-substituents at the position 2.

IN SILICO PREDICTION OF BIOTRANSFORMATION DIRECTIONS OF 3-[(DIMETHYLAMINO)METHYL]-2-METHYL-6-METHOXY-1H-QUINOLIN-4-ONE – A POTENTIAL API WITH NOOTROPIC ACTION

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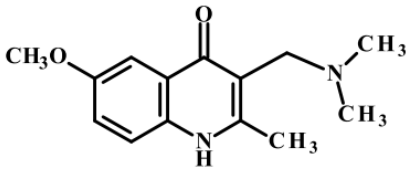
Introduction. The way drugs are processed in the body is largely affected by their metabolism. This means that the drugs can either become inactive or more effective, or even turn into harmful chemicals, depending on how the enzymes in the body break them down. This highlights the significance of drug metabolism in the discovery and development of medicines, and it explains why there are many experimental technologies in use to study how drugs are processed in the body. Since the traditional drug development process is expensive, various computational approaches have been developed to predict the metabolic outcome of drug candidates. This makes it possible to screen a large number of chemical compounds and identify the most promising ones for further development.

Aim. Prediction of probable biotransformation directions of 3-[(dimethylamino)methyl]-2-methyl-6-methoxy-1H-quinolin-4-one – a potential API with nootropic action.

Materials and methods. The object of the study is promising molecule with nootropic action 3-[(dimethylamino)methyl]-2-methyl-6-methoxy-1H-quinolin-4-one:



It was synthesized by Vadym Zubkov, Associate Professor of the Department of Medicinal Chemistry. 3-[(Dimethylamino)methyl]-2-methyl-6-methoxy-1H-quinolin-4-one became a promising object for pharmacological study based on the results of a comprehensive screening study of its psycho- and neurotropic properties performed by Illya Podolsky, Professor of the Department of Medicinal Chemistry.

Xenosite, SMARTCyp, Way2Drug RA, Biotransformer, and GLORYx (online applications) were used in order to predict the possible biotransformation directions of 3-[(dimethylamino)methyl]-2-methyl-6-methoxy-1H-quinolin-4-one.

Results and discussion. A comprehensive analysis of the results of predicting the possible metabolic pathways of 3-[(dimethylamino)methyl]-2-methyl-6-methoxy-1H-quinolin-4-one using five different online systems leads to the conclusion that the molecule can be intensively metabolized by cytochrome P450 enzymes.