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ІНСТИТУТ ОРГАНІЧНОЇ ХІМІЇ НАН УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

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НФаУ

У журналі розглянуто проблеми синтезу й аналізу органічних та елементоорганічних сполук, аналогів природних сполук і лікарських субстанцій, наведено результати фізико-хімічних досліджень у вищезазначених напрямах. Також з погляду (біо)органічної, фармацевтичної, аналітичної та фізичної хімії проаналізовано питання з різних аспектів рослинництва, ґрунтознавства й дослідження навколишнього середовища.

Для працівників науково-дослідних установ, вищих навчальних закладів та фахівців хімічного, фармацевтичного, біологічного, медичного і сільськогосподарського профілів.

«Журнал органічної та фармацевтичної хімії» внесено до затвердженого МОН України Переліку наукових фахових видань України (категорія «Б») для опублікування результатів дисертаційних робіт за спеціальністю 102 – Хімія та 226 – Фармація, промислова фармація (наказ МОН України від 28.12.2019 р. № 1643); індексовано в наукометричних базах даних: Chemical Abstracts (CAS), Index Copernicus; внесено до каталогів та пошукових систем: Directory of Open Access Journals (DOAJ), Bielefeld Academic Search Engine (BASE), Directory of Open Access scholarly Resources (ROAD), PKP Index, Ulrich's periodicals, Worldcat, НБУ ім. В. І. Вернадського і УРЖ «Джерело».

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In Memoriam
Andronati Serhiy Andriyovych

It is with deep sadness that we inform you that on June 29, 2022, at the age of 82, after a long illness, an outstanding scientist in the field of bioorganic and medical chemistry, Doctor of Chemistry, professor, academician of the National Academy of Sciences of Ukraine, adviser to the Presidium of the National Academy of Sciences of Ukraine, honorary director and adviser to the Directorate of the A.V. Bogatsky Physico-Chemical Institute, head of the Department of Medical Chemistry, winner of State prizes of the USSR and Ukraine **Andronati Serhiy Andriyovych** passed away.

Andronati Serhiy Andriyovych was born on September 19, 1940 in Odesa in the family of employees. In 1964, after graduating from the Faculty of Chemistry of the Odesa State University named after I. I. Mechnikov in the specialty "Organic Chemistry" Serhiy Andriyovych began his career. He overcame his career steps confidently and tirelessly: he worked at the Odesa State University (1968–1972) as a senior engineer, senior lecturer and deputy dean of the Faculty of Chemistry. In 1972, S. A. Andronati took the position of senior researcher and later the head of the Department of chemistry of nitrogen-containing heterocycles. After the creation of the Physico-Chemical Institute of the Academy of Sciences of the Ukrainian SSR (the first independent academic institution in Odesa), S. A. Andronati held the position of deputy director for research (1978–1983), and later the director of the Institute (until 2020). He was the head of the Southern Scientific Centre of the National Academy of Sciences and the Ministry of Education and Science of Ukraine; at the same time (since 1998) headed the Department of Pharmaceutical Chemistry of the Odesa State University named after I. I. Mechnikov and was the scientific director of the Chemical and Pharmaceutical Educational Research and Production Complex of the National Academy of Sciences and the Ministry of Education and Science of Ukraine.

The scientific life of Serhiy Andriyovych was brilliant, fast-paced, diverse and extremely fruitful. After completing his postgraduate studies (1970) at the Department of Organic Chemistry of the Odesa State University and in the same year defending his thesis for the degree of Candidate of Chemistry, he has immediately started doing his doctoral investigations and in 1974 successfully defended a thorough research on the problems of synthesis, stereochemistry and biological activity of benzodiazepine derivatives.

The scientific interests of S. A. Andronati involved the issues of bioorganic and medical chemistry. His achievements are the development of methods for the synthesis of biologically active compounds, determination of their structure, conformations, physicochemical, chemical, pharmacological properties, molecular mechanisms of action, the "structure – mechanism of action – activity" relationship, molecular design of potential biologically active compounds, their synthesis. He also made a significant contribution to the development of methods for the synthesis of new derivatives of quinazoline, 1,4-benzodiazepine, 1,3,4-benzotriazepine, 1,5-benzodiazocine, 1,4,5-benzotriazocine, 1,6-benzodiazonines, a number of various macroheterocyclic systems, polynuclear carbo- and heterocyclic compounds, peptidomimetics.

Serhiy Andriyovych also studied the relationship between the structure, stereochemistry, physical, physicochemical, chemical and pharmacological properties of various derivatives of pyrimidine, quinazoline, 1,4-benzodiazepine, 1,5-benzodiazocine, nitrogenous macroheterocycles, polynuclear heterocyclic systems, oligopeptides, peptidomimetics, fluorene, anthracene, etc.

The outstanding scientist was also interested in the problems of fine structure, stereochemistry, and stereodynamics of substances. He studied 1,4-benzodiazepines and related heterosystems in the most detail, finding out that the heteroring of 1,4-benzodiazepines, 1,5-benzodiazocines, and 1,4,5-benzotriazocines had a pseudo-boat conformation. He revealed the main regularities of the relationship between the structure, kinetic and thermodynamic parameters of the inversion of compounds of this class, determined the structure of associates of 1,4-benzodiazepinemolecules, as well as correlations of spectral properties, polarity, basicity, lipophilicity with physicochemical constants characterizing the electronic nature and steric features of substituents.

The works of Serhiy Andriyovych on the search and purposeful synthesis, research on the properties and mechanisms of action of new antihypoxants and actoprotectors aimed at creating effective medicines for the treatment of various diseases accompanied by hypoxic conditions, such as traumatic shock, heart attack, pneumonia, leprosy, etc., are invaluable. The result of these studies was the discovery of a new class of such agents – pyrimidine derivatives, which turned out to be significantly more effective antihypoxants and actoprotectors than the known medicines with similar effects (sodium γ-oxybutyrate, gutimine, dibazole, as well as medicines of the plant origin – ginseng, eleutherococcus).

Of great importance are the scientific achievements of the scientist in creating fundamentally new medicines for the treatment of oncological diseases and acute viral infections belonging to the class of nonspecific immune stimulants. The study of the relationship between the structure, antiviral and interferon-inducing properties of fluorenone, anthracene and acridine derivatives allowed him to find highly effective compounds among them.

S. A. Andronati's work on the synthesis of new regulatory peptides made it possible to obtain new promising modified analogs of tyroliberin and melanostatin. In the course of these studies, new methods of the peptide synthesis based on the use of crown ethers were developed.

The scope of Serhiy Andriyovych's studies is truly impressive. It seems that there is no field of medical chemistry he left overlooked. In particular, he discovered a number of regularities concerning the "structure – psychopharmacological properties" relationship of 1,4-benzodiazepines and their cyclic homologs, as well as other heterocyclic substances related to 1,4-benzodiazepines, the metabolism pathways and pharmacokinetics of compounds with psychotropic, antihypoxic and antiviral properties. S. A. Andronati together with his team obtained important data on the molecular mechanisms of action of neurotropic agents interacting with the GABA-benzodiazepine receptor-ionophore ensemble, serotonin and dopamine receptors. The results of these studies allowed them to form an idea about the nature of the pharmacophore fragment of the substances specified, as well as the influence of conformational factors on their activity.

An important part of Serhiy Andriyovych's scientific heritage is research in the field of biotechnology, in particular, enzymatic and microbial synthesis. Thus, with the participation of the scientist, a convenient method for obtaining optically active 1,4-benzodiazepines was developed and a number of enzymes immobilized on organic and inorganic carriers with a high degree of activity and multiple use were obtained. The processes of hydroxylation, acetylation, and hydrolysis of carbo- and heterocyclic compounds catalyzed by them were studied. As the result of the studies conducted, the medicine "Elastotherase" was created for the treatment of burns and wounds.

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Recently, S. A. Andronati led research in the field of medical chemistry of antithrombotic agents. His research team obtained peptidomimetics promising for the treatment of cardiovascular diseases with a high antithrombotic activity, and discovered the mechanism of action of these substances.

S. A. Andronati is the founder of the scientific school in the field of bioorganic and medical chemistry. Under his supervision, 4 Doctoral theses and 31 PhD theses were defended.

Based on the fundamental research of the scientist and his colleagues in cooperation with pharmacologists, a number of highly effective medicines were created. Among them one should mention the first domestic anxiolytic, hypnotic and anticonvulsant medicine "Phenazepam", the anxiolytic medicine of daytime action "Hidazepam", the first oral interferon inducer with antiviral properties "Amixin", the original hypnotic and the anxiolytic medicine "Levana® IC" (Cinazepam).

The scientific heritage of this outstanding scientist includes 9 monographs, more than 600 articles, 130 patents and author's certificates for inventions. He was a member of the editorial boards of the following journals: "Reports of the National Academy of Sciences of Ukraine", "Ukrainian Chemistry Journal", "Science and Innovation", "Journal of Organic and Pharmaceutical Chemistry", "Bulletin of Psychiatry and Psychopharmacology", "The Odesa Medical Journal". S. A. Andronati was the first president of the Odesa Junior Academy of Sciences "Prometheus".

For his scientific and pedagogical activities, S. A. Andronati was awarded numerous state orders, medals and prizes, government, regional and city diplomas and badges.

S. A. Andronati was not only an outstanding scientist, a recognized luminary in the field of chemical science, but also a unique example of human and scientific ethics for thousands of people who were lucky enough to study and work with him. Colleagues of S. A. Andronati speak of him as an intellectual, a leader, a deeply decent and kind person who was characterized by inexhaustible energy, exceptional diligence and efficiency. He had deep knowledge of many areas of science, culture, and art, was demanding of himself and his employees, always ready to support and help them in solving scientific, organizational tasks, and everyday problems. He was an exemplary family man, a reliable support for parents, a loving husband, a real example for his children and grandchildren.

The death of Serhiy Andriyovych Andronati is a huge, irreparable loss for relatives, colleagues and friends. It is difficult to find words of comfort when the heart of a person who was important in life stops, but the bright memories of Serhiy Andriyovych, who left behind good deeds and lived his life honestly, will always be stronger than death.

We are deeply mourn for this irreparable loss and express our sincere condolences to his family.

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Bianka Tchoubar: A Revolutionary in French Organic Chemistry

Abstract
Virtually unknown in her homeland, Bianka Tchoubar, born in 1910 in Kharkiv, brought about a true paradigm shift in French organic chemistry of the 20th century. Originality of research ideas, scientific rigor and legendary perseverance earned her respect and recognition in the world scientific community. This eccentric Parisian of Ukrainian origin became the first woman to enter the French National Center for Scientific Research (*Centre National de la Recherche Scientifique*, CNRS) upon its creation in 1939. Bianka Tchoubar's contribution to the study of reaction mechanisms and salt effects in organic chemistry were of paramount importance, and so were her efforts to present these novel scientific concepts to the audience of French organic chemists through the clear and concise expression of her books. The name of this great Ukrainian researcher may be found in the pages of French organic chemistry textbooks, where the Demjanov ring expansion reaction is called the Demjanov–Tiffeneau–Tchoubar rearrangement. This article aims at presenting the outstanding scientific legacy and turbulent life path of this researcher to the world scientific community.
Keywords: mechanisms of organic reactions; charged intermediates; salt effects; molecular rearrangements; alicyclic compounds; history of chemistry

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Анотація
Маловідома на Батьківщині уродженка Харкова Б'янка Чубар зуміла створити справжню зміну парадигми у французькій органічній хімії XX століття. Оригінальністю наукових ідей, експериментальною майстерністю та непохитністю власних переконань ця науковиця здобула неабияке визнання у Франції та цілому світі. Ексцентрична парижанка українського походження стала першою жінкою, що увійшла до дослідницького штату Національного центру наукових досліджень Франції (*Centre National de la Recherche Scientifique,* CNRS) під час його створення 1939 року. Внесок науковиці у механістичні дослідження органічних реакцій, а також їх педагогічне опрацювання є неоціненними. Прізвище великої українки гордо майорить у французьких підручниках органічної хімії, які вшановують її експериментальні дослідження, називаючи реакцію розширення аліциклів, відому як перегрупування Дем'янова, перегрупуванням Дем'янова–Тіффано–Чубар. Пропонована стаття має на меті репрезентувати науковій спільноті України та світу видатні здобутки й непростий життєвий шлях цієї дослідниці.
Ключові слова: механізми органічних реакцій; заряджені інтермедіати; сольові ефекти; молекулярні перегрупування; аліциклічні сполуки; історія хімії

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Figure 1. Bianka Tchoubar with a cigarette. The portrait is reproduced from reference [1]

■ Biography

1. Early Life and Education

Bianka Tchoubar was born on the 22nd of October, 1910, in the Ukrainian city of Kharkiv, which was then part of the Russian Empire. The Tchoubar family belonged to the Jewish sect of the Karaites [2].

Bianka's father, Ilya Tchoubar, was a Kharkiv lawyer with Menshevik views. He was a member of the Liberal Party of Constitutional Democrats (Cadets) and a person close to its leaders, Pavel Milyukov and Vladimir Nabokov. To avoid political repression after the advent of Bolshevik power in Russia and the occupation of the Ukrainian People's Republic, the Tchoubar family (a couple with two children, Bianka and her brother Serhiy) were forced to leave Ukraine in 1920. The family settled in Constantinople, then the capital of the still-existing Ottoman Empire. Due to political instability caused by World War I, the collapse of the Ottoman Empire and the Turkish War of Independence, the Tchoubar family only lived in Constatinopole for a short time and moved to Budapest in 1922, where the brother of Ilya Tchoubar lived. The parents then moved to Paris, and the girl and her brother stayed with their uncle, where they were able to study French. The family reunited in Paris in 1924 [3].

After settling in Paris, Bianka Tchoubar converted to Orthodox Christianity in 1925, but soon became disappointed in religion and began to adhere to an agnostic worldview [2].

Bianka's father died of a heart attack in the early 1930s, leaving his wife and two children with little livelihood. To survive, Bianka's mother worked at minimal-wage and non-permanent jobs, selling perfumes during the day and being a cloakroom attendant at a theater in Paris in the evenings. The family lived in extreme poverty [3].

Bianka Tchoubar and her brother began their studies at a Russian school in the 16th arrondissement of Paris, set up by the French government for the children of political migrants from the Russian Empire. It was at this school that Bianka Tchoubar first became interested in the natural sciences, including chemistry, under the tutelage of Mademoiselle Chamier, a Russian chemist who had collaborated extensively with Marie and Pierre Curie in the past. Bianka Tchoubar later reminisced about this teacher as her mother in chemistry [3]. The figure of Marie Curie was extremely important to Bianka Tchoubar; she has repeatedly stated that she had never missed any of Curie's public lectures in Paris [1].

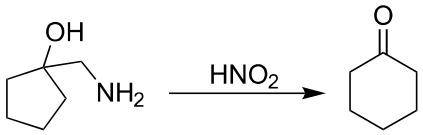
Bianka Tchoubar continued her studies in chemistry, getting admitted to the Sorbonne University in 1929. In 1931, she received the Bachelor of Science Degree and began her research work under the guidance of the then-famous Parisian chemist Professor Paul Freundler, a close friend and colleague of Joseph Achille Le Bel. The choice of the laboratory for her graduate research activities was due to Bianka Tchoubar's interest in asymmetric nitrogen reactions.

In Professor Freundler's group, the young scientist first met with the rejection of her ideas about organic ions by her French colleagues. The conflict of scientific worldviews stemmed from the considerable conservatism of the French scientific community to any new concepts, especially if they came from the so-called "Anglo-Saxon world" [1]. Despite the disagreement of her supervisor, Bianka Tchoubar devoted her Master thesis to the study of charged organic species in tertiary amine reactions with ethyl iodoacetate, which contributed to her Higher Education Degree in Chemistry, awarded in 1932 (*Diplôme d'Études Supérieures de Sciences Chimiques*) [3].

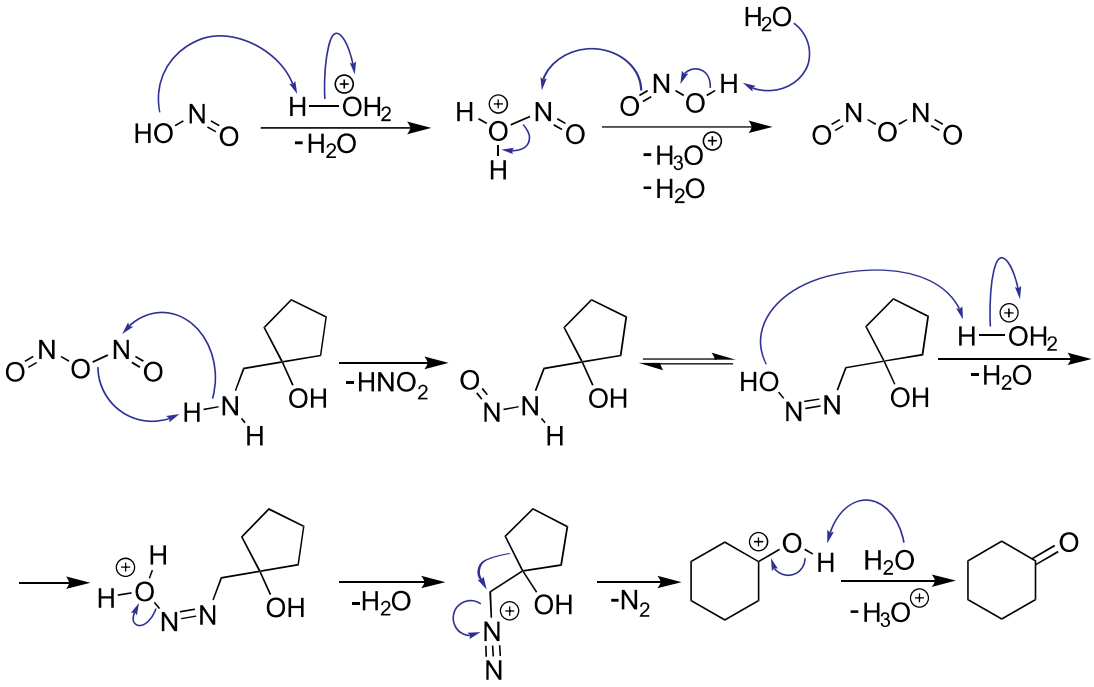
2. Scientific Work with Marc Tiffeneau

In 1933, Bianka Tchoubar began working in the laboratories of the Faculty of Medicine of the University of Paris, under Marc Tiffeneau's supervision. Despite world recognition of his achievements, Marc Tiffeneau had limited recourse to resources in France and was forced to work in the field of pharmaceutical chemistry. He became Bianka Tchoubar's most important mentor. Bianka's first research article on the interaction of Grignard reagents with chlorocyclohexanones was published in 1934 in *Proceedings of the French Academy of Sciences* (*Comptes rendus de l'Académie des Sciences*) [4]. In the laboratory of Marc Tiffeneau, Bianka Tchoubar first met Jeanne Lévy, an organic chemist, who later became one of her research collaborators [3].

In 1937, Bianka Tchoubar established the Laboratory of Organic Chemistry, which studied a variety of atomic transfer reactions. She was appointed also as a trainee researcher at the newly established French National Center for Scientific Research (*Centre national de la recherche scientifique* – CNRS), becoming the first female researcher to enter this institution for science (Figure 1) [2]. She then became interested in molecular rearrangement reactions and proposed a mechanism for nitrite deamination of alicyclic primary amines with ring expansion (Scheme 1) using quantum mechanical theories of chemical bonding. These were considered to be the so-called "English ideas" in France at the time, and viewed as unwelcome. Despite numerous disagreements and criticism of peers, the joint research of Bianka Tchoubar and Marc Tiffeneau made it possible to definitively establish the mechanism of the Tiffeneau–Demjanov rearrangement (or, as it is known in France, the Demjanov–Tiffeneau–Tchoubar rearrangement) (Scheme 2) [5, 6].



Scheme 1. Demjanov–Tiffeneau–Tchoubar rearrangement



Scheme 2. Demjanov–Tiffeneau–Tchoubar rearrangement mechanism

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Bianka Tchoubar's research slowed down significantly in 1939–1945 due to the outbreak of World War II, when she took an active part in the French national resistance to the fascist occupation [2].

The sudden death of Marc Tiffeneau in 1945 further complicated Bianka Tchoubar's scientific career. However, after the war in 1945, Bianka's longtime friend Jeanne Lévy, an Associate Professor of Medicine at the University of Paris at that time, was appointed to establish a new Institute of Medicine in Paris, known today as the Fournier Institute (*Institut Fournier*) [2, 3]. Bianka Tchoubar joined the research department of the newly established Institute, and in 1946 published her thesis "Contribution to the study of alicyclic expansion mechanisms: nitrite deamination of 1-aminomethylcyclohexanols" [2]. Bianka Tchoubar managed to present these radically new ideas in physical organic chemistry while obtaining her doctorate.

The significant delay in defending her doctoral thesis was caused by the political instability in France of 1930s, as well as by the outbreak of World War II. The completion of her PhD was also delayed by the unwillingness of the French scientific community to accept the innovative but "English" concepts Bianka Tchoubar promoted. The young researcher explicitly stated her interest in charged intermediates of organic reactions. Her ambition was to rule the notions of "affinity capacity" and "migratory aptitude" out of organic chemistry as they did not provide satisfactory explanations for the observed patterns in alicyclic expansion reactions. Such ideas of Bianka Tchoubar were severely criticized in Parisian scientific circles of that time [2]. During the defense of the Bianka Tchoubar's thesis, the opponent, French spectroscopist Pauline Ramar-Luca, called the presented explanations of the reaction mechanisms "ephemeral theories". The only supporter of the young scientist's novelties was the chairman of the doctoral jury, Edmond Bauer. The theoretical chemist was impressed by the Bianka's work and accepted her ideas with enthusiasm and encouragement.

The Bianka Tchoubar's doctoral thesis was the first French research work to explain organic reaction mechanisms in terms of mesomerism and the formation of charged intermediates. Bianka Tchoubar was responsible for bringing the widely accepted ideas of Hans Meerwein, who postulated the existence of carbocationic intermediates in the pinacol–pinacolone rearrangement between 1922 and 1927, to French organic chemistry [2].

3. "Anglo-American" Theories and Center No.12 of the French National Center for Scientific Research

French colleagues never refrained from criticism for Bianka Tchoubar's pertinent and witty interpretations of the mechanistic features of chemical reactions "with English accent". Rejection and occasional ridicule of her ideas did not cease even after she was awarded her doctorate degree. It should be noted that French organic chemistry in the twentieth century had a rather strong "chauvinistic inclination" [7]. Achievements of quantum mechanics introduced into chemistry by the American and British chemists led by Linus Pauling and Christopher Ingold were rejected. That the 1912 Nobel Prize in Chemistry that was jointly awarded to two French researchers, Victor Grignard and Paul Sabatier seemed to have an impact to this field of science in France. This gesture of recognition of the French chemical science led to its isolation and Franco-centricity for the decades to come. There was an excessive and sometimes biased emphasis on selected research only because of their performance in France, a politicized approach to research funding and staffing, and deliberate prevention of scientific progress through the introduction of external scientific ideas from the so-called "unfriendly" countries. Moreover, the extremely influential Parisian chemists Charles Prévost and Albert Kirrmann monopolized French organic chemistry in the post-war period. They had been promoting Prévost's theory of organic reactions from their position of power, despite its ridicule at the international level. Prévost's accounts of organic reactions formulated in terms of "syniony" and "metiony", despite their outright inconsistency, had thus become the only acceptable theory in French laboratories and classrooms after World War II. In addition to the anti-British and anti-American sentiments that prevailed in the French society after World War II, as a result of the Vichy regime's propaganda, the French government had also established control over the circulation of English literature, manifested in its artificially limited availability [7].

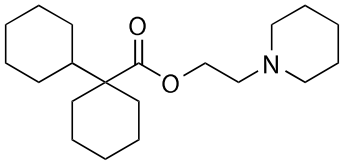
The French reluctance to accept the revolutionary ideas of their British and American counterparts was the main reason of the Bianka Tchoubar's scientific career slowdown at the French National Center for Scientific Research. Due to the inconsistency of political views, her appointment to a full-time research position was delayed until 1955. In 1957–1958, Bianka Tchoubar organized countless seminars, at which

she presented the electronic theories of organic reactions [1–3, 7]. As a summary of seminar reports, in 1960, she wrote a book "*Mechanisms of Organic Reactions*", which was the first French textbook to present the quantum theories of chemical bonding. The famous quotation of Claude Bernard was chosen as the epigraph for this book: "If a theory was considered perfect and was no longer tested, it would have become a dogma" [8, 9]. The book was positively accepted by the world scientific community and translated into six languages [1–3, 7]. The presentation of the already widely accepted quantum-mechanical theories of chemical bonding to the French audience has led to a paradigm shift in the 20th century French organic chemistry [7].

As early as in 1961, Bianka Tchoubar was appointed Director of Research at the French National Center for Scientific Research and headed her first research group at the Institute for Chemistry of Natural Substances in Gif-sur-Yvette, Île-de-France. Bianka Tchoubar's ideas were recognized even more widely in 1968, when she was appointed Director of Research at Center no.12 of the French National Center for Scientific Research in Thiais, Île-de-France. This research group included more than 70 chemists who conducted extensive research in various fields of organic chemistry. It was the largest center for organic chemistry research in France at that time [2].

Scientific research at Center no.12 has focused on the fundamental study of organic reaction mechanisms. Considerable attention was also paid to the synthesis of biologically active substances and drug development. Under the supervision of Bianka Tchoubar at the CNRS Center no.12, the antispasmodic drug *Spasmodex* was developed. The active compound of this drug is dihexyverine (2-(piperidin-1-yl)-ethyl-[1,1'-bi(cyclohexane)]-1-carboxylate) (Figure 2) [3].

Colleagues and students reminisce about Bianka Tchoubar being a gifted research supervisor, whose priority was a friendly atmosphere in the group. Bianka Tchoubar took each and every aspect of the research papers published under the CNRS Center no.12 affiliation as her personal responsibility. For these reasons, no research work in these laboratories was published without prior review by Bianka, even as she did not claim co-authorship in these works, thereby paving the path for young scientists. That is why, in 10 years of managing the laboratories of Center no.12, she agreed to put her name in 5 publications only, instead giving the real distinction to her colleagues [2, 3].



Dihexyverine (Spasmodex)

Figure 2. Dihexyverine structure

Monday seminars of young researchers were another tradition at Center no.12, led by Bianka Tchoubar. Former graduate students of Bianka recall that she appeared to be always shrouded in a thick cloud of cigarette smoke at the seminars. Her presence in the front rows of the auditorium was ubiquitous, where she actively and passionately discussed research results. Not self-serving, this scientist never used her established authority to dominate scientific discussions, which earned her the respect of colleagues [1–3].

Bianka Tchoubar tirelessly incorporated the ideas of quantum chemistry into the interpretation of the results obtained in her own laboratories. Despite the experimental nature of her own research, she was also interested in the achievements of theoretical branches of chemistry, which were developing extremely rapidly in that period [2].

4. Scientific Research in the USSR and Last Years of Life

Bianka Tchoubar first met with Yevhen Shilov at the IUPAC International Congress of Applied Chemistry in Paris in 1957. Dr. Shilov was the Head of the Laboratory of Organic Reaction Mechanisms of the Institute of Organic Chemistry of the Ukrainian Soviet Socialist Republic Academy of Sciences (Figure 3). Having common interests and like-minded scientific views, a deep friendship grew between Bianka Tchoubar and Yevhen Shilov. Likewise, these same commonalities led to the subsequent friendship with his son, Oleksandr Shilov, who later became an academician of the USSR and of the Soviet Academy of Sciences [10]. The friendship with the Shilovs was the impetus for Bianka Tchoubar's research in the field of coordination chemistry.

In 1974, Bianka Tchoubar began studying the reduction of molecular nitrogen in coordination compounds of iron. She conducted such research in collaboration with colleagues and friends from the former Soviet Union, including Yevhen Shilov from the Academy of Sciences of Ukraine, with whom she met several times at the Institute

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Figure 3. Bianka Tchoubar with the Head of the Laboratory of Organic Reaction Mechanisms of the Institute of Organic Chemistry of the Ukrainian Soviet Socialist Republic Academy of Sciences Dr. Yevhen Shilov in Paris (1957).
The picture is reproduced from reference [10]

of Organic Chemistry of the National Academy of Sciences of Ukraine in Kyiv. Much of the research in this period of her life was conducted with the leading Soviet organic chemists Alla and Oleksandr Shilov (Figure 4), as well as with the 1956 Nobel Laureate in Chemistry Nikolai Semenov [1–3]. The results of iron complexes investigation led to amassing of a significant array of data on the salt effect influence on organic reaction kinetics [2, 11].

During her last years at the French National Center for Scientific Research, Bianka Tchoubar actively collaborated with the French spectroscopists Didier Astruc and Georges Bram. Their joint research was dedicated to the study of microwave activation effects on organic reactions, as well as to the organic reactions occurring in the absence of the solvent [3].

Bianka Tchoubar officially retired from the French National Center for Scientific Research in 1978, but never left her love of science behind, continuing to be active in new for her fields of organic chemistry.

In 1981, Bianka Tchoubar was awarded the Louis Jecker Prize (*Prix Jecker*) of the French Academy of Sciences. Interestingly, Bianka's mentor, Marc Tiffeneau, received this award three times: in 1911, 1922 and 1923 [3].

Together with André Loupy in 1988, she wrote a book "*Salt Effects in Organic and Organometallic Chemistry*", which was translated into several foreign languages [2, 11].

The last experiments Bianka Tchoubar conducted were designed to study solvent effects on the competition of S_N2 and $E2$ reactions [2]. Her last paper was a literature review "*Salt Effects as a Result of Ion Vapor Exchange*" published in the *Chemical Review*, co-authored with André Loupy and Didier Astruc [12]. In total, Bianka Tchoubar authored some 140 scientific papers [3].

Bianka Tchoubar passed away on April 24, 1990, and was buried at the Sainte-Geneviève-des-Bois cemetery in the Essonne department, Île-de-France.

■ Books

Mechanisms of organic reactions (1960)

This textbook easily fitting in a lab coat pocket was a huge success and quickly earned the nickname of "The Little Tchoubar".

Figure 4. Bianka Tchoubar with the Shilovs. Left to right: Alla Shilova, Bianka Tchoubar, and Oleksandr Shilov. The figure is reproduced from reference [2]

The first four chapters of the book are dedicated to molecular orbital hybridization, bond polarity and polarizability, inductive and mesomeric electronic effects, and modern theories of acids and bases. These sections reflected the influence of Christopher Ingold's research in the field of organic chemistry [9].

The remaining nine chapters cover some issues of chemical kinetics and transition states, as well as individual aliphatic substitution reactions, elimination and addition reactions, as well as prototropic processes, carbonyl group reactions, and aromatic substitutions [9].

There were several mechanistic interpretations that turned out to be proven wrong with time. In particular, the textbook proposes a carbanion mechanism of deuteroexchange with halogenated substrates, instead of *E*2. However, the overall content of the textbook has withstood the test of time, with such inaccuracies being rare occurrences [13].

Salt effects in organic and organometallic chemistry (1988)

This book was co-authored with André Loupy.

The first chapters of the book discuss the basics of the theory of Lewis acids and bases, ion pairs, salt effects in chemical bond cleavage, electrophilic and nucleophilic induction of heterolytic bond cleavage in halogenated substrates, specific salt effects in S_N2 reactions, salt effects during multiple bonding reactions in ketones, esters, and nitriles, drying effects, bifunctional catalysis and electrophilic addition to carbon-carbon double bonds.

Subsequent sections examine salt control over regioselectivity, stereoselectivity of substitution, addition, and elimination reactions, as well as the means of controlling the chemical equilibrium by salt and solvent effects.

The final section of the book analyzes the role of the aforementioned phenomena in organometallic chemistry [11, 14].

■ Acknowledgments

The author expresses her gratitude to Roald Hoffmann, 1981 Nobel Laureate in Chemistry, Emeritus Professor at Cornell University, and a dear friend of Bianka Tchoubar's, for relating his unique memories about Tchoubar's life and work. Additional thanks go to Professor Mykola Obushak, Head of the Organic Chemistry Department at Ivan Franko National University of Lviv, for supporting the author in the writing of this article.

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Synthesis and the Antimicrobial Activity of Salt Carbenoid Compounds

Abstract

Aim. To synthesize aliphatic and aromatic derivatives of salt carbenoid compounds of the series of imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole containing fluorophenyl, cetyl or adamantyl substituents, and study their antimicrobial (antibacterial and antifungal) activities.

Results and discussion. New derivatives of heterocyclic carbenoid salts and zwitterions based on the imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole heterocyclic systems containing fluorophenyl, cetyl or adamantyl substituents were synthesized. For this purpose, reactions of cyclization of the corresponding diimines with ethoxymethyl chloride (imidazolium salts), quaternization of the corresponding azoles with cetyl bromide or 1-adamantyl bromide in organic solvents (benzimidazolium, pyridinium and 1,3,4-oxadiazolium salts), cyclization of di(1-adamantylamino)alkanes hydrobromides with the orthoformic ester (4,5-dihydroimidazolium and tetrahydropyridinium salts) were used. Zwitterionic compounds were obtained by the reaction of the corresponding azolium salts with phenyl isothiocyanate in the presence of potassium carbonate. Some macrocyclic and adamantyl substituted heterocyclic compounds showed antifungal and antibacterial activities.

Experimental part. The structure of the compounds synthesized was proven by ^{1}H and ^{13}C NMR spectroscopy methods. The antimicrobial activity was studied out by the agar diffusion method to determine diameters of the growth inhibition zones of microorganisms (bacteria and fungi) and by the method of serial dilutions to determine the minimum inhibitory concentration and minimum bactericidal and fungicidal concentrations.

Conclusions. The synthesis of new heterocyclic carbenoid salts and zwitterions based on the imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole heterocyclic systems containing fluorophenyl, cetyl or adamantyl substituents has been performed. Compounds of macrocyclic and adamantyl heterocyclic series with antifungal and antibacterial activities have been found. 1,3-Dicetylimidazolium bromide, macrocyclic *bis*(decylenebenzimidazolium) bromides, azolium-N-phenylthiocarboximides have been proven to be the most active.

Keywords: fluoroaryl, cetyl-, 1-adamantyl substituted heterocyclic salts; antimicrobial activity

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Синтез і антимікробна активність сольових карбеноїдних сполук

Анотація

Мета. Синтезувати аліфатичні й ароматичні похідні сольових карбеноїдних сполук ряду імідазолу, бензімідазолу, піридину, піримідину та 1,3,4-оксадіазолу, що містять флуорофенільні, цетильний або адамантильний замісники, та дослідити їхню антимікробну (антибактеріальну й протигрибкову) активність.

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Результати та їх обговорення. Синтезовано нові похідні гетероциклічних карбеноїдних солей і цвітеріонів на основі систем імідазолу, бензімідазолу, піридину, піримідину та 1,3,4-оксадіазолу, що містять флуорофенільні, цетильний або адамантильний замісники. Для цього застосовано реакції циклізації відповідних діімінів дією етоксиметилхлориду (імідазолієві солі), кватернізації відповідних азолів цетилбромідом або 1-адамантилбромідом в органічних розчинниках (бензімідазолієві, піридинієві та 1,3,4-оксадіазолієві солі), циклізації гідробромідів ді(1-адамантиламіно)-алканів ортоформіатним естером (4,5-дигідроімідазолієві та тетрагідропіримідинієві солі). Цвітеріонні сполуки отримано реакцією відповідних азолієвих солей з фенілізотіоціанатом у присутності калій карбонату. Виявлено речовини макроциклічного й адамантилгетероциклічного ряду з протигрибковою та антибактеріальною активністю.
Експериментальна частина. Будову синтезованих сполук доведено методами ^{1}H та ^{13}C ЯМР-спектроскопії. Антимікробну активність досліджували методом дифузії речовини в агар з визначенням діаметрів зон затримки зростання мікроорганізмів (бактерій і грибів) та методом серійних розведень із визначенням мінімальної інгібувальної та мінімальних бактерицидної і фунгіцидної концентрацій.
Висновки. Здійснено синтез нових гетероциклічних карбеноїдних солей і цвітеріонів на основі систем імідазолу, бензімідазолу, піридину, піримідину та 1,3,4-оксадіазолу, що містять флуорофенільні, цетильний або адамантильний замісники. Виявлено речовини макроциклічного й адамантилгетероциклічного ряду з протигрибковою та антибактеріальною активністю. Найбільш активними виявилися 1,3-дицетилімідазолій бромід, макроциклічні *біс*-децилен-бензімідазолій броміди, азолій-N-фенілтіокарбоксіміди.
Ключові слова: флуороарил-, цетил-, 1-адамантилзаміщені гетероциклічні солі; антимікробна активність

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■ Introduction

Heterocyclic salts have recently attracted researchers' attention with their biological activity (see, for example, a detailed review [1]). Compounds with antimicrobial, antitumor, antiprotozoal and other types of activity were found. The bactericidal activity has been determined for derivatives of ionic liquids [2–6], which are mostly imidazolium salts with one long aliphatic N-substituent. Oligomeric imidazolium salts with the antimicrobial activity are described in the works [1, 7]. The authors of the article have been studying the antimicrobial activity of both organic salts and carbene complexes of silver, copper(I), nickel, cobalt and palladium, and have found a particularly highly active derivatives of adamantyl-containing 1,2,4-triazolium salts [8–12]. In the research [10], a highly active antimicrobial substance belonging to macrocyclic salts of the imidazole series has also been revealed.

This study aims to synthesize aliphatic and aromatic derivatives of a series of imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole with fluorophenyl, cetyl and adamantyl substituents and study their antimicrobial (antibacterial and antifungal) activities. It is also important to compare active carbenoid salts and their methyl-substituted (non-carbenoid) analogs.

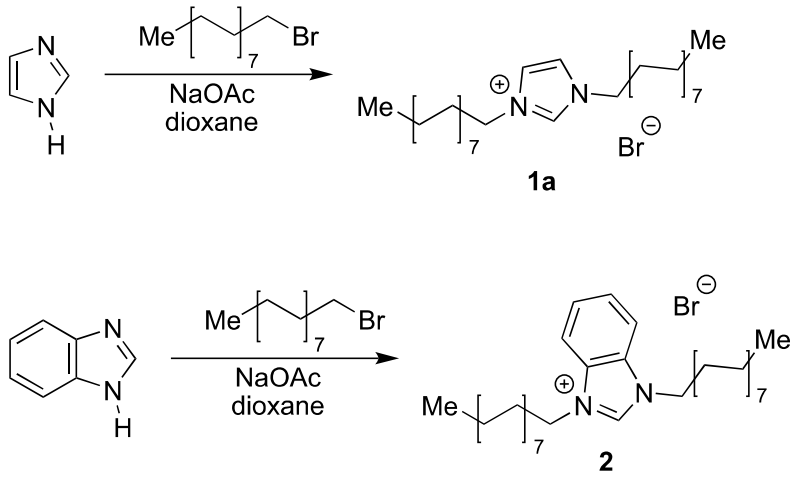
■ Results and discussion

1. The synthesis of imidazolium salts with cetyl and fluorophenyl substituents

A number of known antimicrobial compounds have long aliphatic substituents or fragments in their structure (e.g. 1-cetylpyridinium chloride, undecylenic acid and their derivatives). The effect of aliphatic groups on the antimicrobial activity of these compounds has not been fully elucidated though.

We have synthesized ionic compounds with cetyl substituents based on imidazole and benzimidazole, which are analogs of ionic liquids of the imidazole series. The reactions were carried out with the corresponding azoles and cetyl bromide in dioxane in the presence of sodium acetate. As a result, salts **1a** and **2** were formed with the yields of 40–75% as colorless substances, which themselves might be of interest as potential biologically active compounds (Scheme 1).

The structures of salts **1a** and **2** were confirmed by ^{1}H and ^{13}C NMR spectroscopy. Typical C^2H proton signals in the region of 10.1–10.3 ppm can be found in the ^{1}H NMR spectra of the compounds. The signals of the aliphatic fragment are observed in the region of 0.71–0.82 ppm (CH_3C), 1.08–1.40 ppm (CH_2C), 1.76–1.94 ppm (CH_2CN), 4.20–4.47 ppm (CH_2N). Resonances of imidazole



Scheme 1. The synthesis of cetyl substituted ionic compounds **1a** and **2**

$C^{4,5}H$ protons of compound **1a** are at 7.49 ppm. The ^{13}C NMR spectra of compounds **1a** and **2** contain signals of C^2 carbon atoms in the range of 136.5–142.6 ppm, $C^{4,5}$ atoms of the imidazole ring of compound **1a** at 122.41 ppm. The resonances of aliphatic fragments are at 14.08–14.19 (CH_3C), 22.64–22.75 (C^2H_2C), 26.22–26.63 (C^3H_2C), 29.01–29.76 (other CH_2C), 31.87–31.98 (CH_2CN) and 47.79–49.99 ppm (CH_2N).

The synthesis of fluorine-containing imidazolium salts **1b**,**c** was carried out by the reaction of glyoxal with the corresponding amines and the subsequent cyclization of the diimines **1A** obtained under the action of ethoxymethyl chloride (Scheme 2). The salt yields are low (21–31%).

In the ^1H NMR spectra of salts **1b,c** the characteristic signals of $C^{4,5}H$ protons at 7.87 and 8.43 ppm, and C^2H protons at 10.08–10.35 ppm are observed.

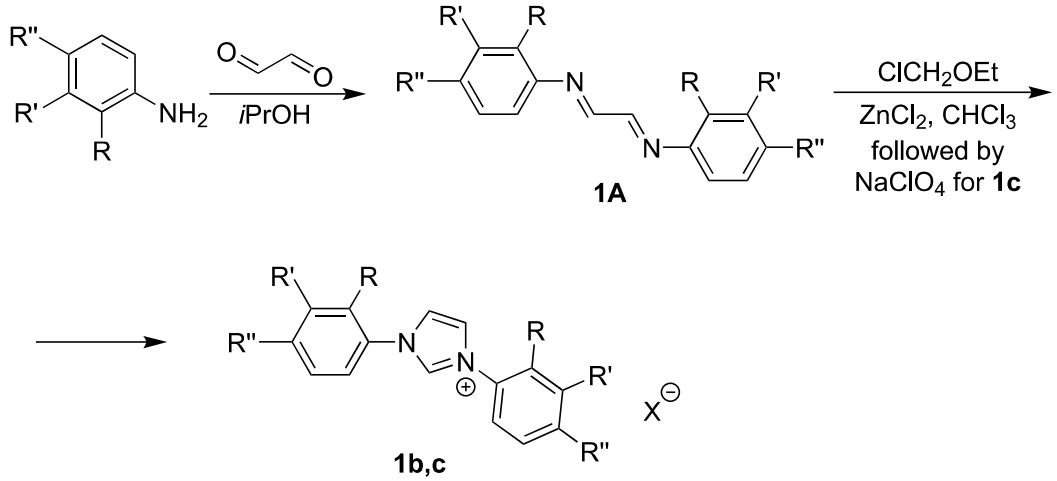
Thus, new imidazolium and benzimidazolium salts with cetyl groups (**1a, 2**) and imidazolium salts with fluorophenyl substituents (**1b,c**) were synthesized.

2. The synthesis of macrocyclic ionic compounds of the imidazole and benzimidazole series

In the work [10], we synthesized macrocyclic ionic compounds from imidazole, which proved to be effective as antimicrobial agents. Therefore, it was promising to synthesize related compounds, particularly from other azoles.

In this section, we describe the synthesis of macrocyclic analogs of the above carbenoids, which were obtained by quaternization of *bis*-azolylalkanes with dihaloalkanes. In this case, decane units were used.

Initial compounds **3A**, **4A** were prepared *in situ* by heating the corresponding benzimidazoles and imidazoles with 1,10-dibromodecane in *o*-dichlorobenzene followed by deprotonation of the *bis*-imidazolylalkane salts obtained by sodium acetate



b: R = R' = R'' = F, X = Cl;
c: R = CF_3, R' = R'' = H; X = ClO_4

Scheme 2. The synthesis of fluorine-containing imidazolium salts

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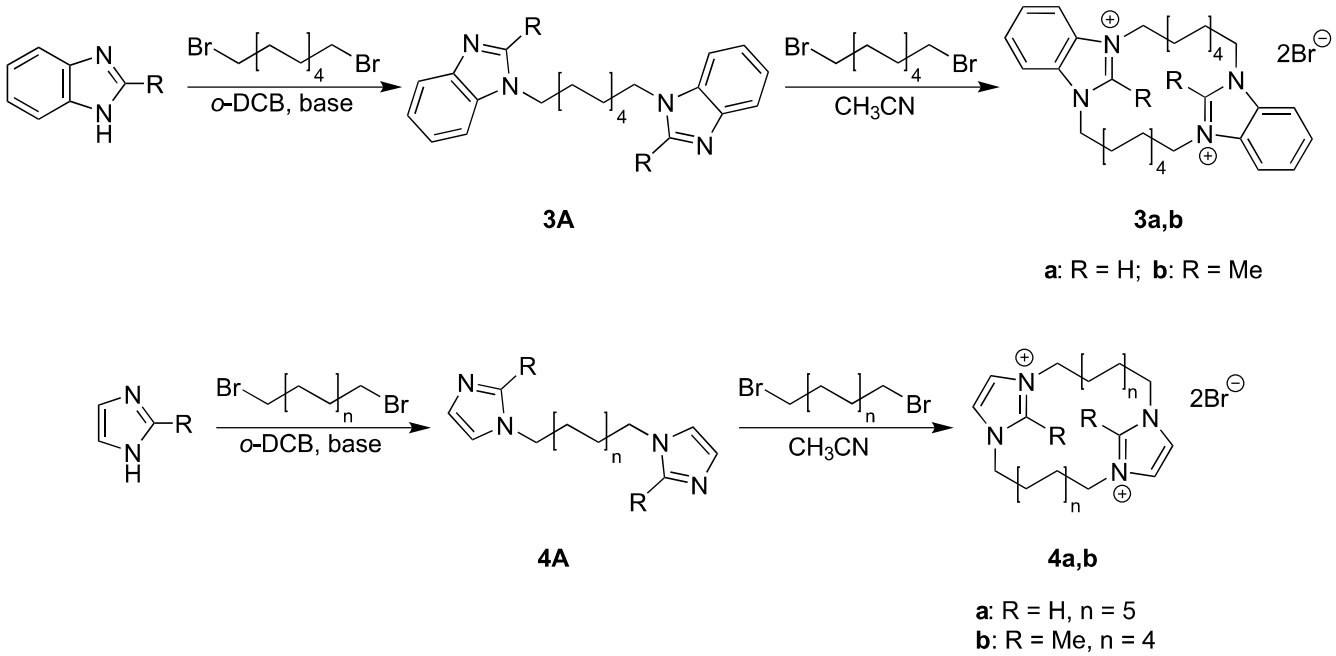


Scheme 3. Formation of macrocyclic salts **3a,b** and **4a,b**

in acetonitrile similarly to the methods of works [10, 11]. The interaction of 1,10-*bis*(1-benzimidazolyl)decanes **3A** with 1,10-dibromodecane in acetonitrile yielded macrocyclic salts **3a,b** with the yields of 93–98% (Scheme 3). Compound **3a** crystallized well from acetonitrile. Compound **4b** was similarly prepared from 2-methylimidazole in the yield of 32%. The latter is analogous to compound **4a** synthesized in the work [10]. Methyl-substituted compounds **3b** and **4b** are hygroscopic.

The ^{1}H NMR spectra of compounds **3a,b**, **4b** contain specific resonances of aliphatic bridges in the ranges of 0.91–1.33 ppm (CH_2C), 1.68–1.73 ppm (CH_2CN), 4.13–4.27 ppm (CH_2N), signals of aromatic protons, and for **3a** proton signal at 10.62 ppm (C^2H). In the ^{13}C NMR spectra of compounds **3a,b**, **4b**, signals of C^2N carbon atoms in the region of 141.78–147.06 ppm, resonances of aliphatic units of CH_2N groups at 47.35–52.22 ppm and other atoms of these units at 26.07–33.96 ppm are observed.

3. The synthesis of adamantyl-containing heterocyclic compounds

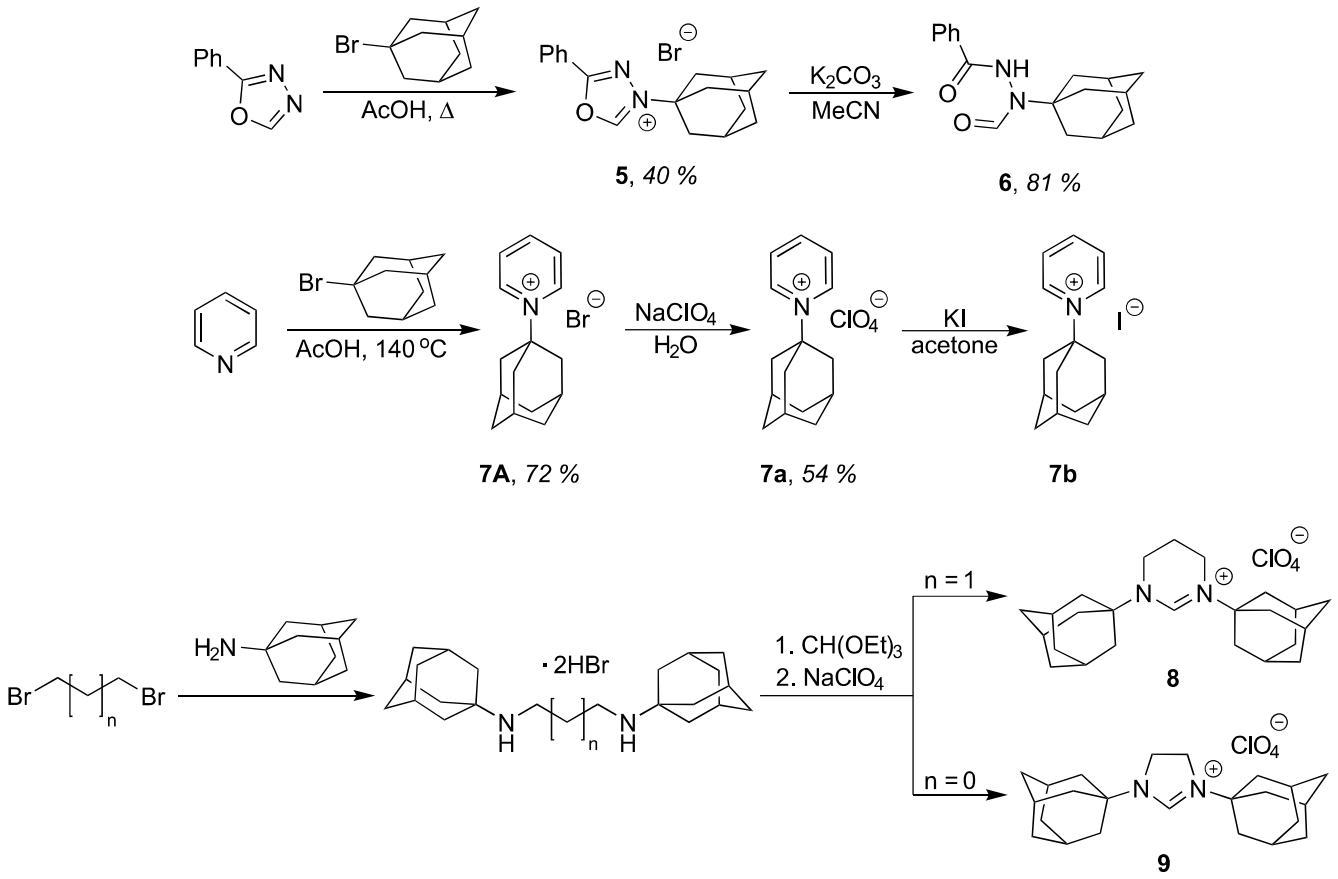
It is well known that adamantane derivatives have been proven to be effective antiviral agents, for instance the influenza A M2 ion channel protein inhibitors rimantadine and amantadine. The latter is also used as an antiparkinsonian agent inhibiting a NMDA-type glutamate receptor, increasing the dopamine release, and blocking the dopamine reuptake. Adamantyl-containing heterocyclic salts and their complexes have already been studied by the authors of the articles [8–10, 12, 13], which allowed to find new effective antimicrobial agents. In this paper, we continue our investigations aiming at synthesizing similar salt systems with the adamantane group.

Thus, we have found out that heating of 2-phenyl-1,3,4-oxadiazole with 1-adamantyl bromide in acetic acid leads to the formation of salt **5** with the yield of 40%, which is very labile under the action of even weak alkalis (potassium carbonate or acetate) and gives the product of hydrolysis of an intermediate carbene (due to the presence of a minute amount of water) – acyclic hydrazide **6** with the yield of 81% (Scheme 4).

The structure of salt **5** was confirmed by ^{1}H NMR spectroscopy. Characteristic signals in the spectrum are the *meso*-proton signal C^2H (11.76 ppm), as well as the resonances of CH_2-protons (1.58 and 1.89 ppm) and CH-protons (2.10 ppm) of the adamantyl ring. Proton signals of aromatic nucleus are observed at 7.63 and 7.65 ppm. Characteristic signals of adamantyl (1.58, 2.00, 2.54 ppm) and formyl (9.79 ppm) protons are observed in the ^{1}H NMR spectrum of compound **6**.

A similar adamantyl derivative **7a** was also obtained by heating pyridine and 1-bromoadamantane in acetic acid, followed by the ion exchange to perchlorate with the yield of 54% (Scheme 4). The subsequent exchange of a perchlorate ion to iodide gives the corresponding salt **7b**.

In the ^{1}H NMR spectrum of compound **7a** proton signals of adamantyl groups at 1.75–2.30 ppm, as well as the resonance of $C^{2,6}H$ protons (9.31 ppm), $C^{3,5}H$-protons (8.16 ppm) and C^4H-proton (8.59 ppm) of the pyridinium cycle are present.



Scheme 4. Synthetic approaches to the adamantyl-containing salts

To study the antimicrobial activity, the six- and five-membered formamidinium salts (tetrahydropyrimidinium **8** and 4,5-dihydroimidazolium **9**) recently described [14, 15] were also obtained by the condensation of the corresponding dibromoalkanes with 1-aminoadamantane and the subsequent cyclization of the intermediate diaminoalkanes with the orthoformic ester (Scheme 4).

Zwitterionic compounds also have an ionic structure although they do not contain external anions. Fluorine-containing zwitterion **10** (94% yield) and for comparison the known compound **11** (81% yield) were both synthesized by *in situ* conversion of the corresponding salts in the reaction with phenylisothiocyanate in the presence of potassium carbonate in acetonitrile at room temperature. Previously, compound **11** was also obtained by the reaction of the corresponding carbene with phenylisothiocyanate [16]. It should be noted that obtaining carbene from salt **1c** is impossible due to its easy dimerization. Only *in situ* the approach was realized (Scheme 5).

4. The antimicrobial activity of the compounds synthesized

In this work, the antimicrobial activity of the compounds synthesized against bacterial strains of *Escherichia coli* 67, *Staphylococcus aureus* 209 P and *Mycobacterium luteum* VKM B-868, as well as fungi strains of *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119, was studied. The study was carried out by two methods [17, 18]: 1) the agar diffusion method to determine diameters of the growth inhibition zones of microorganisms (*Method A*), and 2) the serial dilutions method to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal (MBC) and fungicidal (MFC) concentrations (*Method B*) (see *Experimental part*). The activities of the compounds synthesized were compared to the activity of a known broadly used antimicrobial drug 1-cetylpyridinium chloride **12**, which characteristics are given under the same conditions in the article [16], and with the activity of selected compounds **13**, **14** synthesized in the work [8].

The diameters of the growth inhibition zones of microorganisms are given in Table 1. The results obtained indicate that compounds **3a**, **4b**, **8**, **9** are among the most active in the concentration of 0.5%, but further dilution nullifies the activity. A comparison of the properties of compound **4a** synthesized earlier [10] with the compounds studied, in particular, macrocyclic ones **3a,b** and **4b**, also shows greater activity of the former derivative **4a**. The same can be said about diadamantyl-containing salts **8**, **9**, which

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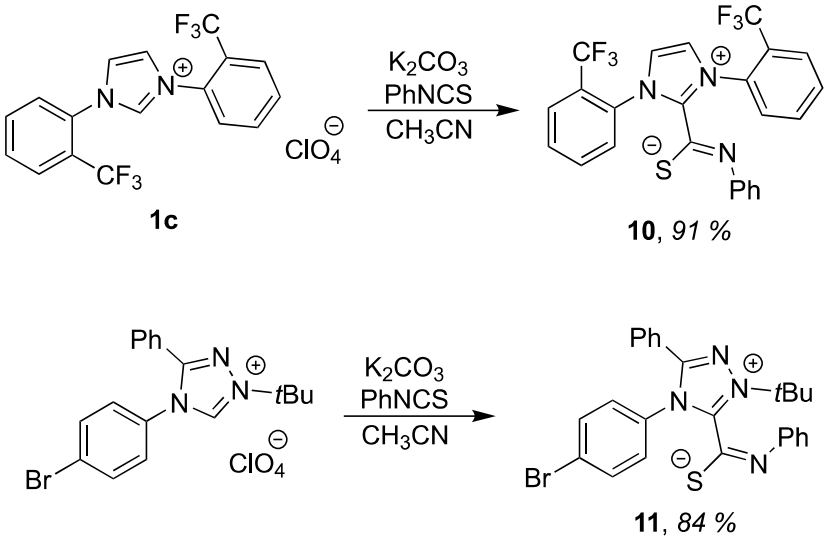
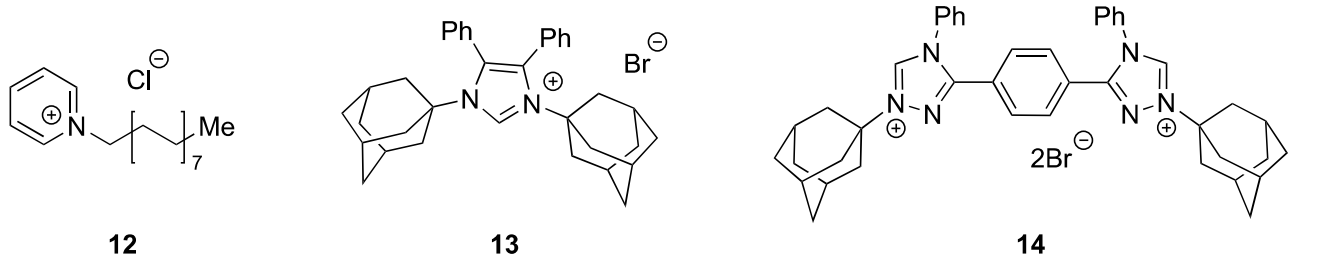


Scheme 5. The synthesis of zwitterionic compounds **10**, **11** from azolium salts

Table 1. The antimicrobial activity determined by the agar well diffusion method (*Method A*)

Compound[a]	Concentration, %	The diameter of the growth inhibition zones[b] (n = 3), mm				
		E. coli 67	*S. aureus* 209 P	*M. luteum* VKM B-868	*C. tenuis* VKM Y-70	*A. niger* VKM F-1119
1a	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
1b	0.5	0	0	0	0	8.0 ± 0.2
	0.1	0	0	0	0	0
1c	0.5	0	0	0	0	7.0 ± 0.1
	0.1	0	0	0	0	0
3a	0.5	0	10.0 ± 0.3	10.0 ± 0.1	0	10.0 ± 0.1
	0.1	0	0	0	0	0
3b	0.5	0	0	0	0	7.0 ± 0.2
	0.1	0	0	0	0	0
4a [10]	0.5	15.4 ± 0.4	21.4 ± 0.2	23.0 ± 0.3	18.0 ± 0.2	9.7 ± 0.2
	0.1	7.0 ± 0.1	15.0 ± 0.2	14.0 ± 0.3	12.0 ± 0.2	6.0 ± 0.1
4b	0.5	8.4 ± 0.2	10.0 ± 0.2	15.0 ± 0.4	10.0 ± 0.2	7.0 ± 0.2
	0.1	0	0	0	0	0
5	0.5	0	0	0	0	10.0 ± 0.1
	0.1	0	0	0	0	0
7a	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
7b	0.5	0	0	0	0	8.0 ± 0.2
	0.1	0	0	0	0	0
8	0.5	0	15.0 ± 0.3	12.0 ± 0.2	0	10.0 ± 0.1
	0.1	0	0	0	0	0
9	0.5	10.0 ± 0.2	15.0 ± 0.4	15.0 ± 0.2	0	0
	0.1	0	0	0	0	0
10	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
11	0.5	0	0	0	0	7.0 ± 0.1
	0.1	0	0	0	0	0
12 [10]	0.5	0	0	14.4 ± 0.3	0	10.0 ± 0.2
	0.1	0	0	12.0 ± 0.2	0	7.0 ± 0.1
13 [8]	0.5	0	22.3 ± 0.3	39.3 ± 0.2	0	15.6 ± 0.3
	0.1	0	19.6 ± 0.2	32.3 ± 0.3	0	10.0 ± 0.1
14 [8]	0.5	11.3 ± 0.2	23.6 ± 0.4	35.6 ± 0.2	0	0
	0.1	0	16.0 ± 0.2	24.0 ± 0.3	0	0

Notes: [a] compound **2** could not be studied due to its low solubility; [b] control values correspond to 0 mm



12 **13** **14**

Figure. The known compounds with a significant antimicrobial activity

are less active than diadamantyl-containing salts **13**, **14** (Figure) synthesized in [8].

Table 2 shows the data of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the compounds synthesized against the bacterial strains determined by the method of serial dilutions (*Method B*).

As one can see from Table 2, for most compounds the antibacterial activity is low or absent in the concentrations studied. But macrocyclic compounds **3a,b** have good indicators of both MIC and MBC (not more than 62.5 μg mL^{-1}). The activity of compound **3a**, for which the MIC reaches 7.8 μg mL^{-1}, and MBC 15.6 μg mL^{-1} on the culture of *M. luteum*, is particularly high. For compound **11**, the MIC observed is 7.8 μg mL^{-1}. It should be noted that in most cases, a high activity of the compounds studied is observed for only one culture – *M. luteum*. For comparison, the activity of compound **4a** [10] previously synthesized is much higher (MIC and MBC reaches 3.9 μg mL^{-1} against the *E. coli* and *M. luteum* cultures). Compared to the activity of compound **12** (MIC 3.9 and 7.8 μg mL^{-1} and MBC 7.8 and 15.6 μg mL^{-1} on the cultures of *S. aureus* and *M. luteum*, respectively), the related imidazolium salt **1a** showed a substantially lower antimicrobial action.

Table 3 shows similar indicators of MIC and MFC determined by the *Method B* on the cultures of fungi *C. tenuis* and *A. niger*.

As can be seen from these data, a sufficiently high activity is observed for compound **1a** on the *C. tenuis* culture (MIC 15.6 μg mL^{-1}, MFC 31.2 μg mL^{-1}), however, these values indicate a slightly lower fungicidal effect than that for pyridinium salt **12** (MIC 3.9 μg mL^{-1}, MFC 7.8 μg mL^{-1}) and especially for macrocyclic salt **4a** (MIC 1.9 μg mL^{-1}, MFC 3.9 μg mL^{-1}). The culture of *C. tenuis* is more sensitive to the action of carbenoid compound **3a** (MIC 7.8 μg mL^{-1}, MFC 15.6 μg mL^{-1}) compared to that of non-carbenoid compound **3b** (MIC 31.2 μg mL^{-1}, MFC 62.5 μg mL^{-1} against the culture of *C. tenuis* and MIC 62.5 μg mL^{-1} against the culture of *A. niger*).

Table 2. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)[a],[b] of the compounds determined by the serial dilutions method (*Method B*)

Compound[c]	Bacteria cultures					
	E. coli 67		*S. aureus* 209 P		*M. luteum* VKM B-868	
	MIC, μg mL^{-1}	MBC, μg mL^{-1}	MIC, μg mL^{-1}	MBC, μg mL^{-1}	MIC, μg mL^{-1}	MBC, μg mL^{-1}
1a	+	+	250.0	500.0	250.0	500.0
1b	+	+	+	+	250.0	500.0
1c	+	+	250.0	500.0	125.0	500.0
3a	+	+	+	+	7.8	15.6
3b	+	+	+	+	31.2	62.5
4a [10]	3.9	3.9	31.2	62.5	3.9	3.9
4b	+	+	+	+	250.0	500.0
5	+	+	+	+	+	+
7a	+	+	+	+	250.0	500.0
7b	+	+	250.0	500.0	250.0	500.0
8	+	+	31.2	62.5	31.2	62.5
9	125.0	250.0	31.2	62.5	15.6	62.5
10	+	+	+	+	+	+
11	+	+	+	+	7.8	N[d]
12 [10]	31.2	125	3.9	7.8	7.8	15.6

Notes: [a] "+" means no antibacterial effect was observed in the concentrations studied (growth of the microorganisms); [b] control values correspond to "+"; [c] Compound **2** could not be tested due to its low solubility; [d] no indicator of bactericidal effect was found in the concentrations studied

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Table 3. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC)[a],[b] of the compounds determined by method of serial dilutions (*Method B*)

Compound[c]	Fungi cultures			
	C. tenuis VKM Y-70		*A. niger* VKM F-1119	
	MIC, μg mL^{-1}	MFC, μg mL^{-1}	MIC, μg mL^{-1}	MFC, μg mL^{-1}
1a	15.6	31.2	+	+
1b	+	+	250.0	500.0
1c	125.0	250.0	500.0	N[d]
3a	7.8	15.6	125.0	250.0
3b	31.2	62.5	62.5	250.0
4a [10]	1.9	3.9	3.9	62.5
4b	500.0	N	500.0	N
5	+	+	500.0	N
7a	+	+	+	+
7b	250.0	500.0	250.0	N
8	250.0	500.0	500.0	N
9	125.0	250.0	+	+
10	+	+	+	+
11	+	+	62.5	N
12 [10]	3.9	7.8	7.8	62.5

Notes: [a] "+" means no antifungal effect was observed in the concentrations studied (growth of the microorganisms was observed); [b] control values correspond to "+"; [c] compound **2** could not be tested due to its low solubility; [d] no indicator of fungicidal effect was found in the concentrations studied

Zwitterion **10** also noticeably inhibits the growth of *A. niger* (MIC 62.5 μg mL^{-1}).

Thus, we have found new compounds **1a**, **3a,b**, **10**, **11** with the antimicrobial activity, which can be used as a basis for new improved series of compounds for biological research.

Conclusions

The synthesis of new heterocyclic carbenoid salts and zwitterions based on the imidazole, benzimidazole, pyridine, pyrimidine and 1,3,4-oxadiazole heterocyclic systems containing fluorophenyl, cetyl or adamantyl substituents has been performed. Compounds of macrocyclic and adamantyl heterocyclic series with antifungal and antibacterial activities have been found. 1,3-Dicetylimidazolium bromide, macrocyclic *bis*(decylenebenzimidazolium) bromides, azolium-N-phenylthiocarboximides have been proven to be the most active.

Experimental part

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance II 400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectra) in DMSO-d_6 or $CDCl_3$ solution. The ^{1}H NMR and ^{13}C NMR chemical shifts are reported relative to tetramethylsilane (TMS) (solution). To assess purity of the compounds synthesized, thin-layer chromatography was performed on silica gel with chloroform or the mixture of chloroform and methanol (10:1) as an eluent, followed by development with iodine. Melting points were measured on a Boethius chair (Nagema, Germany). The elemental analysis was performed in the analytical laboratory of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine. Commercial solvents and reagents were used in the syntheses, except specially indicated cases.

1,3-Dicetylimidazolium bromide (1a)

The mixture of imidazole (0.68 g, 10 mmol, 1.0 equiv) and hexadecyl bromide (7.32 g, 24 mmol, 2.4 equiv) in anhydrous dioxane (3 mL) was stirred at 100°C for 1 h. Then anhydrous sodium acetate (0.821 g, 10 mmol, 1.0 equiv) was added to the solution and stirred at 100°C for 16.5 h. The precipitate of inorganic salts was filtered off. The solution was heated to boiling and cooled to room temperature. A colorless precipitate formed was filtered off, washed with hexane and dried.

Yield – 4.47 g (75%). M. p. 65°C. Anal. Calcd for $C_{35}H_{69}BrN_2$, %: C 70.32; H 11.63; Br 13.37; N 4.69. Found, %: C 70.40; H 11.65; Br 13.29; N 4.67. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.71 (6H, s, 2×CH_3C); 1.08 (52H, m, 26×CH_2C); 1.76 (4H, s, 2×CH_2CN); 4.20 (4H, s, 2×CH_2N); 7.49 (2H, s, C^{4,5}H$_{Im}$); 10.07 (1H, s, C^{2}HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 14.08 (CH_3C); 22.64 ($C^{2}H_2$C); 26.22 ($C^{3}H_2$C); 29.01 ($C^{4}H_2$C); 29.32 ($C^{5}H_2$C); 29.40 ($C^{6}H_2$C); 29.51 ($C^{7}H_2$C); 29.60, 29.62, 29.63, 29.66 ($C^{8\text{-}13}H_2$C); 30.32 (CH_2CCN);

31.87 (*C*H_2CN); 49.99 (*C*H_2N); 122.41 ($C^{4,5}_{Im}$); 136.48 (C^2N).

1,3-*Bis*(2,3,4-trifluorophenyl)imidazolium chloride (1b)

Step 1. N,N'-Bis(2,3,4-trifluorophenyl)glyoxaldiimine. The solution of 2,3,4-trifluoroaniline (4.9 g, 33.3 mmol) and 40% glyoxal solution (4.83 g, 33.3 mmol) in 20 mL of isopropyl alcohol was stirred at room temperature for 7 days. The solvent was evaporated, and the resulting residue containing diimine **1A** was used without purification in the next step.

Step 2. The cyclization reaction. Anhydrous zinc chloride (4.09 g, 30 mmol) and ethoxymethyl chloride (5.67 g, 60 mmol) were added to the solution of diimine **1A** obtained in the previous step, in chloroform (50 mL) and stirred at room temperature for 3 days. The solution was evaporated, and the organic salt was extracted with hot water (100 mL). The water solution was evaporated to a small volume. A colorless precipitate formed was filtered off and dried.

Yield – 3.1 g (27% based on the starting aniline). When conducting the experiment at a ratio of aniline/glyoxal of 2:1 the salt yield was 31%. M. p. 244–246°C (water). Anal. Calcd for $C_{15}H_7ClF_6N_2$, %: C 49.40; H 1.93; Cl 9.72; N 7.68. Found, %: C 49.52; H 1.89; Cl 9.69; N 7.63. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 7.58–7.65 (2H, m, ArH); 7.92–7.97 (2H, m, ArH); 8.43 (2H, s, $C^{4,5}$H); 10.35 (1H, s, C^2HN).

1,3-*Bis*(2-trifluoromethylphenyl)imidazolium perchlorate (1c)

Step 1. N,N'-Bis(2-trifluoromethylphenyl)glyoxaldiimine. 40% Solution of glyoxal (4.85 g, 33.4 mmol) was added to the solution of 2-trifluoromethylaniline (4.66 g, 28.89 mmol) in isopropyl alcohol (18 mL). The mixture was stirred for 5 days at room temperature, the solution was evaporated and the resulting residue containing diimine **1A** was used without purification for the synthesis of salt **1c**.

Step 2. The cyclization reaction. Anhydrous zinc chloride (1.98 g, 14.5 mmol) and ethoxymethyl chloride (1.88 g, 21.7 mmol) were added successively to a solution of diimine **1A** in chloroform (40 mL), and the mixture was stirred at room temperature for 3 days. The solution was evaporated. The residue was extracted with hot water. The excess of sodium perchlorate was added to the water solution, and the colorless precipitate was filtered off.

Yield – 2.66 g (50% based on the starting aniline). M. p. 255–257°C (water). Anal. Calcd for $C_{17}H_{11}ClF_6N_2O_4$, %: C 44.71; H 2.43; Cl 7.76; N 6.13. Found, %: C 44.65; H 2.44; Cl 7.81; N 6.17. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 7.84–7.90 (3H, m, ArH); 7.92–7.95 (3H, m, ArH); 8.08–8.10 (2H, m, ArH); 8.14 (2H, d, J = 8.0 Hz, $C^{4,5}$HN); 10.08 (1H, s, C^2HN).

1,3-Dicetylbenzimidazolium bromide (2)

The mixture of benzimidazole (1.18 g, 10 mmol), cetyl bromide (6.41 g, 21 mmol) and sodium acetate (0.82 g, 10 mmol) in anhydrous dioxane (4 mL) was stirred at 100°C for 4 h. The solution was filtered from the inorganic precipitate in a hot state and evaporated to give colorless salt **2**, which was recrystallized from acetonitrile.

Yield – 2.6 g (40%). M. p. 116–118°C (acetonitrile). Anal. Calcd for $C_{39}H_{71}BrN_2$, %: C 72.30; H 11.05; Br 12.33; N 4.32. Found, %: C 72.42; H 11.03; Br 12.26; N 4.29. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.82 (6H, t, J = 6.8 Hz, 2×CH_3C); 1.18–1.20 (48H, m, 24×CH_2C); 1.24–1.40 (4H, m, 2×CH_2C); 1.94 (4H, t, J = 6.4 Hz, 2×CH_2CN); 4.47 (4H, t, J = 6.8 Hz, 2×CH_2N); 7.61–7.64 and 7.68–7.71 (4H, m, ArH); 11.31 (1H, s, C^2HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 14.19 (*C*H_3C); 22.75 (*C*H_2C); 26.63 (*C*H_2C); 29.13; 29.42; 29.46; 29.58; 29.62; 29.66; 29.72; 29.76 (*C*H_2C); 31.98 (*C*H_2CN); 47.79 (*C*H_2N); 113.21 (*ipso*-C); 127.20 ($C^{5,6}$); 131.28 ($C^{4,7}$) (Ar); 142.64 (C^2).

1,3-*Bis*(1,10-decylenebenzimidazolium) bromide (3a)

The solution of benzimidazole (1.50 g, 12.72 mmol) and 1,10-dibromodecane (1.91 g, 6.36 mmol) in *o*-dichlorobenzene (4 mL) was stirred at 130°C for 8 h, then anhydrous sodium acetate (1.04 g, 12, 72 mmol) was added, and the stirring was continued under the same conditions for 4 h. A precipitate was filtered off, the mother liquor containing 1,10-di(benzimidazol-1-yl)decane of type **3A** with the additional portion of 1,10-dibromodecane (1.91 g, 6.36 mmol) was stirred at 130°C for 8 h. Then acetonitrile (10 mL) was added, and the solution was refluxed for 24 h. A colorless precipitate was filtered off, washed with acetonitrile and hexane, dried and recrystallized from acetonitrile.

Yield – 4.0 g (93%). M. p. 122–124°C (acetonitrile). Anal. Calcd for $C_{34}H_{50}Br_2N_4$, %: C 60.54; H 7.47; Br 23.69; N 8.31. Found, %: C 60.68; H 7.41; Br 23.58; N 8.34. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 0.91 (10H, m, 5×CH_2C); 1.01 (14H, m, 7×CH_2C); 1.68 (8H, s, 4×CH_2CN); 4.27 (8H, s, 4×CH_2N); 7.34 (4H, s, $ArH^{5,6}$); 7.55 (4H, s, $ArH^{4,7}$); 10.62 (2H, s, C^2HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 26.07, 28.49, 28.76, 29.94 (*C*H_2C, *C*H_3C);

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47.35 (CH_2N); 113.33 ($C^{4,7}$, Ar); 127.12 ($C^{5,6}$, Ar); 131.11 (*ipso*-C, Ar); 141.78 (C^2N).

1,3-*Bis*(1,10-decylene-2-methylbenzimidazolium) bromide (3b)

The solution of 1,10-*bis*(2-methylbenzimidazol-1-yl)decane of type **3A** obtained from 2-methylbenzimidazole (1.64 g, 12.46 mmol) and 1,10-dibromodecane (1.87 g, 6.23 mmol), similarly to the preparation of salt **3a**, was washed by hexane (15 mL) threefold, another portion of 1,10-dibromodecane (1.87 g, 6.23 mmol) in acetonitrile (8 mL) was added and refluxed for 8 h. Then another portion of acetonitrile (10 mL) was added, and the solution was refluxed for 24 h. The mother liquor was evaporated, and a colorless solid residue was dried.

Yield – 4.29 g (98%). M. p. 167–170°C. Anal. Calcd for $C_{36}H_{54}Br_2N_4$, %: C 61.54; H 7.75; Br 22.74; N 7.97. Found, %: C 61.64; H 7.76; Br 22.69; N 7.92. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 1.01–1.49 m (24H, $12CH_2C$); 1.81 (6H, s, $2\times CH_3C$); 3.06 (8H, s, $4\times CH_2CN$); 4.47 (8H, s, $4\times CH_2N$); 7.50–7.80 (4H, m, ArH); 7.80–8.04 (4H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 31.14, 33.67, 33.96, 33.04 (CH_2C+CH_3C); 52.22 (CH_2N); 118.58; 131.93; 136.23; 147.06 (C^2N).

1,3-*Bis*(1,10-decylene-2-methylimidazolium) bromide (4b)

The solution of 1,10-*bis*(2-methylimidazol-1-yl)decane of type **4A** obtained from 2-methylimidazole (1.02 g, 12.46 mmol) and 1,10-dibromodecane (1.87 g, 6.23 mmol) similarly to the preparation of salt **3A** was washed by hexane (15 mL) threefold, another portion of 1,10-dibromodecane (1.87 g, 6.23 mmol) in acetonitrile (8 mL) was added, and the mixture obtained was refluxed for 8 h. The resulting solution was evaporated to dryness, and an oily colorless residue was dried and solidified while standing.

Yield – 1.20 g (32%). M. p. 124–127°C. Anal. Calcd for $C_{28}H_{50}Br_2N_4$, %: C 55.82; H 8.36; Br 26.52; N 9.30. Found, %: 55.88; H 8.32; Br 26.60; N 9.20. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 1.20 (10H, m, $5\times CH_2C$); 1.25 (6H, m, $3\times CH_2C$); 1.73 (8H, m, $4\times CH_2C$); 2.66 (6H, s, $2\times CH_3C$); 3.17 (8H, s, $4\times CH_2CN$); 4.13 (8H, s, $4\times CH_2N$); 7.60 (4H, s, $C^{4,5}HN$). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 9.87 (CH_3C); 25.78, 28.47, 28.72, 29.40 (CH_2C); 48.18 (CH_2N); 121.39 ($C^{4,5}$); 142.63 (C^2N).

4-(1-Adamantyl)-2-phenyl-1,3,4-oxadiazolium bromide (5)

The solution of 2-phenyl-1,3,4-oxadiazole (2.93 g, 20 mmol) and 1-bromoadamantane (4.73 g, 22 mmol) in glacial acetic acid (3 mL) was stirred at 120°C for 1 day. The mixture of methyl *tert*-butyl ether/acetic acid (10:1) (10 mL) was added to the solution, and a colorless precipitate formed was filtered off and dried.

Yield – 2.9 g (40%). M. p. > 250°C. Anal. Calcd for $C_{18}H_{21}BrN_2O$, %: C 59.84; H 5.86; Br 22.12; N 7.75. Found, %: C 59.72; H 5.88; Br 22.20; N 7.77. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.58 (6H, m, CH_2 Ad); 1.89 (6H, m, CH_2 Ad); 2.10 (3H, m, CH Ad); 7.55 (2H, dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, ArH); 7.65 (1H, dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, ArH); 7.95 (2H, d, J = 7.6 Hz), 11.76 (1H, s, C^5HN).

1-(1-Adamantyl)-1-formyl-2-benzoylhydrazine (6)

Anhydrous potassium carbonate (0.70 g, 1.94 mmol) was added to a solution of salt **5** (0.3 g, 0.83 mmol) in acetonitrile (2 mL) and stirred at 35–40°C for 12 h. The solution was filtered from inorganic substances and evaporated to dryness to give a colorless compound **6**.

Yield – 0.2 g (81%). M. p. 154–156°C. Anal. Calcd for $C_{18}H_{22}N_2O_2$, %: C 72.46; H 7.43; N 9.39. Found, %: C 72.38; H 7.40; N 9.50. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.48–1.66 (12H, m, CH_2 Ad); 2.00 (3H, m, CH Ad); 7.46 (2H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, ArH); 7.50 (1H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, ArH); 7.84 (2H, d, J = 7.2 Hz, ArH); 9.79 (1H, s, CHO), NH (in exchange).

1-(1-Adamantyl)pyridinium perchlorate (7a)

Anhydrous pyridine (0.8 mL, 10 mmol) was added to a suspension of 1-bromoadamantane (2.15 g, 10 mmol) in acetic acid (2 mL). The mixture was heated at 140°C for 24 h under the nitrogen atmosphere and cooled to room temperature. Acetic acid was extracted with hexane, the precipitate was triturated with hexane and then with methyl *tert*-butyl ether. The precipitate (2.12 g, 72%) of bromide **7A** was filtered off, dissolved by heating in water (5 mL), and filtered after the treatment with activated carbon. The excess of sodium perchlorate (1.47 g, 12 mmol) was added to the hot solution. After cooling, a colorless precipitate was filtered off and dried.

Yield – 1.59 g (54%). M. p. 238–240°C. Anal. Calcd for $C_{15}H_{20}ClNO_4$, %: C 57.42; H 6.42; Cl 11.30; N 4.46. Found, %: C 57.35; H 6.40; Cl 11.41; N 4.44. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.75 (6H, s, CH_2 Ad); 2.30 (9H, s, CH_2+CH Ad); 8.16 (2H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, $C^{3,5}H_{pyr}$); 8.59 (1H, dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, C^4H_{pyr}); 9.31 (2H, d, J = 7.2 Hz, $C^{2,6}H_{pyr}$).

1-(1-Adamantyl)pyridinium iodide (7b)

The salt was obtained by the exchange of ions from perchlorate **7a** and potassium iodide in acetone.

Yield – 94%. M. p. 249–250°C. Anal. Calcd for $C_{15}H_{20}IN$, %: C 52.80; H 5.91; I 37.19; N 4.10. Found, %: C 52.87; H 5.90; I 37.10; N 4.13. The compound has similar spectral characteristics to perchlorate **7a**.

1,3-*Bis*(2-trifluoromethylphenyl)imidazolium-2-(N-phenylthiocarboximide) (10)

The mixture of 1,3-*bis*(2-trifluoromethylphenyl)imidazolium perchlorate (**1c**) (0.30 g, 0.66 mmol) and anhydrous potassium carbonate (0.182 g, 1.32 mmol, 2 equiv) in anhydrous acetonitrile (3 mL) was stirred at room temperature under the nitrogen atmosphere for 10–15 min, phenyl isothiocyanate (0.08 mL, 0.66 mmol, 1 equiv) was then added, and the mixture was stirred at room temperature for 20 h. The precipitate of inorganic salts was filtered off and washed with hot anhydrous acetonitrile. The mother liquor was evaporated *in vacuo*, the residue was triturated with hexane. A pale yellow precipitate was filtered off, washed with hexane and dried.

Yield – 0.29 g (91%). M. p. 165°C. Anal. Calcd for $C_{24}H_{15}F_6N_3S$, %: C 58.66; H 3.08; N 8.55; S 6.52. Found, %: C 58.85; H 3.01; N 8.49; S 6.46. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 6.41 (2H, s, $C^{4,5}H_{Im}$); 6.73 (1H, t, J = 6.4 Hz, ArH); 6.96 (2H, t, J = 6.4 Hz, ArH); 7.53–7.94 (10H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 120.81; 121.50; 123.60; 126.33; 126.97; 127.57; 130.10; 130.82; 132.04; 132.53; 147.71 (*ipso*-C, PhN); 150.89 (C^2N); 162.77; 163.79 (N*C*S).

1-*tert*-Butyl-3-phenyl-4-(4-bromophenyl)-1,2,4-triazolium-5-(N-phenylthiocarboximide) (11)

A mixture of 1-*tert*-butyl-4-(4-bromophenyl)-3-phenyl-1,2,4-triazolium perchlorate (0.30 g, 0.66 mmol) and anhydrous potassium carbonate (0.192 g, 1.39 mmol, 2.11 equiv) in anhydrous acetonitrile (3 mL) was stirred under nitrogen atmosphere at room temperature for 10 min, and then phenyl isothiocyanate (0.08 mL, 0.66 mmol, 1 equiv) was added. The mixture was additionally stirred at room temperature for 4 h. A precipitate of inorganic salts was filtered off and washed with anhydrous acetonitrile. The filtrate was evaporated *in vacuo*, the solid residue was triturated with hexane. A pale yellow precipitate was filtered off, washed with hexane and dried.

Yield – 0.27 g (84%). M. p. 198–199°C (benzene). Anal. Calcd for $C_{25}H_{23}BrN_4S$, %: C 61.10; H 4.72; Br 16.26; N 11.40; S 6.52. Found, %: C 61.31; H 4.54; Br 16.22; N 11.44; S 6.49. ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 2.00 (9H, s, *t*Bu); 7.02–7.57 (14H, m, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz), δ, ppm: 28.7 (CH_3C, *t*Bu); 67.4 (*ipso*-C, *t*Bu); 121.4; 123.7; 129.2; 128.6; 128.8; 129.1; 131.1; 131.9; 123.1; 125.4; 132.8; 149.9; 150.6; 150.0 (C^3_{triaz}), 166.5 (N*C*S).

Procedures for assessing the antimicrobial activities of the compounds synthesized

Method A. 0.5% and 0.1% solutions of the test substances in DMSO were prepared and introduced to the culture medium. The antimicrobial activity of the compounds synthesized was studied on test bacteria cultures of *Escherichia coli* 67, *Staphylococcus aureus* 209 P and *Mycobacterium luteum* VKM B-868 and fungi *Candida tenuis* VKM Y-70 and *Aspergillus niger* VKM F-1119 by the agar diffusion method on a solid nutrient medium – meat-peptone agar (MPA) for bacterial strains and wort agar (WA) for fungi. The microbial load was 10^9 colony-forming units (CFU) in 1 mL. The 0.5 McFarland standard test of turbidity was used to make the bacterial suspension. Counting of cells (spores) of fungi was carried out in the Goryaev's chamber. The duration of incubation of bacteria was 24 h at 35°C, fungi – 48–72 h at 28–30°C. The degree of the activity of the compounds studied was assessed by the diameters of the growth inhibition zones for test cultures of microorganisms, assuming that at a diameter of 11–15 mm a microorganism is insensitive to the drug, it is sensitive at 16–25 mm, and is highly sensitive at > 25 mm. Each experiment was repeated thrice.

Method B. The minimum inhibitory (MIC), bactericidal (MBC) and fungicidal (MFC) concentrations were determined by the serial dilution method in a liquid nutrient medium. The initial solution of a substance was prepared in DMSO in the concentration of 10000 μg mL^{-1}. The solution was then two-fold serially diluted with DMSO, and 0.1 mL of each dilution was then transferred to tubes and diluted to the volume of 1 mL with the nutrient medium reaching a concentration of the substance from 0.9 to 500 μg mL^{-1}. The meat peptone broth was used as a nutrient medium for bacteria and the untouched beer wort of 6–8°Blg – for fungi. Bacterial and fungal inocula were sown in the culture medium (the microbial load – 10^6 CFU in 1 mL). The seeded tubes were kept in a thermostat at the appropriate temperature (37°C – for the bacterial strains; 30°C – for fungal strains) for 24–72 h. The results were evaluated for the presence or absence of growth of microorganisms, the visual inspection was performed in transmitted light, comparing the degree

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of microbial turbidity of the nutrient medium with the "negative control".

To determine the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) from tubes, in which the medium solutions were visually transparent, 0.02 mL of the medium was taken and applied to a sterile MPA (for bacterial strains) or WA (for fungal strains) in sterile Petri dishes incubated in a thermostat. The results were evaluated for testing bacteria in 24 h, for testing fungi in 48–72 h. In the absence of growth of the microorganism colonies on the incubated Petri dishes, MBC or MFC of the test substance was determined. Each experiment was repeated thrice.

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1,2,3-Triazole-4(5)-amines – Convenient Synthetic Blocks for the Construction of Triazolo-Annulated Heterocycles

Abstract

Aim. To analyze and summarize the synthetic potential of 1,2,3-triazole-4(5)-amines as efficient building blocks in the synthesis of triazolo-annulated pyridine, azine and azepine systems.

Results and discussion. Original literature sources revealing the synthetic potential of 4(5)-amino functionalized 1,2,3-triazoles as convenient and available building blocks for the preparation of triazolo-annulated pyridines, azines and azepines were analyzed and systematized. Condensation of 1,2,3-triazole-4(5)-amines with methylene active compounds was shown to be a powerful tool for the synthesis of versatile triazolo[4,5-*b*]pyridines. In turn, the cyclocondensation based on 5-amino-1,2,3-triazole-4-carboxylic acids and their structurally modified derivatives was proven to be a general way for obtaining a number of triazolo[4,5-*d*]pyrimidine systems. Few representatives of triazolo-annulated pyridazines, 1,3-oxazines and 1,3-thiazines were synthesized by the intramolecular cyclization of the corresponding 4-aryl(carboxy-, aminomethyl)-5-amino-1,2,3-triazoles. The cyclocondensation involving 4,5-diamino-, 4-carbofunctionalized 5-amino-1,2,3-triazoles and 4-amino-5-thiocarboxamido-1,2,3-triazoles was successful for the construction of di-, oxa- and thiazepino-annulated triazoles.

Conclusions. The analysis, systematization and summary of the literature regarding the synthetic potential of 1,2,3-triazole-4(5)-amines conclusively demonstrate that these structures are easily available and convenient molecular blocks for the construction of triazolo-annulated pyridine, azine and azepine systems that are important for synthetic and biomedical research.

Keywords: 4(5)-amino-1,2,3-triazoles; triazolo[4,5-*b*]pyridines; triazolo[4,5-*d*]pyridines; triazoloannulated azepines; cyclocondensation

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1,2,3-Триазол-4(5)-аміни – зручні синтетичні блоки для конструювання триазолоанельованих гетероциклів

Анотація

Мета. Проаналізувати та узагальнити синтетичний потенціал 1,2,3-триазол-4(5)-амінів як ефективних білдинг-блоків у синтезі триазолоанельованих піридинових, азинових та азепінових систем.

Результати та їх обговорення. Проаналізовано та систематизовано оригінальні літературні джерела, які розкривають синтетичні можливості 4(5)-амінофункціоналізованих 1,2,3-триазолів як зручних і доступних будівельних блоків для одержання триазолоанельованих азинів та азепінів. Доведено, що конденсація 1,2,3-триазол-4(5)-амінів із метиленактивними сполуками є потужним інструментом синтезу різноманітних триазоло[4,5-*b*]піридинів. Зі свого боку для отримання низки триазоло[4,5-*d*]піримідинових систем досить загальними виявились циклоконденсації на основі 5-аміно-1,2,3-триазол-4-карбонових кислот та їхніх структурно модифікованих похідних. Нечисленних представників триазолоанельованих піридазинів, 1,3-оксазинів та 1,3-тіазинів було синтезовано внутрішньомолекулярними циклізаціями відповідних 4-арил(карбокси-, амінометил)-5-аміно-1,2,3-триазолів. Для конструювання ді-, окса- та тіазепіноанельованих триазолів вдалими виявились циклоконденсації за участю 4,5-діаміно-, 4-карбофункціоналізованих 5-аміно-1,2,3-триазолів та 4-аміно-5-тіокарбоксамідо-1,2,3-триазолів.

Висновки. Аналіз, систематизація та узагальнення літературних джерел, які стосуються синтетичного потенціалу 1,2,3-триазол-4(5)-амінів, переконливо засвідчують, що такого типу структури є доступними й зручними молекулярними блоками для конструювання важливих для синтетичних і біомедичних досліджень триазолоанельованих піридинових, азинових та азепінових систем.

***Ключові слова*:** 4(5)-аміно-1,2,3-триазоли; триазоло[4,5-*b*]піридини; триазоло[4,5-*d*]піримідини; триазолоанельовані азепіни; циклоконденсація

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■ Introduction

1,2,3-Triazole-4(5)-amines, including those additionally modified with other functional substituents and heterocyclic rings, occupy their rightful place in the chemistry of azole compounds and are of great interest to researchers due to their pronounced synthetic capabilities. Particular interest in heterocyclic systems annulated with a triazole ring arose with the discovery of the drug "Ticagrelor" **I** (Figure) indicated to prevent or reduce the risk of coronary thrombosis in patients with the acute coronary syndrome and patients undergoing the percutaneous coronary intervention or coronary artery bypass grafting [1].

Inhibitors of the human carbonic anhydrase isoenzyme type hCA IX **II** [2] and calcium/calmodulin-regulated kinases PIM **III** [3], a potent antagonist of the Dengue virus **IV**, were found in a number of triazoloanelated pyridines [4].

A low molecular weight agonist of cannabinoid receptor 2 (CB2) **V** [5], inhibitors of replication of the Chikungunya virus (CHIKV) **VI** [6] and a reversible inhibitor of lysine-specific demethylase 1 (LSD1) **VII** [7], compounds with the antitumor activity against breast cancer cells MCF-7, lungs A549 **VIII** [8] and lungs H1650 **IX** have been identified among the functionalized triazolo[4,5-*d*]pyrimidines [9].

The bioscreening results of triazolo[4,5-*b*]-[1,5]benzodiazepine **X** showed the antidopaminergic and anticholinergic activity to bind [^{3}H]spiperone and [^{3}H]QNB receptors. The neuroleptic potential of derivatives **X** was evaluated in terms of their ability to induce hypothermia and catalepsy in mice and to block conditioned avoidance reactions in rats [10].

At the same time, despite the wide pharmaceutical profile of heteroannulated 1,2,3-triazole systems, the original works related to the methods of their synthesis based on functionalized 4(5)-aminotriazoles were not subjected to systematic analysis. Thus, it seemed appropriate to comprehensively summarize the published literature on the use of 1,2,3-triazole-4(5)-amines for the preparation of triazoloannulated six- and seven-member heterocyclic systems.

■ Results and discussion

1. The synthesis of triazolo[4,5-*b*]pyridines

5-Aminotriazoles **1** as heterocyclic analogs of enamines were successfully used in the three-component condensation with 5-chloroisatin (**2**) and Meldrum's acid (**3**) to obtain a series of spirotriazolopyridones **4** and **5** (Scheme 1) [4, 11, 12]. Thus, using (*S*)- and (*R*)-1-[1-(4-chlorophenyl)ethyl]-1*H*-1,2,3-triazole-5-amines **1**, diastereomeric mixtures of optically pure spiro-derivatives **4** and **5** were synthesized. Instead, the condensation of 1-(4-chlorobenzyl)- and 1-[(5-chloropyridin-2-yl)-methyl]-1*H*-1,2,3-triazole-5-amines **1** led to the formation of a mixture of enantiomers, of which the preparative high-performance liquid chiral chromatography yielded only (*R*)-diastereomer **4**. In the case of 1-[1-(4-chlorophenyl)ethyl(propyl)]-1*H*-1,2,3-triazole-5-amines **1**, racemate reaction products were isolated.

4-Functionalized 5-amino-1,2,3-triazoles also proved to be convenient building blocks for the synthesis of substituted triazolo[4,5-*b*]pyridine derivatives. Thus, the treatment of 5-amino-4-formyltriazoles **6** with an excess of acetone or ethyl acetoacetate **7** in an aqueous solution of NaOH or

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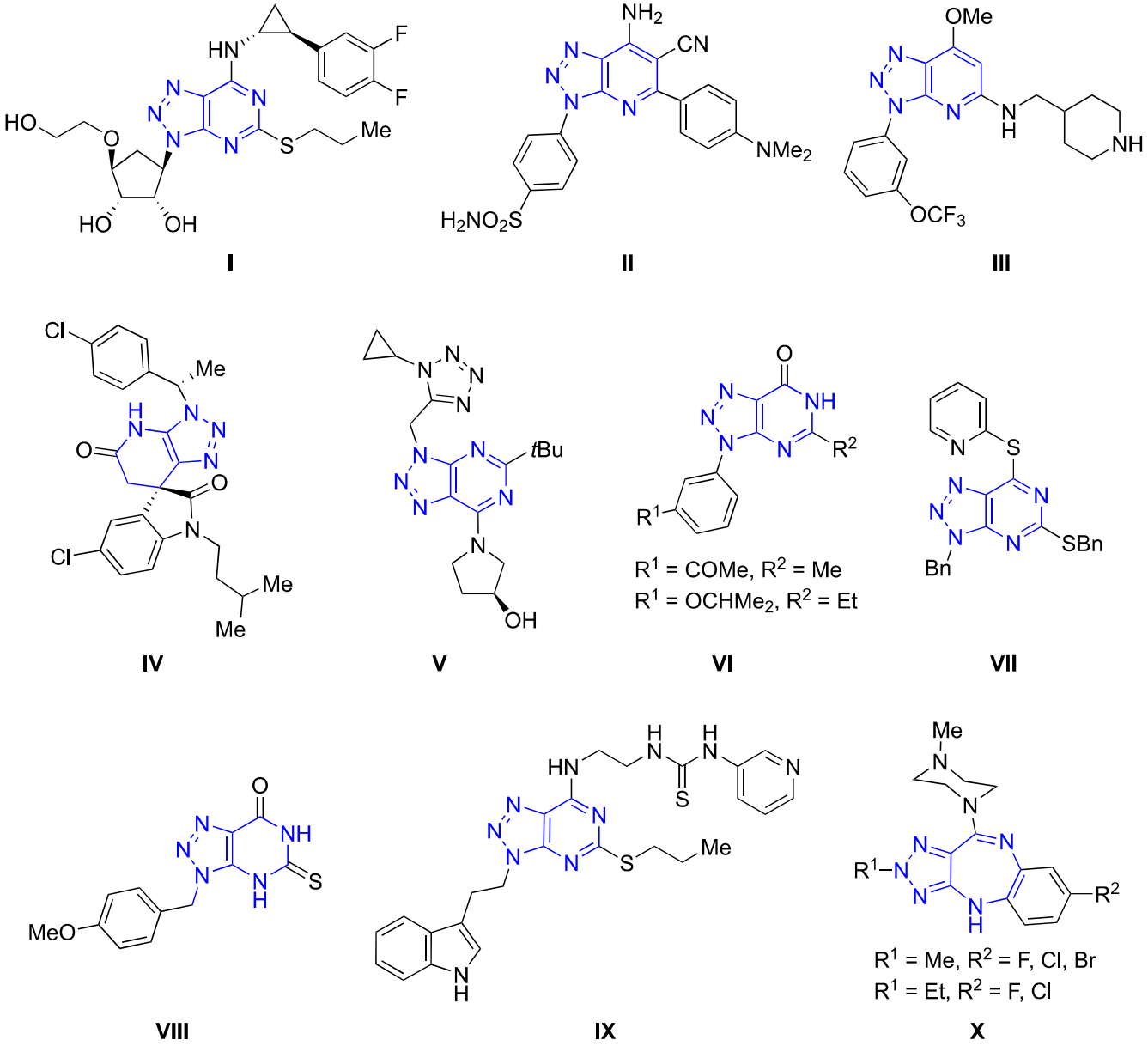
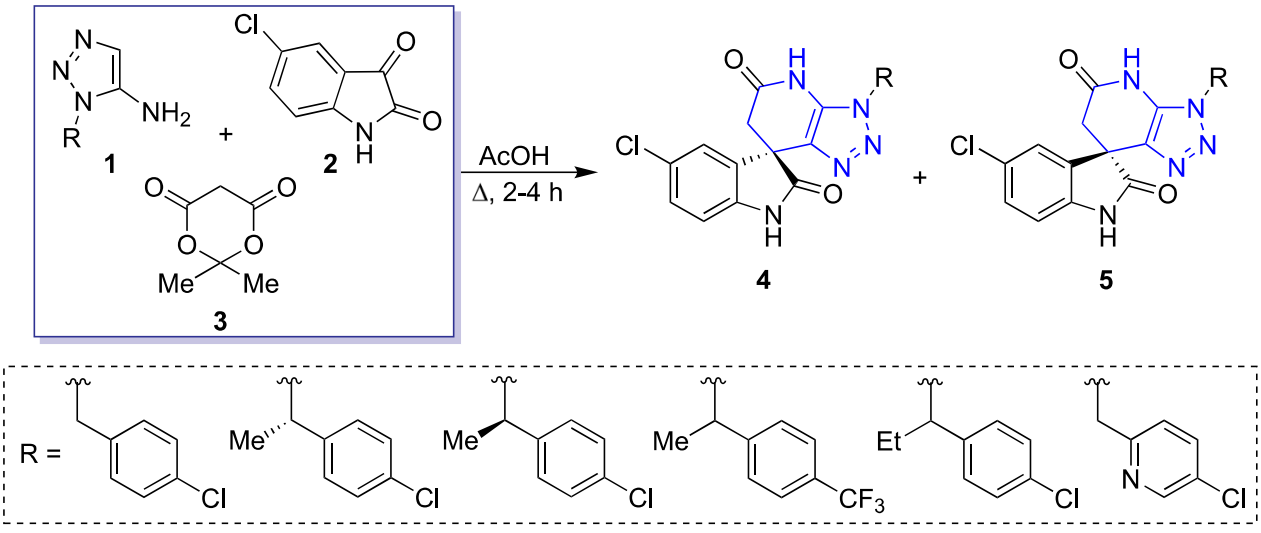
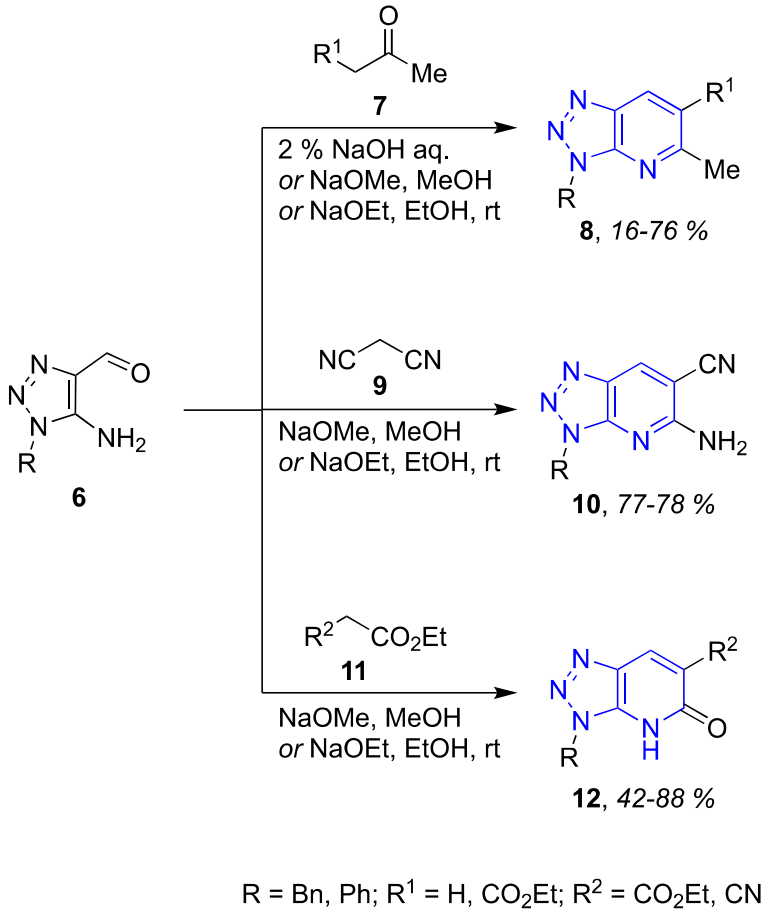


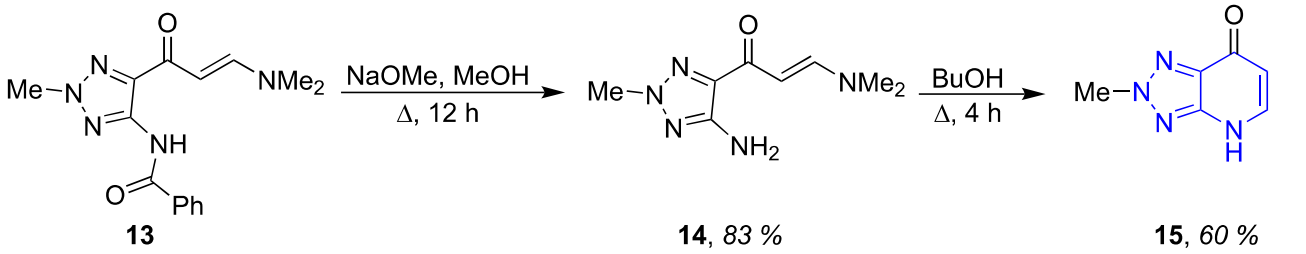
Figure. The structures of the drug «Ticagrelor» **I** and bioactive triazoloannulated heterocycles **II-X**



Scheme 1. The synthesis of spirotriazolopyridones **4, 5**



Scheme 2. The cyclocondensation of 5-amino-4-formyltriazoles **6** with active methylene compounds



Scheme 3. The synthesis of 2-methyltriazolo[4,5-*b*]pyridin-7-one **15**

in an alcoholic solution of sodium alkoxide yielded di- and trisubstituted 1,2,3-triazolo[4,5-*b*]pyridine **8** (Scheme 2) [13]. In turn, their cyclocondensation with malononitrile (**9**) led to the formation of 5-amino-1,2,3-triazolo[4,5-*b*]pyridine-6-carbonitriles **10**, and with ethyl malonate or ethyl cyanoacetate **11** produced 3,6-disubstituted 1,2,3-triazolo[4,5-*b*]pyridine-5(4*H*)-ones **12**.

The cyclization of 5-amino-4-[3-(dimethylamino)acryloyl]-2-methyl-1,2,3-triazole (**14**) obtained from the corresponding *N*-{5-[3-(dimethylamino)-acryloyl]-2-methyl-2*H*-1,2,3-triazol-4-yl}benzamide (**13**) proved to be effective for the preparation of 2-methyltriazolo[4,5-*b*]pyridin-7-one (**15**) (Scheme 3) [14].

The interaction of 4-acetyltriazole-2-phenyl-5-amine (16) with malononitrile (**9**) in boiling DMF led to the formation of 5-aminotriazolo[4,5-*b*]pyridine-6-carbonitrile (**17**), while the acetylation with acetic anhydride yielded derivative **18** condensed with dimethylformamide dimethylacetal (DMFDMA) to give *cis*-enaminone **19** (Scheme 4). The latter underwent the cyclization upon the treatment with phenyldiazonium chloride (**20**) under basic conditions, followed by the deacylation, and formed [1,2,3]triazolo[4,5-*b*]pyridin-7-one (**21**) [15].

The authors of [16] have developed an easy variant of the synthesis of triazolo[4,5-*b*]pyridin-5-ones **24**, which includes the interaction of 4-acyltriazole-5-amines **16** with carboxylic acids or esters **22** under the microwave irradiation with the formation of the corresponding acetamides **23**; the cyclization of the latter in boiling DMF yields target products **24** (Scheme 5).

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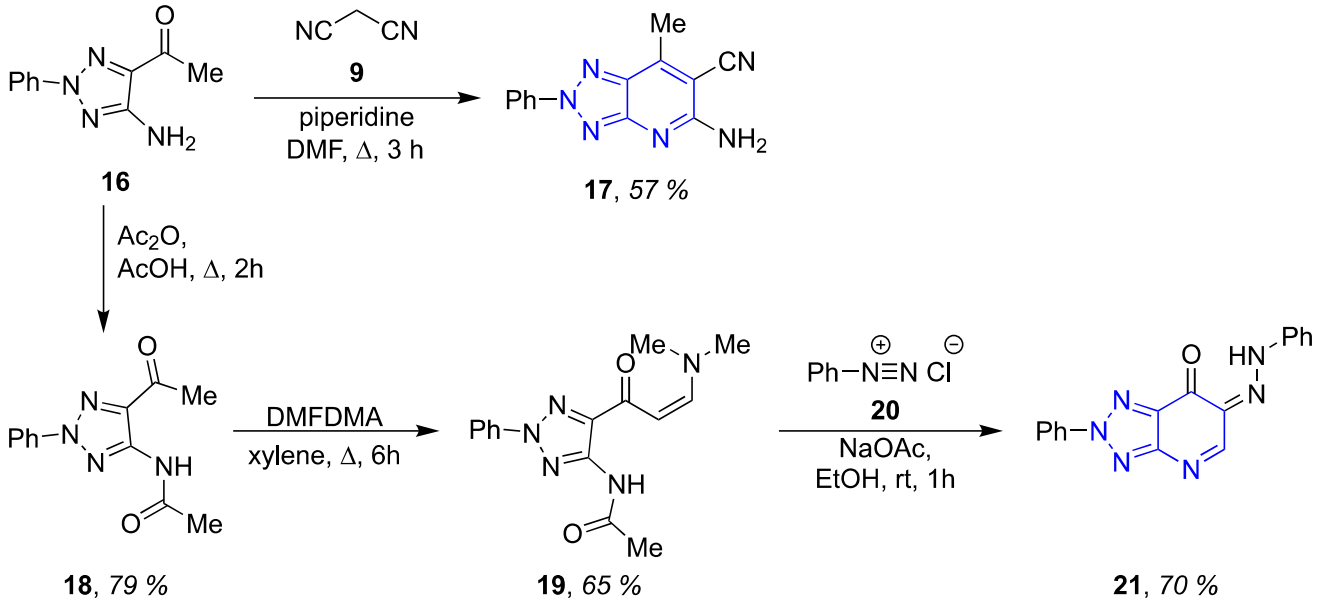
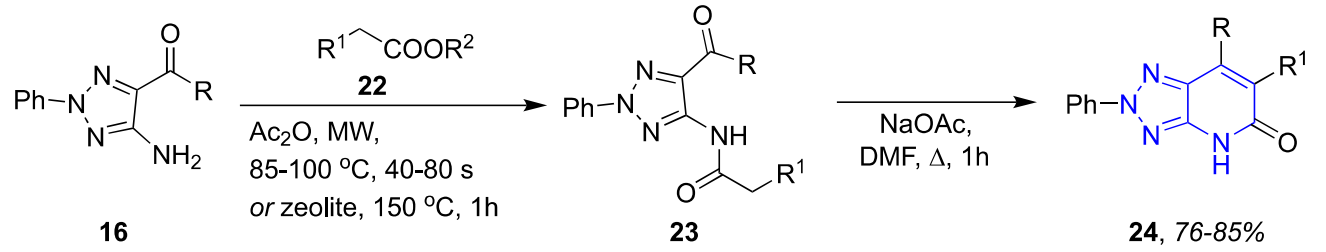


Scheme 4. Synthetic possibilities of 4-acetyltriazole-5-amine **16**



R = Me, Ph
R^1 = CN, CO_2Et, MeCO, PhCO, 4-NO_2-C_6H_4
R^2 = H, Et

Scheme 5. The method for the synthesis of trisubstituted triazolo[4,5-*b*]pyridin-5-ones **24**

The condensation of 5-aminotriazole-4-carbonitrile **25** with ethyl cyanoacetate (**11**) led to 7-amino-5-oxotriazolo[4,5-*b*]pyridine-6-carbonitrile **26**, and with benzylidene derivatives **27** to 7-aminotriazolo[4,5-*b*]pyridine-6-carbonitriles **28** (Scheme 6) [2].

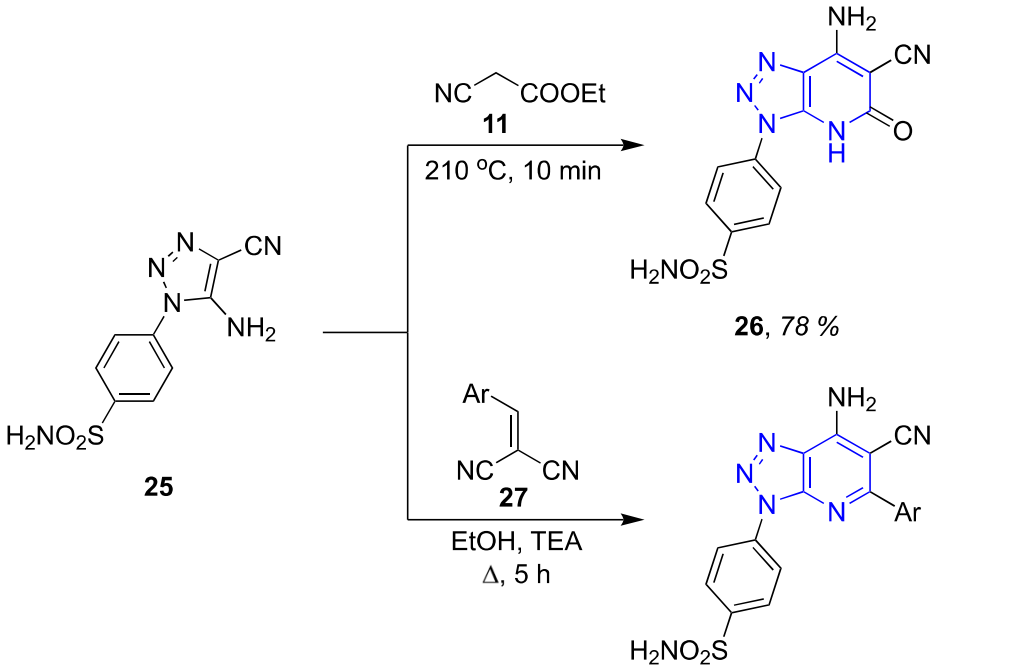
A convenient method for the synthesis of 7-aminotriazolo[4,5-*b*]pyridine-6-carboxylates **31** is based on the reaction of aminonitriles **29** with acetoacetic ester (**30**) in the presence of a Lewis acid (Scheme 7) [17].

The reaction of triazolylaminonitrile **32** with nickel complexes of 1,3-dicarbonyl compounds **33** proved to be successful in the preparation of triazoloannulated pyridines **34** (Scheme 8) [18].

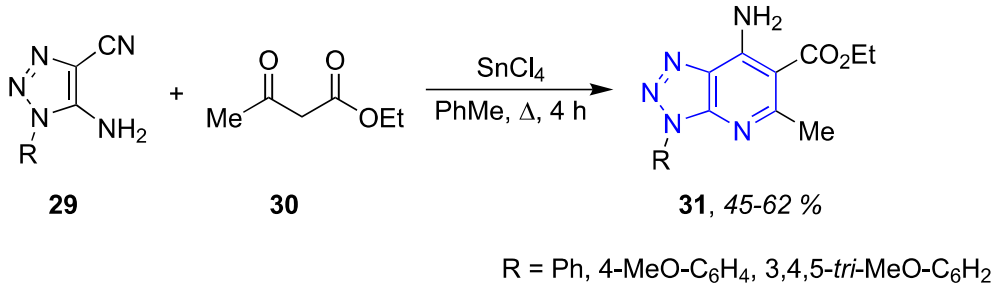
The authors [19] proposed effective conditions for the Friedlaender reaction of *N*-Boc-4-aminotriazole-5-carbaldehydes **35** with acetylacetone (**36**) or malononitrile (**9**) which resulted in the formation of target 6-acetyltriazolo[4,5-*b*]pyridines **37** and 5-aminotriazolo[4,5-*b*]pyridine-6-carbonitriles **38**, respectively (Scheme 9).

It was found that heating of *N*-Boc-4-aminotriazole-5-carbaldehydes **35** with malonic acid (**39**) in acetic acid at 100°C in the presence of catalytic amounts of pyrrolidine led to the formation of 5-oxo-4,5-dihydro-1*H*-[1,2,3]triazolo[4,5-*b*]pyridine-6-carboxylic acids **40** previously undescribed in 61–66% yields (*Method A*) (Scheme 10). However, the use of Meldrum's acid (**3**), a synthetic equivalent of malonic acid, in this process under similar reaction conditions is much more productive since it increases the yield of the target compounds to 91–94% (*Method B*). The likely transformation scheme in the case of malonic acid is through intermediate products **A** and **B**, while in the case of Meldrum's acid it is through **C** and **D**. Indeed, the efficiency of the latter is due to the structure of intermediate **D** which, in contrast to intermediate **B**, is characterized by much higher selectivity of further transformation [20].

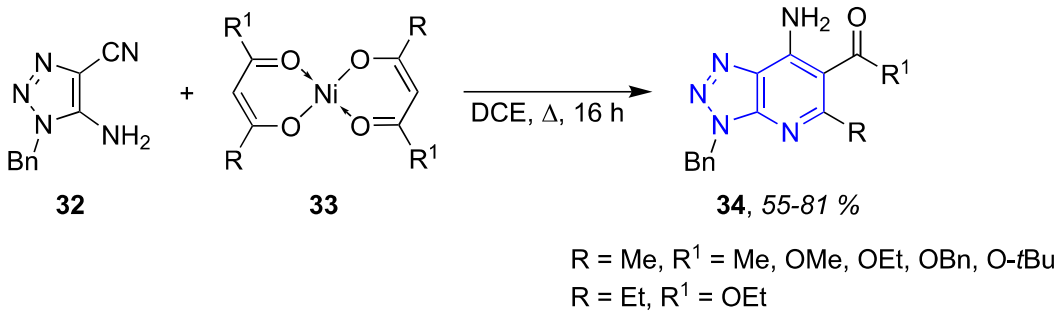
To obtain new heterocyclic analogs of carboannulated triazolopyridines as promising bioactive



Scheme 6. The synthesis of 7-aminotriazolo[4,5-*b*]pyridine-6-carbonitriles **26** and **28**



Scheme 7. The synthesis of 7-aminotriazolo[4,5-*b*]pyridine-6-carboxylates **31**



Scheme 8. The reaction of 5-aminotriazole-4-carbonitrile **32** with nickel complexes

compounds, aminoaldehydes **35** were tested in the cyclocondensation with cycloalkanones **41** and 1,3-cyclohexanediones **43**, which made it possible to isolate carbocyclic derivatives **42** and hydrogenated 1,2,3-triazolo[4,5-*b*]quinolines **44**, respectively (Scheme 11) [19].

Another method for the formation of the triazolo[4,5-*b*]quinoline core reported by the authors of the patent [21] was the use of the intramolecular cyclization of 4-*N*-arylamino-substituted 1,2,3-triazole-5-carboxylic acids **45** by their heating in polyphosphoric acid (Scheme 12).

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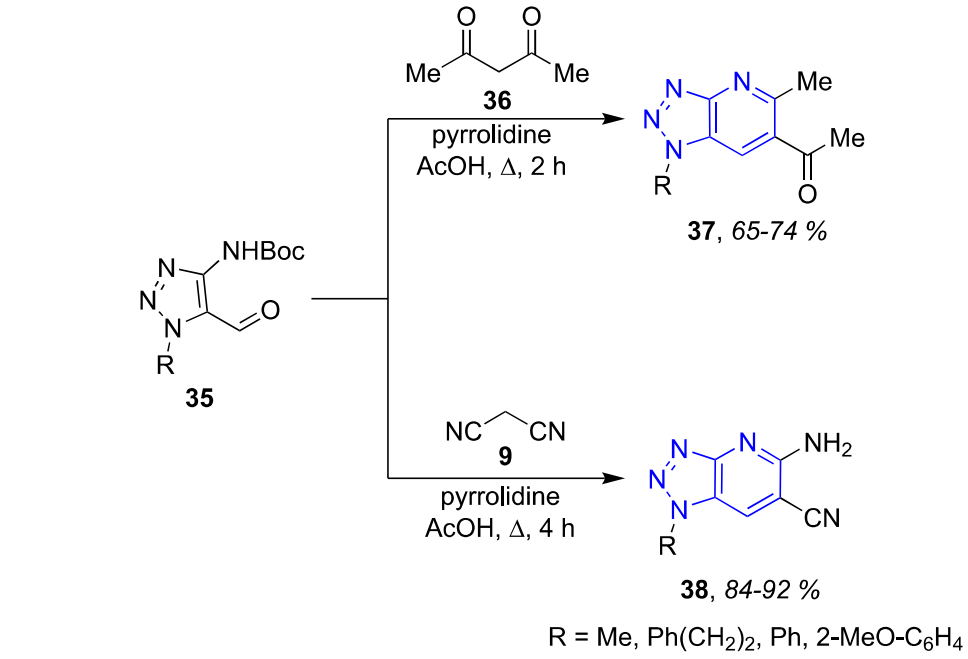
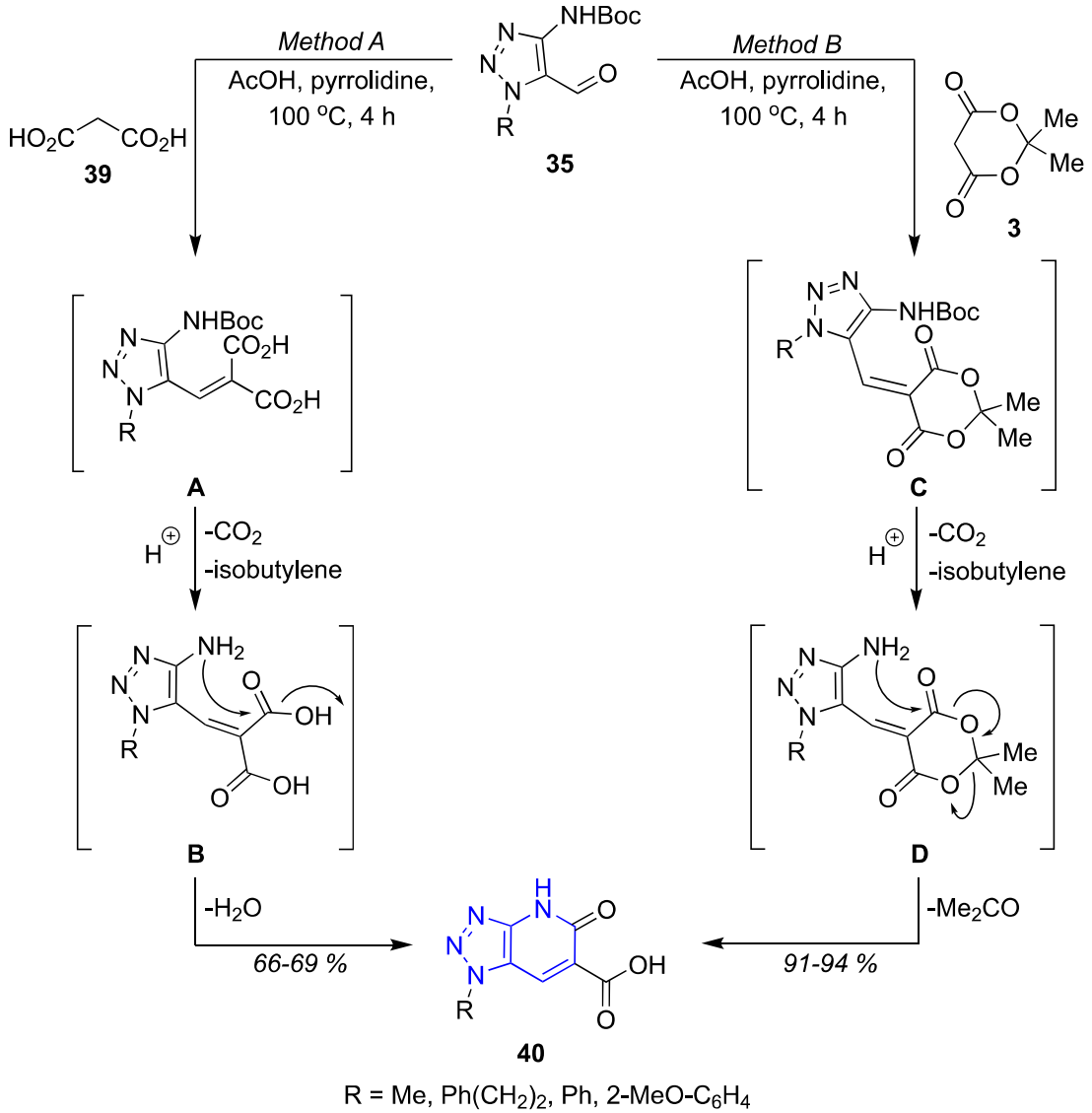
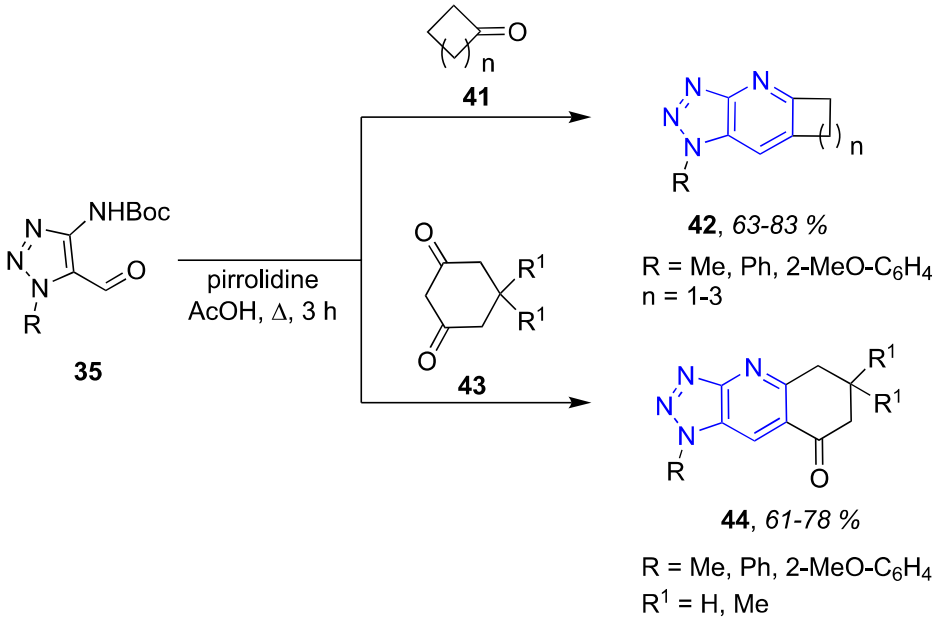


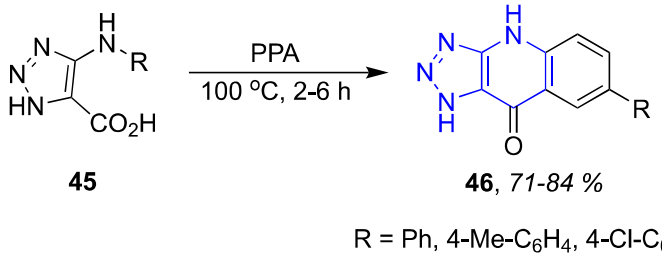
Scheme 9. The reaction of *N*-Boc-4-aminotriazole-5-carbaldehydes **35** with active methylene compounds



Scheme 10. The synthesis of 5-oxo-4,5-dihydro-1*H*-[1,2,3]triazolo[4,5-*b*]pyridine-6-carboxylic acids **40**



Scheme 11. The synthesis of carbocyclic triazolopyridines **42** and **44**



Scheme 12. The intramolecular cyclization of 4-*N*-arylamino-substituted 1,2,3-triazole-5-carboxylic acids **45**

2. The synthesis of triazolo[4,5-*d*]pyrimidines

2.1. Reactions involving 5-amino-1,2,3-triazole-4-carboxylates

An important field of application of amino-functionalized 1,2,3-triazoles has become the development of a method for the synthesis of triazolo[4,5-*d*]pyrimidines, which can be considered as isosteres of biologically promising purines.

Thus, the reaction of carboxylates **47** with triethyl orthoformate gave the corresponding 5-ethoxymethyleneamino-1,2,3-triazoles **48**, which were easily cyclized to 6-aminotriazolo[4,5-*d*]pyrimidin-7-ones **49** by the action of hydrazine hydrate (Scheme 13) [22]. Instead, the reaction of triazoles **47** in the hydrazine solution after 3 h of boiling led to 5-amino-1,2,3-triazole-4-carbohydrazides **50**, which heterocyclization with triethyl orthoformate proved to be effective for obtaining (triazolo[4,5-*d*]pyrimidine-6-yl)formimidates **51**. In turn, the treatment of triazoles **47** with an excess of primary amine **52** in the presence of NH_4Cl at 200°C led to aminoamides **53**, which upon prolonged heating with triethyl orthoformate yielded the target triazolo[4,5-*d*]pyrimidines **54**.

The reaction of aminoester **55** with imidoyl chloride **56** catalyzed by a Lewis acid under the microwave irradiation resulted in the synthesis of triaryl-substituted triazolo[4,5-*d*]pyrimidinone **57** (Scheme 14) [23, 24].

The high-temperature cyclocondensation of 1-hetaryl-substituted 5-amino-1,2,3-triazole-4-carboxylate **58** with urea or thiourea resulted in the formation of triazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione and its 5-thio analog **59**, respectively (Scheme 15) [25].

The authors [26] developed a two-stage method for the synthesis of *bis*[1,2,3]triazolo[1,5-*a*:4',5'-*e*]pyrimidinones **62**, the first stage of it was azidation of amines **60** to the corresponding 5-azido-1,2,3-triazolocarboxylates **61**, and the second stage was their cyclocondensation with active methylene nitriles **9** leading to the target products (Scheme 16).

A number of works [27–33] describe an approach that is widely used to activate the triazole amino group with reduced nucleophilicity. For this purpose, 5-amino-1-aryl-1,2,3-triazole-4-carboxylates **63** were converted by the action of Ph_3P into the corresponding iminophosphoranes **64**.

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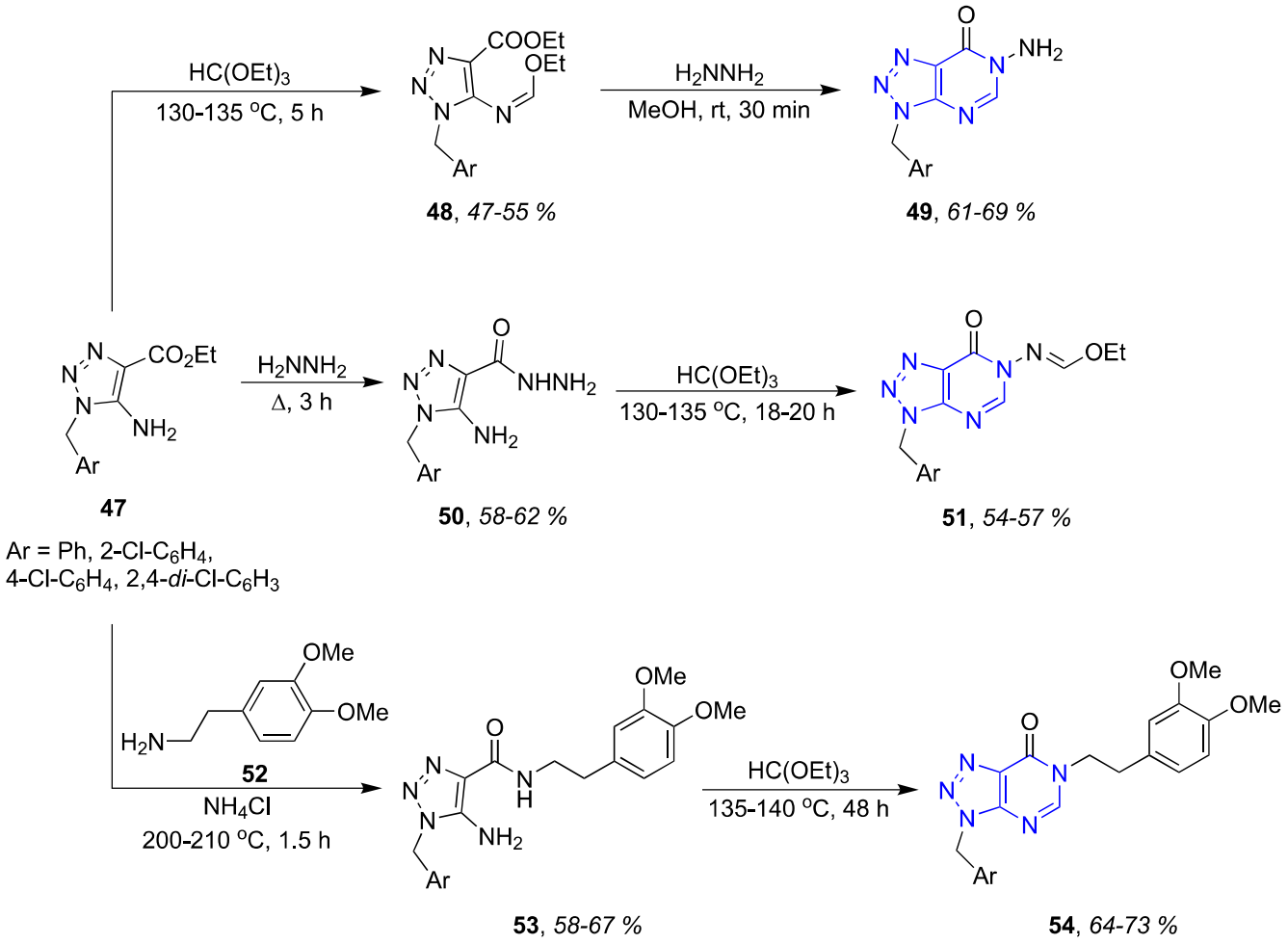
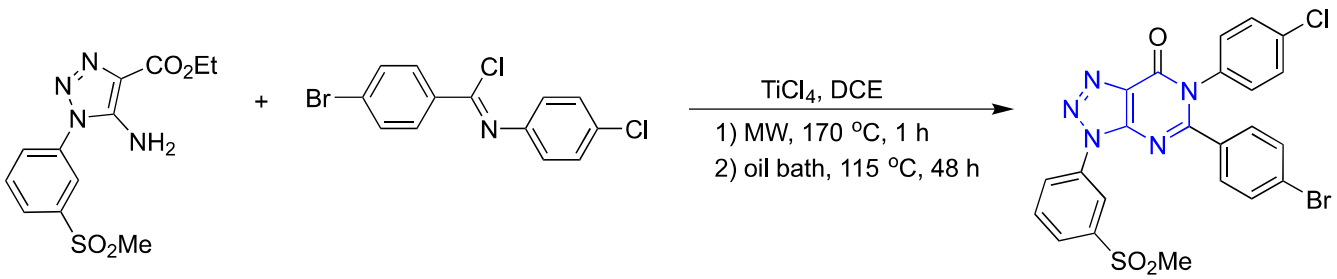
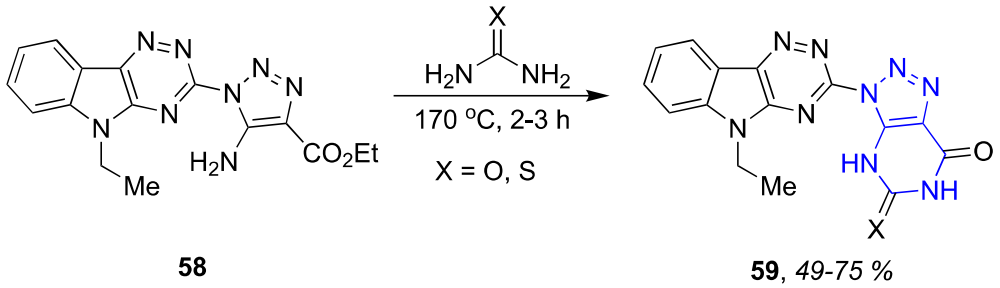


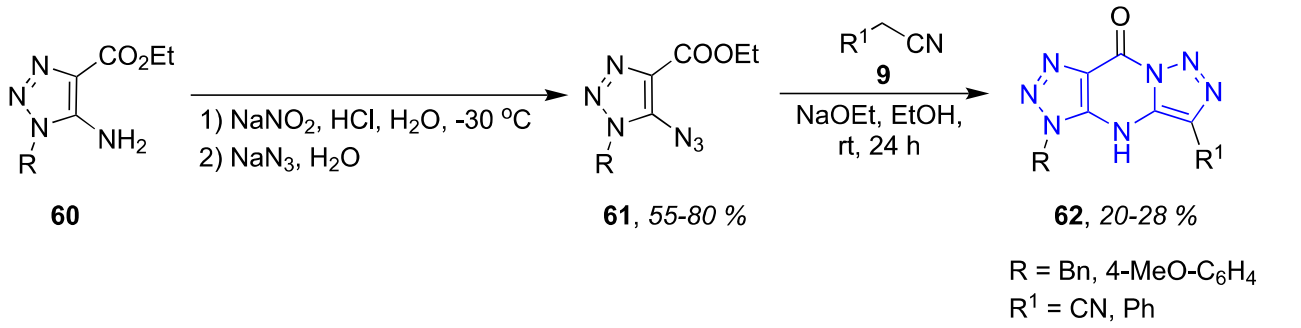
Scheme 13. Preparation of 6-functionalized triazolo[4,5-*d*]pyrimidin-7-ones **49**, **51**, **54**



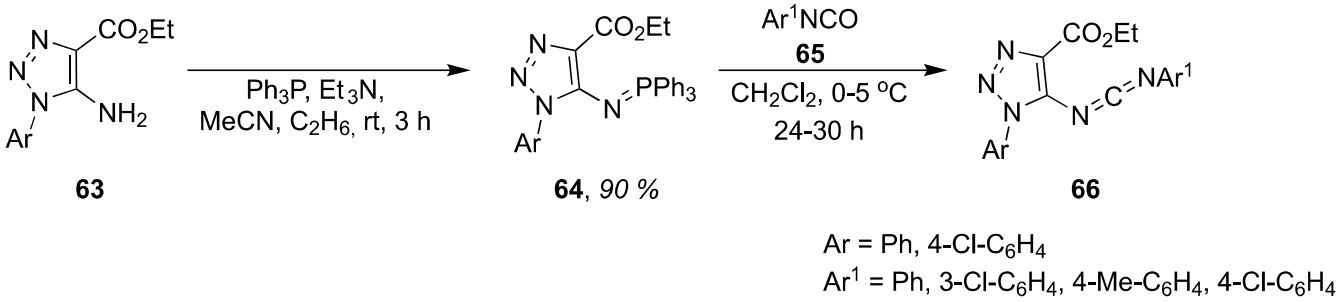
Scheme 14. The microwave synthesis of triazolo[4,5-*d*]pyrimidin-7-one **57**



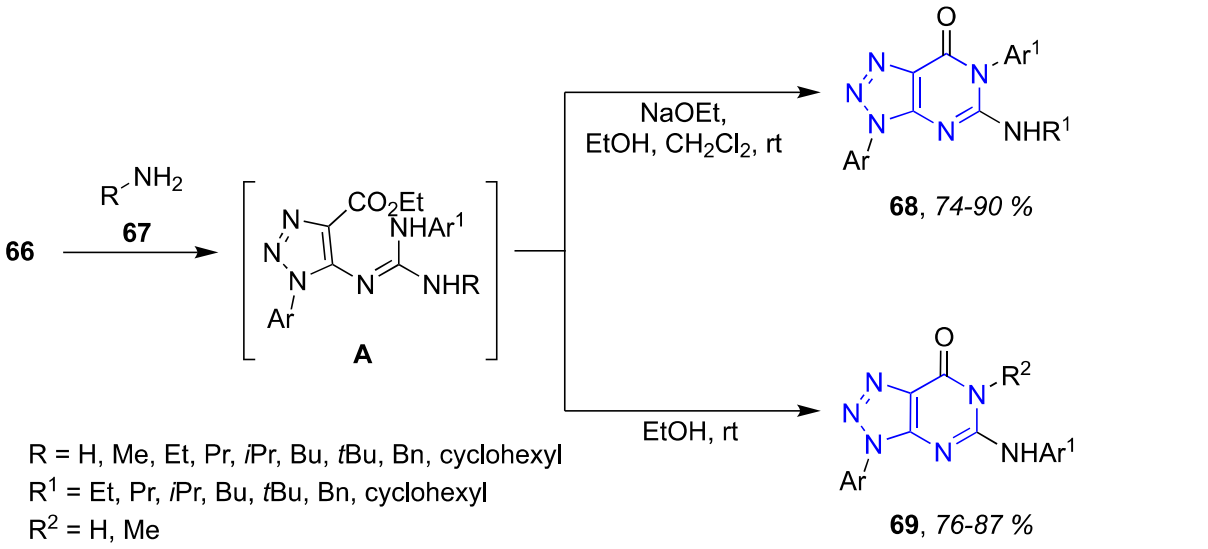
Scheme 15. The cyclocondensation of aminoester **58** with (thio)urea



Scheme 16. The synthesis of *bis*[1,2,3]triazolo[1,5-*a*:4',5'-*e*]pyrimidinone **62**



Scheme 17. The synthesis of triazolyl-containing carbodiimides **66**

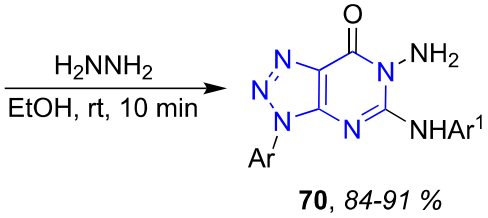


Scheme 18. Preparation of isomeric 5-alkylamino- and 5-arylaminotriazolo[4,5-*d*]pyrimidin-7-ones **68** and **69**

The latter easily underwent *aza*-Wittig reaction with aromatic isocyanates **65** to form triazolyl-containing carbodiimides **66** which had found wide application as effective precursors for the synthesis of triazolo[4,5-*d*]pyrimidines (Scheme 17).

The authors of [27] showed that the interaction of carbodiimides **66** with a number of alkylamines **67** in the presence of NaOEt led to the selective formation of 5-alkylaminotriazolo[4,5-*d*]pyrimidin-7-ones **68** (Scheme 18). At the same time, the formation of regioisomeric 5-arylaminoderivatives **69** was observed under the action of ammonia or methylamine in the absence of a base.

The reaction of triazolylcarbodiimides **66** with hydrazine hydrate in alcohol at room temperature also led to 5-arylamino-6-aminotriazolo[4,5-*d*]pyrimidin-7-ones **70** (Scheme 19) [29].



Scheme 19. The synthesis of 5-arylamino-6-aminotriazolo[4,5-*d*]pyrimidin-7-ones **70**

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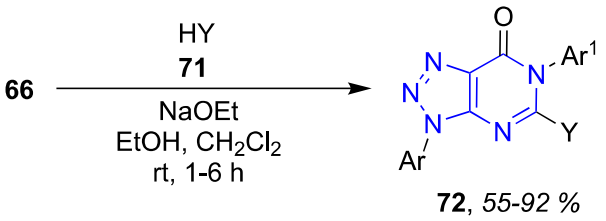


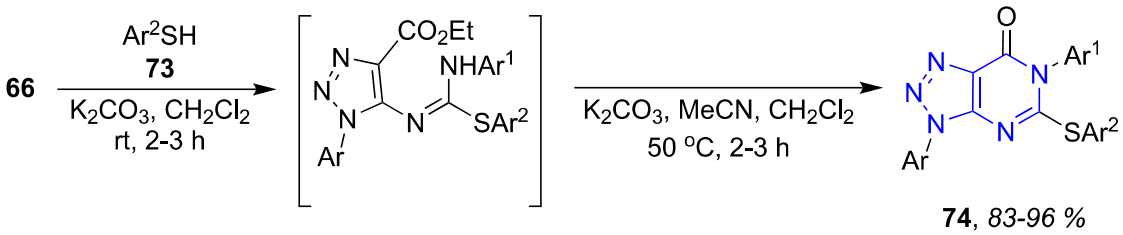
72, *55-92 %*

Y = NEt_2, NPr_2, NBu_2, $N(nC_5H_{11})_2$, $N(nC_6H_{13})_2$, $N(CH_2)_5$, $N(CH_2)_4O$, $N(iBu)_2$, NMe(Ph), $N(iPr)_2$, OMe, OEt, OPr, OBu, O-*i*Pr, OCH_2CCH, $OCH_2CH{=}CH_2$, OPh, 4-Me-C_6H_4O, 4-MeO-C_6H_4O, 4-Cl-C_6H_4O

Ar = Ph, 4-Cl-C_6H_4

Ar^1 = Ph, 4-Cl-C_6H_4, 3-Me-C_6H_4

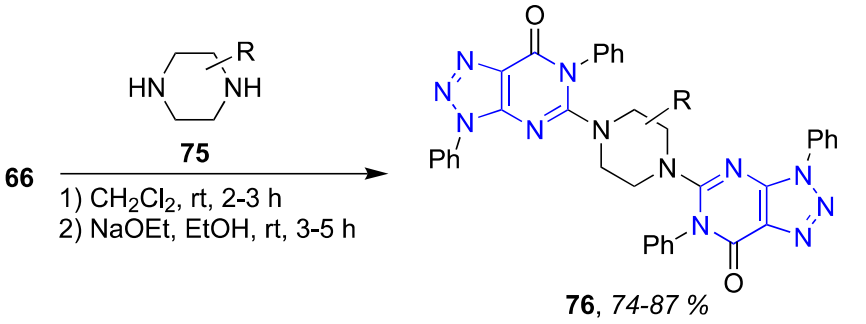
Scheme 20. The synthesis of 5-substituted triazolo[4,5-*d*]pyrimidin-7-ones **72**



74, *83-96 %*

Ar^2 = Ph, 2-Cl-C_6H_4, 4-Me-C_6H_4, 4-F-C_6H_4, 4-Cl-C_6H_4

Scheme 21. The synthesis of 5-arylthiotriazolo[4,5-*d*]pyrimidin-7-ones **74**



76, *74-87 %*

R = H, 2-Me, 2,5-*di*-Me, 2,6-*di*-Me, 2-Et

Scheme 22. The synthesis of 1,4-*bis*[triazolo[4,5-*d*]pyrimidin-7(6*H*)-one]piperazines **76**

To expand the boundaries of the reaction and synthesize various 5-substituted triazolo[4,5-*d*]-pyrimidin-7-ones **72**, *N,N*-dialkylamines, secondary amines and phenols **71** were used as nucleophilic reagents for the formation of a pyrimidine ring based on carbodiimides **66** (Scheme 20) [28, 30].

The reaction of carbodiimides **66** with thiophenols **73** at room temperature did not give the expected cyclization products, whereas at 50°C it yielded 5-arylthiotriazolo[4,5-*d*]pyrimidin-7-ones **74** [32, 33], among which compounds with high herbicidal activity against rapeseed and common flatweed were found (Scheme 21).

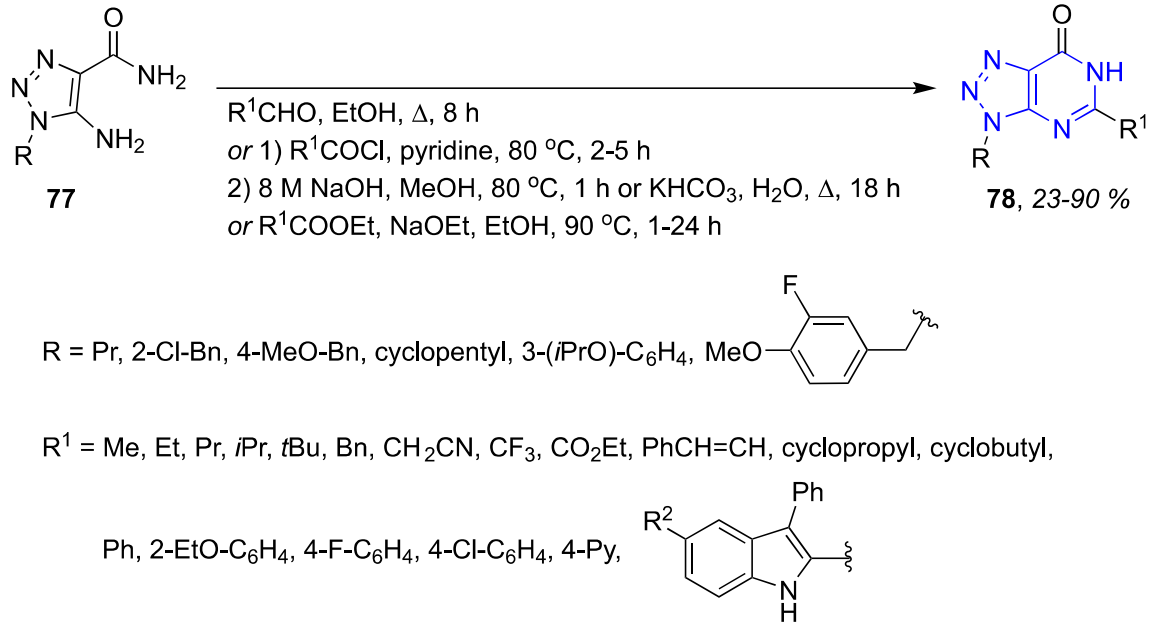
1,4-*Bis*[triazolo[4,5-*d*]pyrimidin-7(6*H*)-one]-piperazines **76** were readily prepared by reacting carbodiimides **66** with substituted piperazines **75** (Scheme 22) [31].

2.2. The cyclization of 5-amino-1,2,3-triazole-4-carboxamides

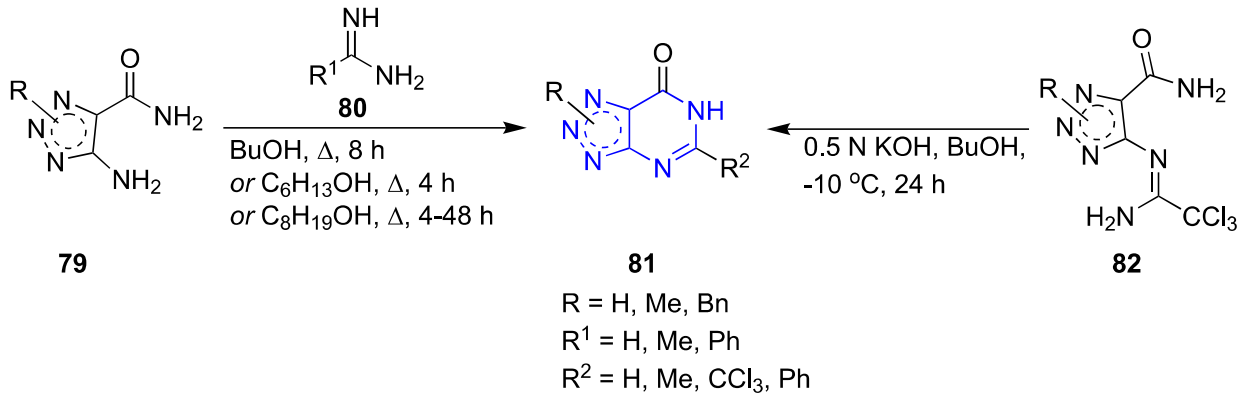
An effective approach to the synthesis of 3,5-disubstituted 1,2,3-triazolo[4,5-*d*]pyrimidin-7-ones **78** is the cyclocondensation of 5-amino-triazole-4-carboxamides **77** with benzaldehydes [8, 34], acyl chlorides [5, 35–39] and esters of mono- and dicarboxylic acids [6, 40–45] (Scheme 23).

The authors of [46] used the cyclocondensation of triazoloaminoamides **79** with amidines **80** to synthesize triazolo[4,5-*d*]pyrimidinones **81**, as well as the intramolecular cyclization of [(1-amino-2,2,2-trichloroethylidene)amino]triazolocarboxamides **82** under basic conditions (Scheme 24).

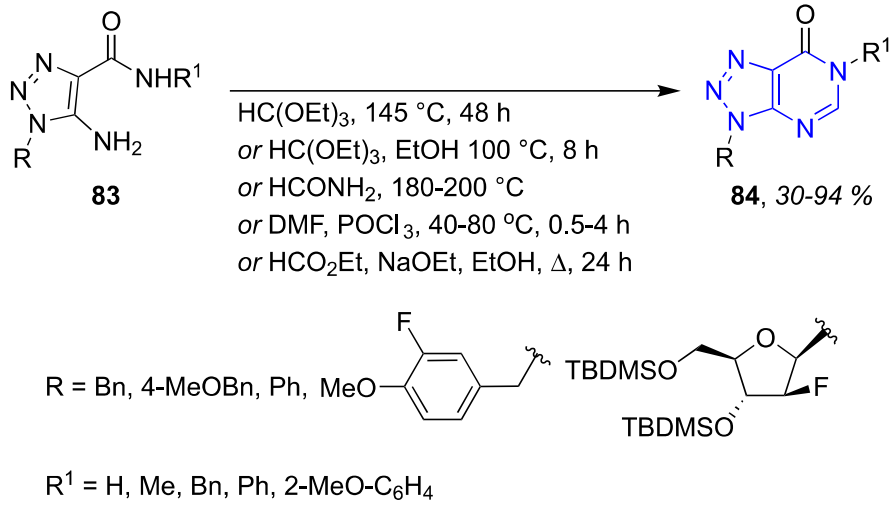
To build a pyrimidine ring based on 5-amino-4-triazolocarboxylic acid amides **83** and to form 3,6-disubstituted triazolo[4,5-*d*]pyrimidin-7-ones **84**



Scheme 23. 5-Aminotriazole-4-carboxamides **77** in the synthesis of triazolo[4,5-*d*]pyrimidin-7-ones



Scheme 24. Approaches to the construction of a triazolo[4,5-*d*]pyrimidine core based on triazoloaminoamides



Scheme 25. The synthesis of 3,6-disubstituted triazolo[4,5-*d*]pyrimidin-7-ones **84**

orthoesters [8, 34, 47, 48], amides and formic acid esters were used as one-carbon reagents [41, 49–51], as well as Vilsmeier-Haack reagent [50] (Scheme 25).

A similar scheme of a high-temperature condensation of 4-aminotriazole-5-carboxamide **85** with formamide was used to obtain 1-methyltriazolo[4,5-*d*]pyrimidin-7-one (**86**) (Scheme 26) [52].

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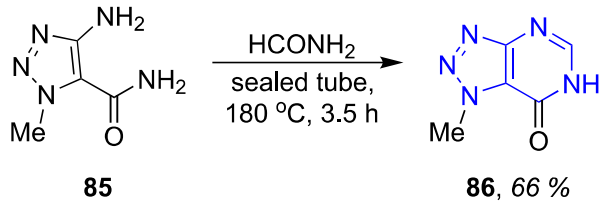
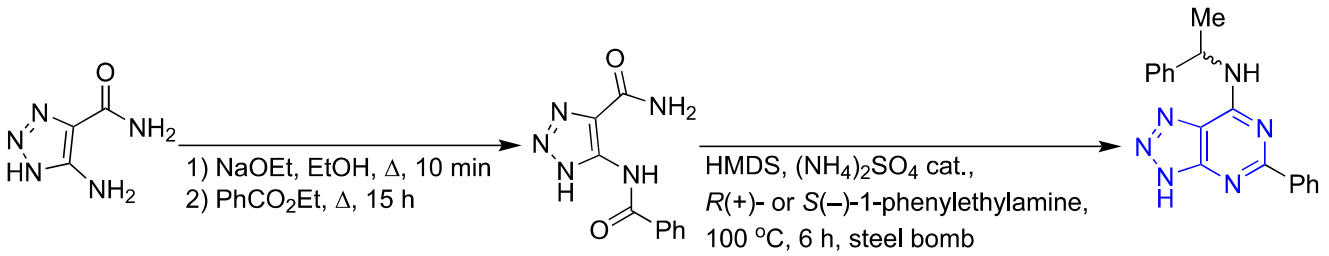


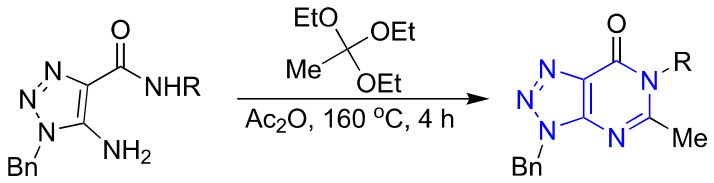
85 **86**, *66 %*

Scheme 26. The synthesis of 1-methyltriazolo[4,5-*d*]pyrimidin-7-one **86**



87 **88**, *65 %* **R-89**, *7.7 %* **S-89**, *5.4 %*

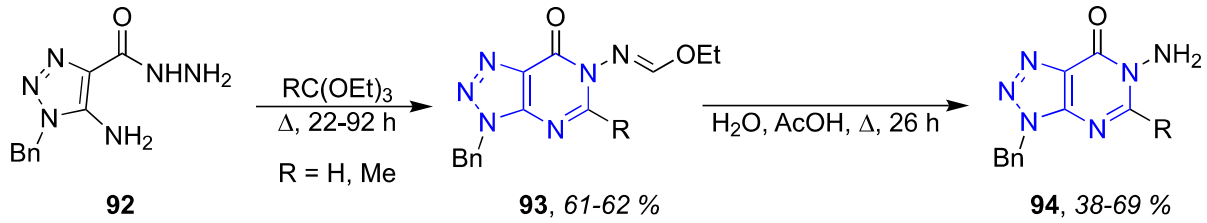
Scheme 27. The synthesis of triazolo[4,5-*d*]pyrimidines **89**



90 **91**, *40-90 %*

R = H, Me, Bn, Ph

Scheme 28. The synthesis of 6-alkyl(aryl)substituted triazolo[4,5-*d*]pyrimidines **91**



92 **93**, *61-62 %* **94**, *38-69 %*

Scheme 29. Preparation of 6-aminotriazolo[4,5-*d*]pyrimidine **94**

5-Benzamidotriazole-4-carboxamide (**88**) obtained from 5-amino-4-carbamoyl-1,2,3-triazole (**87**) by the action of hexamethyldisilazane (HMDS), catalytic amounts of $(NH_4)_2SO_4$ and *R*- or *S*-1-phenylethylamine was converted into 6-aminotriazolo[4,5-*d*]pyrimidines **89** (Scheme 27) [53].

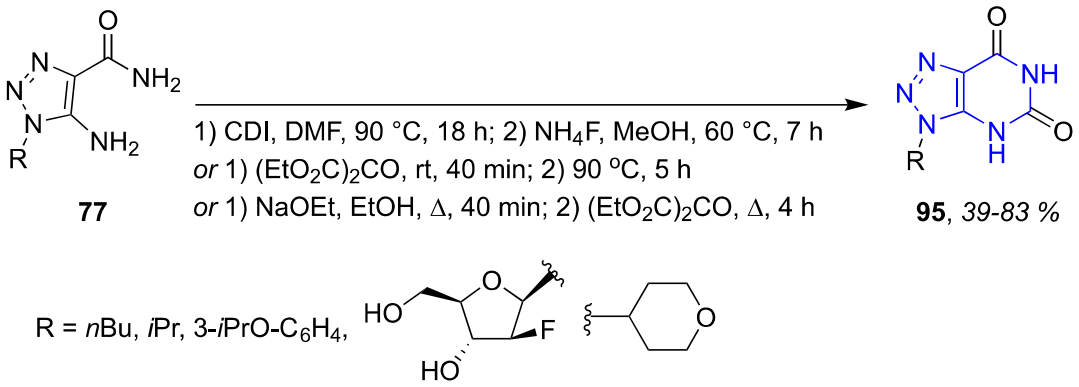
Heating of amides **90** with triethyl orthoacetate in the presence of acetic anhydride proved to be successful to obtain 6-alkyl(aryl)substituted triazolo[4,5-*d*]pyrimidines **91** (Scheme 28) [50].

The cyclocondensation of 5-aminotriazol-4-carbohydrazide **92** with an excess of triethyl orthoformate or triethyl orthoacetate led to ethyl-*N*-(triazolo[4,5-*d*]pyrimidin-6(7*H*)-yl)formimidates **93** undergoing the hydrolysis under acidic conditions to the corresponding 6-aminoderivatives **94** (Scheme 29) [54].

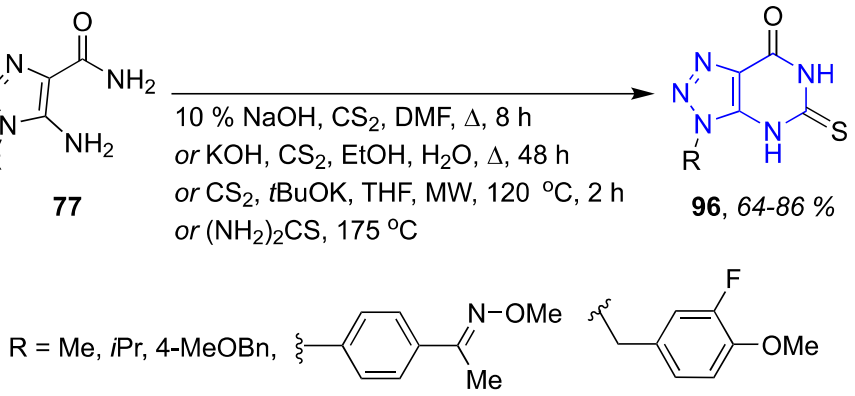
The synthesis of triazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-diones **95** was successful by heating triazolilaminoamides **77** with *N*,*N*'-carbonyldiimidazole (CDI) in DMF [6, 40, 47] or with diethyl carbonate in ethanol [55–60] (Scheme 30).

The condensation of aminoamides **77** with carbon disulfide under alkaline conditions [8, 34, 40, 61–63] or co-melting with thiourea [64] led to 3-substituted 5-thioxotriazolo[4,5-*d*]pyrimidine-7(4*H*)-ones **96** (Scheme 31).

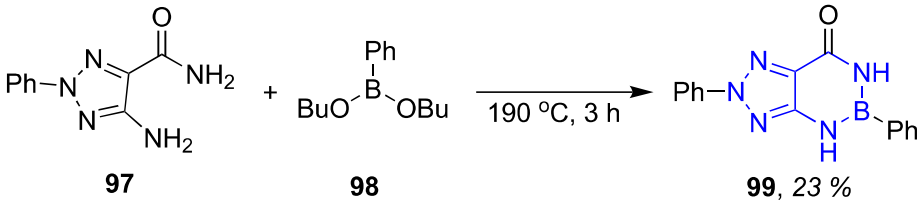
The high-temperature reaction of 5-amino-2-phenyltriazole-4-carboxamide (**97**) with dibutyl phenylboronate (**98**) turned out to be a convenient



Scheme 30. The synthesis of triazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-diones **95**



Scheme 31. The synthesis of 5-thioxotriazolo[4,5-*d*]pyrimidine-7(4*H*)-ones **96**



Scheme 32. The synthesis of triazolo[4,5-*d*][1,3,2]diazaborinin-7(4*H*)-one **99**

method for the preparation of triazolo[4,5-*d*]-[1,3,2]diazaborinin-7(4*H*)-one **99** (Scheme 32) [65].

2.3. The cyclocondensation of triazolilaminonitrites

Amidines or their salts **80** were used as 1,3-binucleophilic reagents to complete the pyrimidine ring to 5-aminotriazole-4-carbonitrile **100** in order to synthesize triazolo[4,5-*d*]pyrimidine-7-amines **101** (Scheme 33) [66].

A similar reaction of 5-(methylamino)triazole-4-carbonitrile **102** with acetimidate **80** produced 4-methyl-4*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine-7-amine **103** (Scheme 34) [45].

A convenient method for the preparation of triazolo[4,5-*d*]pyrimidine-7-amine **105** was heating aminonitrile **104** in an excess of diethylmethylamine (DEMA) followed by the treatment with ammonia in MeOH (Scheme 35) [67].

The condensation of aminonitriles **106** with phenylisothiocyanate led to 7-anilinotriazolo[4,5-*d*]pyrimidine-5-thiones **107**, while **106** with potassium *O*-ethyldithiocarbonate or carbon disulfide followed by the treatment of the reaction mixture with methyl iodide yielded 5,7-*bis*(methylthio) derivatives **108** (Scheme 36). The intramolecular cyclocondensation of 5-cyano-4-ethoxymethyleneamino-1,2,3-triazoles **106** by 10 h reflux in NaHS solution proved to be convenient for the synthesis of triazolo[4,5-*d*]pyrimidine-7-thiones **109** [68].

The cyclization of 5-aminotriazole-4-carbonitrile **25** with phenylisocyanate, isothiocyanates, or thiourea at elevated temperatures was successfully used to obtain triazolo[4,5-*d*]pyrimidin-

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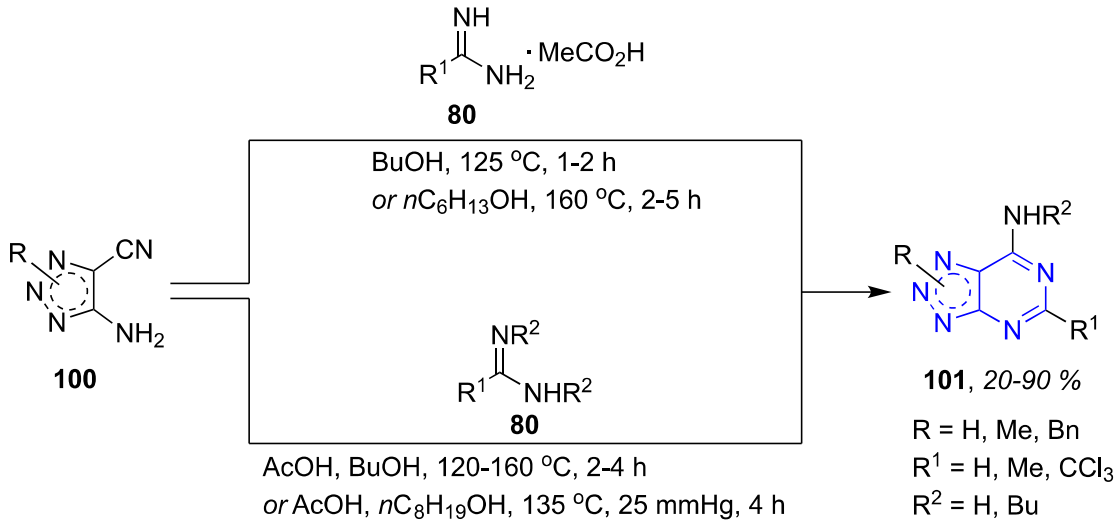
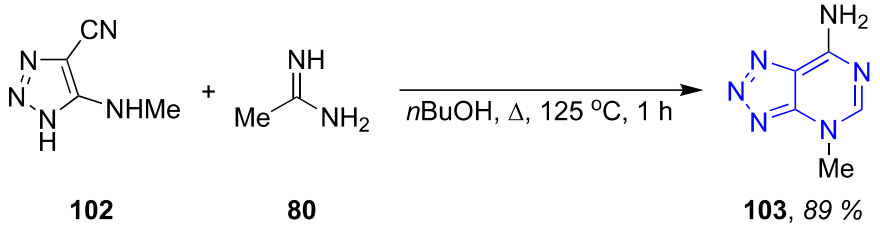
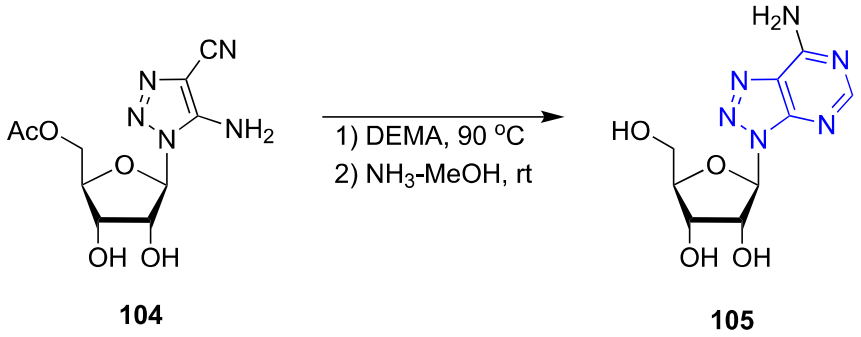


Scheme 33. The synthesis of 5-substituted triazolo[4,5-*d*]pyrimidine-7-amines **101**



Scheme 34. The synthesis of 4-methyl-4*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine-7-amine **103**



Scheme 35. The synthesis of triazolo[4,5-*d*]pyrimidine-7-amine **105**

5-ones **110** and triazolo[4,5-*d*]thiones **111**, **112**, respectively (Scheme 37) [2].

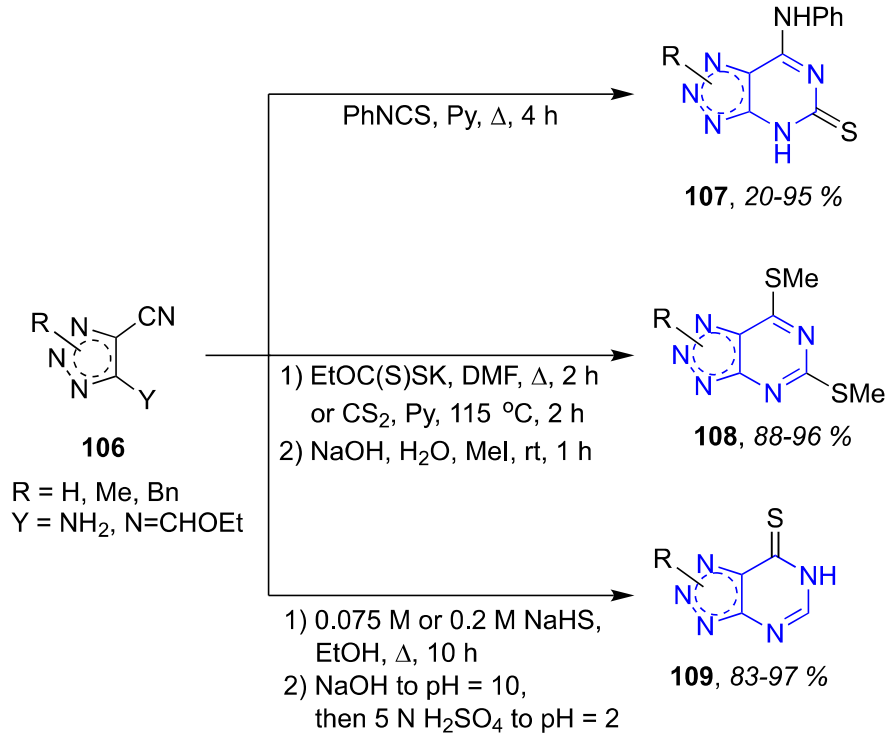
Isomeric 4-aminotriazole-5-carbonitrile **113** was subjected to the cyclization to triazolopyrimidine systems **114-116** in the reaction with formamide, phenylisothiocyanate, or carbon disulfide in an alcoholic solution of KOH. Its interaction with ethylenediamine formed imidazoyl-1,2,3-triazole **117**, which was converted to imidazo[1,2-*c*]-[1,2,3]triazolo[4,5-*e*]pyrimidine **118** by the action of triethyl orthoformate (Scheme 38) [69].

The authors of the work [70] successfully used the condensation of 4-hetarylsubstituted triazole-5-amines **120** (obtained from triazole-4-carbonitriles **119**) with orthoesters to synthesize imidazo-[1,2-*c*][1,2,3]triazolo[4,5-*e*]pyrimidines, pyrimido-[1,2-*c*][1,2,3]triazolo[4,5-*e*]pyrimidines, triazolo-[4',5':4,5]pyrimido[1,6-*a*][1,3]diazepines **121** (Scheme 39).

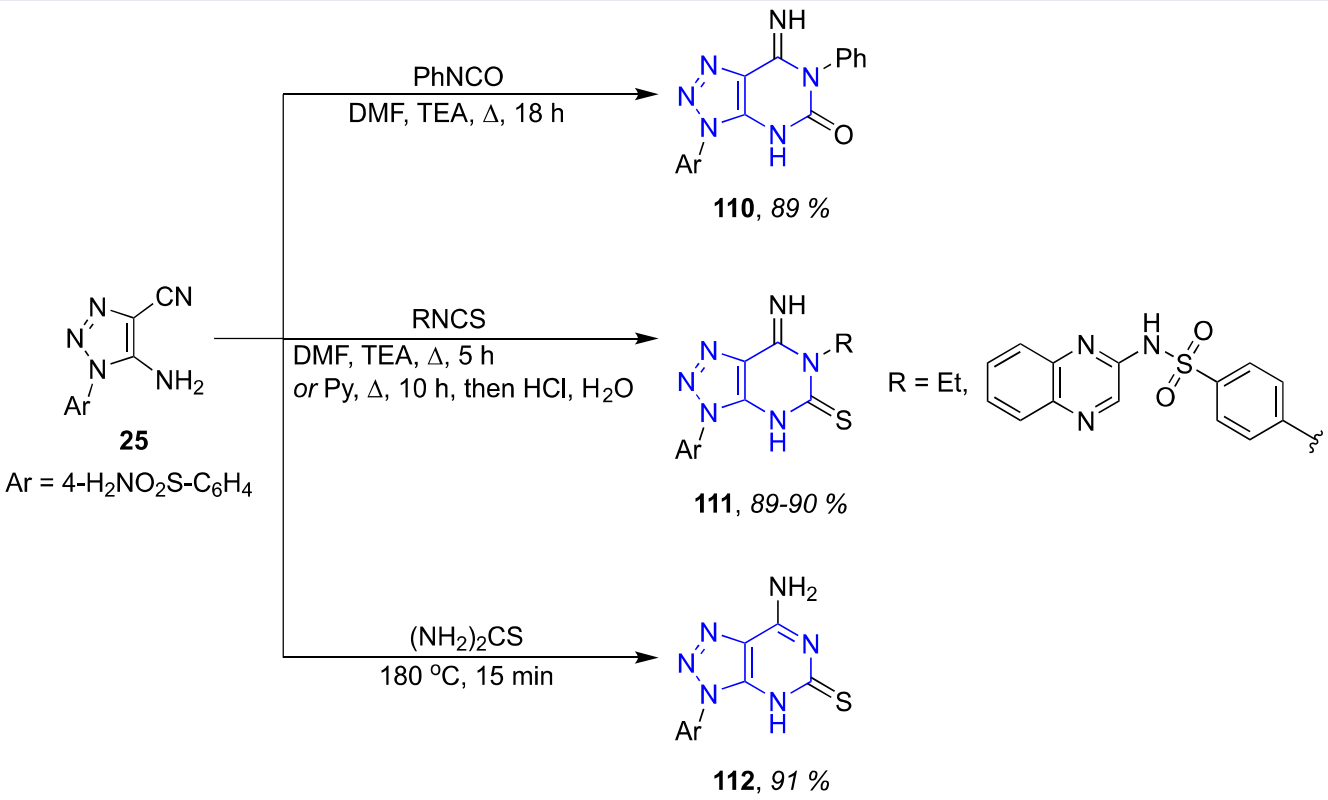
Hydrogenated analogs of imidazo[1,2-*c*][1,2,3]-triazolo[4,5-*e*]pyrimidines **122** were obtained by reacting imidazoyl-1,2,3-triazoles **120** with aromatic aldehydes (Scheme 39).

3. The synthesis of triazoloannulated pyridazines, oxazines and thiazines

Despite the relative ease of fusion of the pyridine and pyrimidine nuclei to the triazole ring, obtaining polycyclic systems with other heteronuclei proved to be a more difficult task. However, the authors of [71] succeeded in synthesizing 3*H*-[1,2,3]triazolo[4,5-*c*]cinoline **124** by the nitrosation



Scheme 36. Preparation of sulfur-containing triazolo[4,5-*d*]pyrimidines **107-109**



Scheme 37. The synthesis of triazolo[4,5-*d*]pyrimidin-5-(thi)ones **110-112**

of the amino group of 1,4-diaryl-substituted 5-aminotriazole **123** followed by the intramolecular azo coupling (Scheme 40).

The cyclocondensation of 1-heteryl-substituted 5-aminotriazole-4-carboxylic acid **125** with acetic anhydride proved to be effective for the preparation of the triazolo[4,5-*d*][1,3]oxazine-7-one derivative **126** (Scheme 41) [25].

The cyclization of sodium carbamodithioate **128** (synthesized from triazolodiamine **127**) with an excess of CS_2 yielded triazolo[4,5-*d*][1,3]thiazine-5-thione **129** (Scheme 42) [72].

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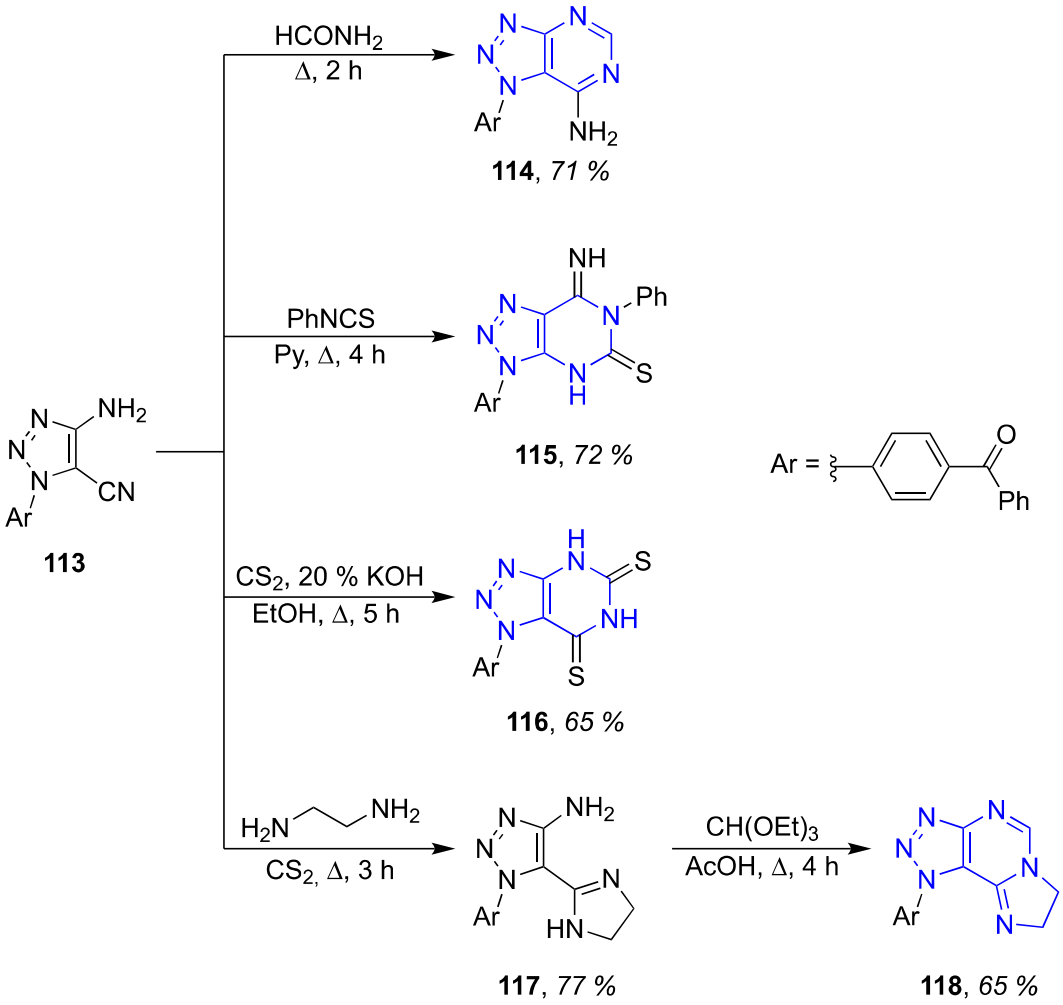
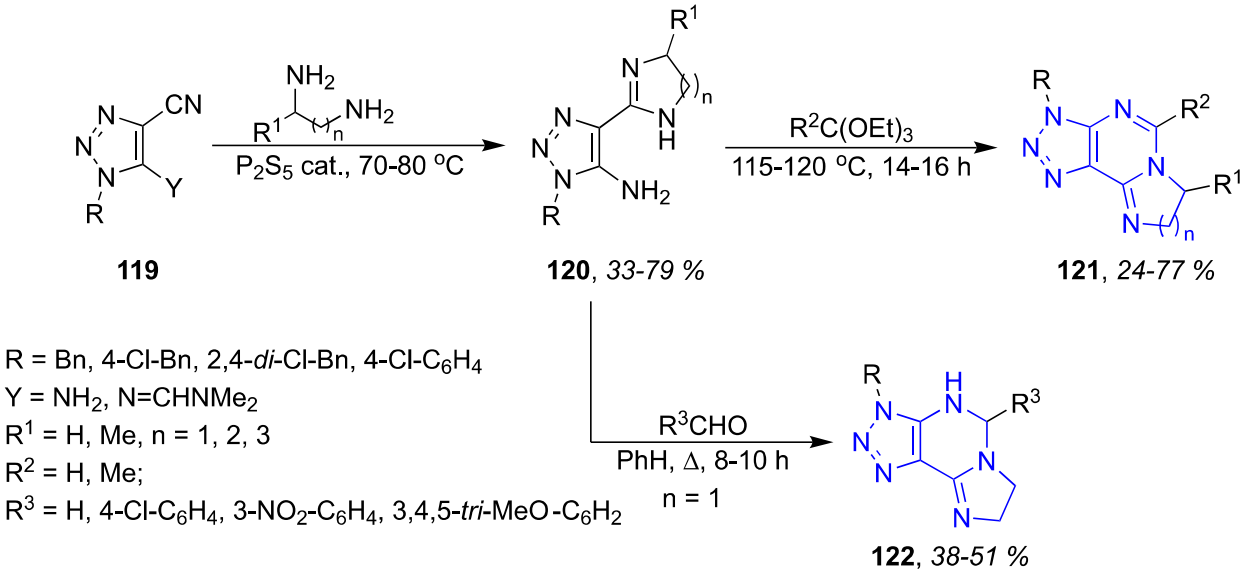
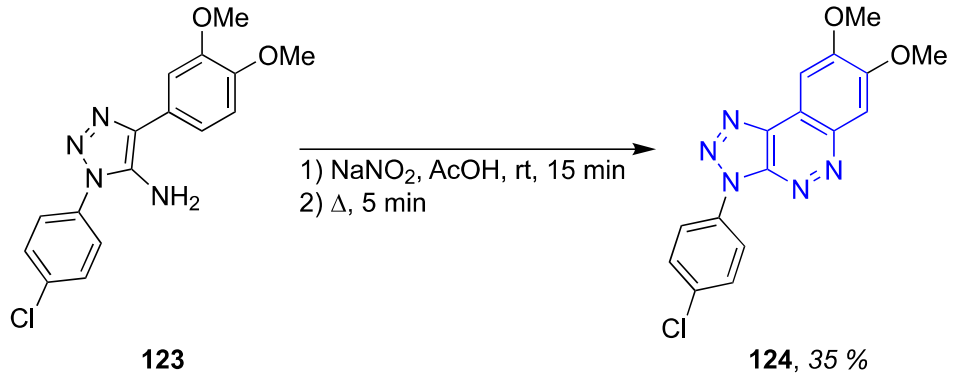


Scheme 38. The synthesis of bi- and tricyclic triazoloannulated pyrimidine systems

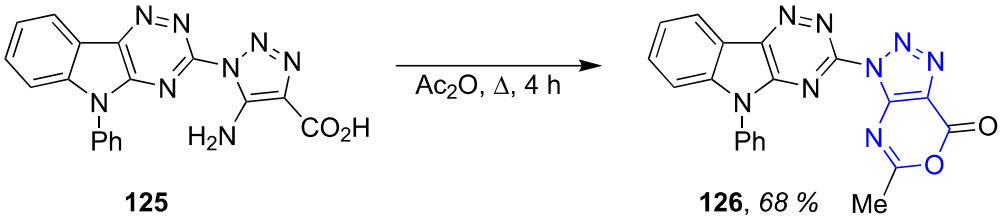


Scheme 39. The synthesis of tricyclic triazole-containing pyrimidines **121-122**



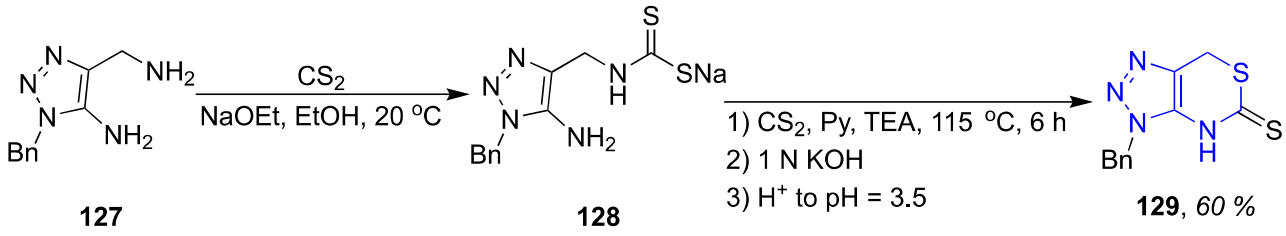
123 **124**, *35 %*

Scheme 40. The synthesis of 3*H*-[1,2,3]triazolo[4,5-*c*]cinoline **124**



125 **126**, *68 %*

Scheme 41. The synthesis of triazolo[4,5-*d*][1,3]oxazine-7-one **126**



127 **128** **129**, *60 %*

Scheme 42. The synthesis of triazolo[4,5-*d*][1,3]thiazine-5-thione **129**

4. The synthesis of triazoloannulated di-, oxa-, and thiazepines

In addition to the triazoloannulated azine structures described above, aminotriazoles also turned out to be important substrates for the synthesis of triazolodi(oxa-, thi)azepine systems.

The condensation of 4,5-diaminotriazoles **130** with β-dicarbonyl compounds proved to be a convenient tool for constructing a triazolo[4,5-*b*][1,4]-diazepine core with varying degrees of saturation (Scheme 43) [73]. Thus, a series of 1,5,7-substituted 1,6-dihydrotriazolo[4,5-*b*][1,4]diazepines **133** was obtained by the reaction of triazoles **130** with dibenzoylmethane, benzoylacetone and acetylacetone **131**. In turn, the reaction with ethyl acetoacetate and ethyl butyroacetate **133** proceeded through the step of forming enamino derivatives **134**, which were cyclized under basic conditions to 3,7-disubstituted triazolo[4,5-*b*][1,4]diazepin-5-ones **135**. In the case of benzoyl acetate **136**, the initially formed amides **137** were cyclized under acidic conditions to 1,7-disubstituted triazolodiazepin-5-ones **138**. Finally, 1,6-disubstituted triazolo[4,5-*b*][1,4]diazepine-5,7-diones **140** were obtained by the cyclocondensation of triazoles **130** with diethyl 2-methyl(2-phenyl)-malonate **139**.

For the synthesis of triazolo[4,5-*b*][1,5]benzodiazepines **144**, aminonitriles **141** were subjected to *N*-arylation with *ortho*-halogenonitrobenzenes **142** to derivatives **143**, their reduction and subsequent cyclization were done by the action of anhydrous $SnCl_2$ in an alcoholic solution of HCl (Scheme 44) [10, 74].

For the synthesis of optically active triazolo-[4,5-*d*][1,3]diazepin-8-oles **148** and **149**, *N*′-(4-formyltriazol-5-yl)-*N*,*N*-dimethylformimidates **145** were converted into trimethylsilylcyanohydrins **146**, then the reduction of the nitrile group with Raney nickel was accompanied by fusion of the diazepine ring and the formation of triazolodiazepine **147** (Scheme 45). The deprotection of the β-*D*-ribofuranosyl fragment and the subsequent chromatographic separation of racemates **147** yields target products with a high optical purity [75].

A convenient method for the synthesis of isoelectronic analogs of isoazepinomycin, triazolo-[4,5-*e*][1,4]diazepine derivatives, was developed

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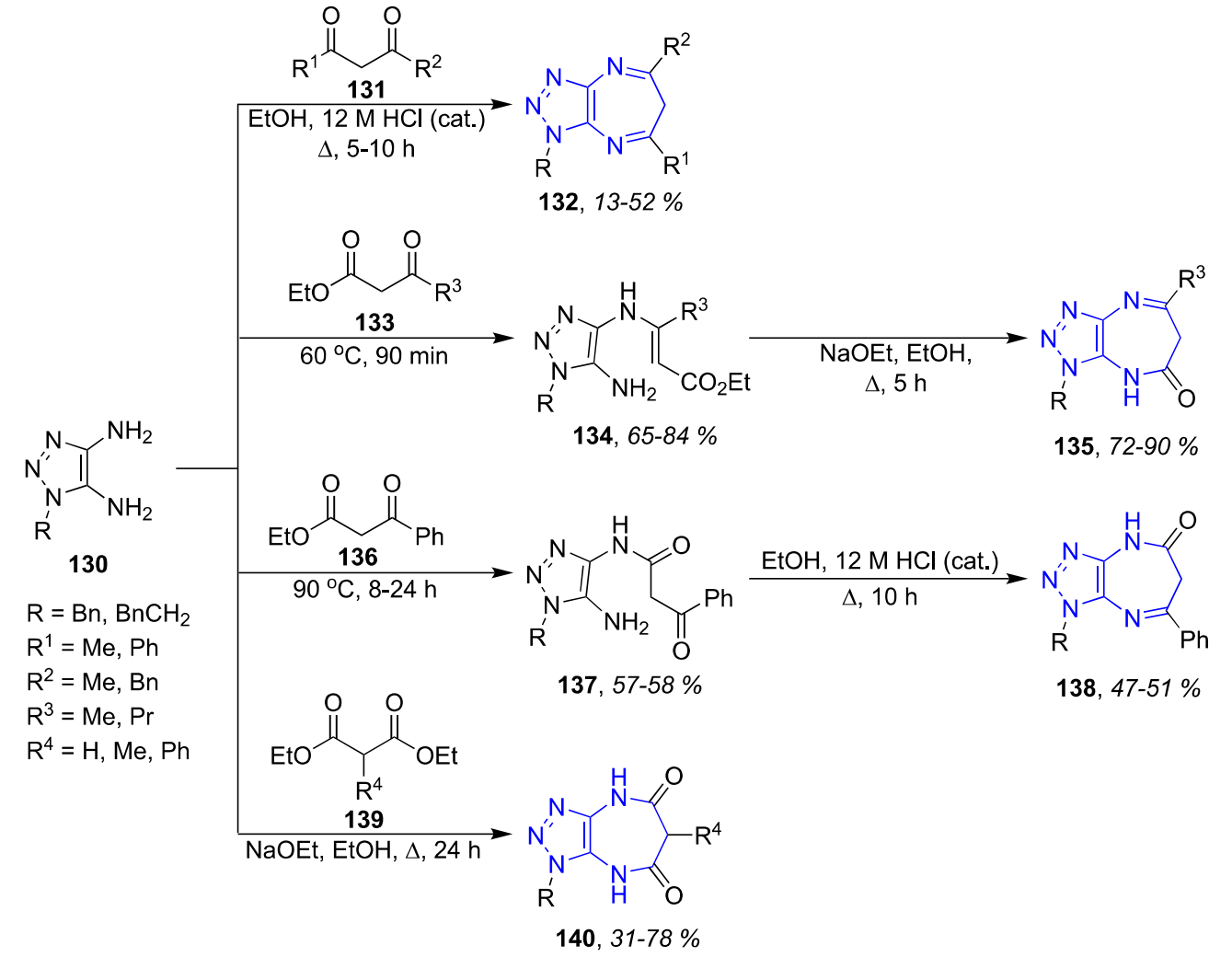
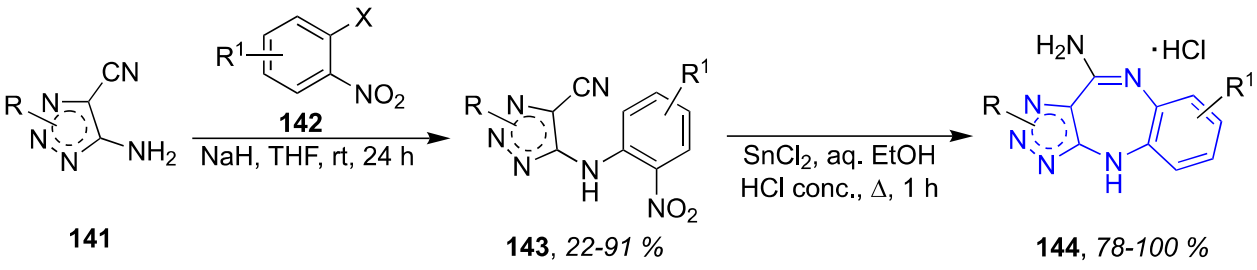


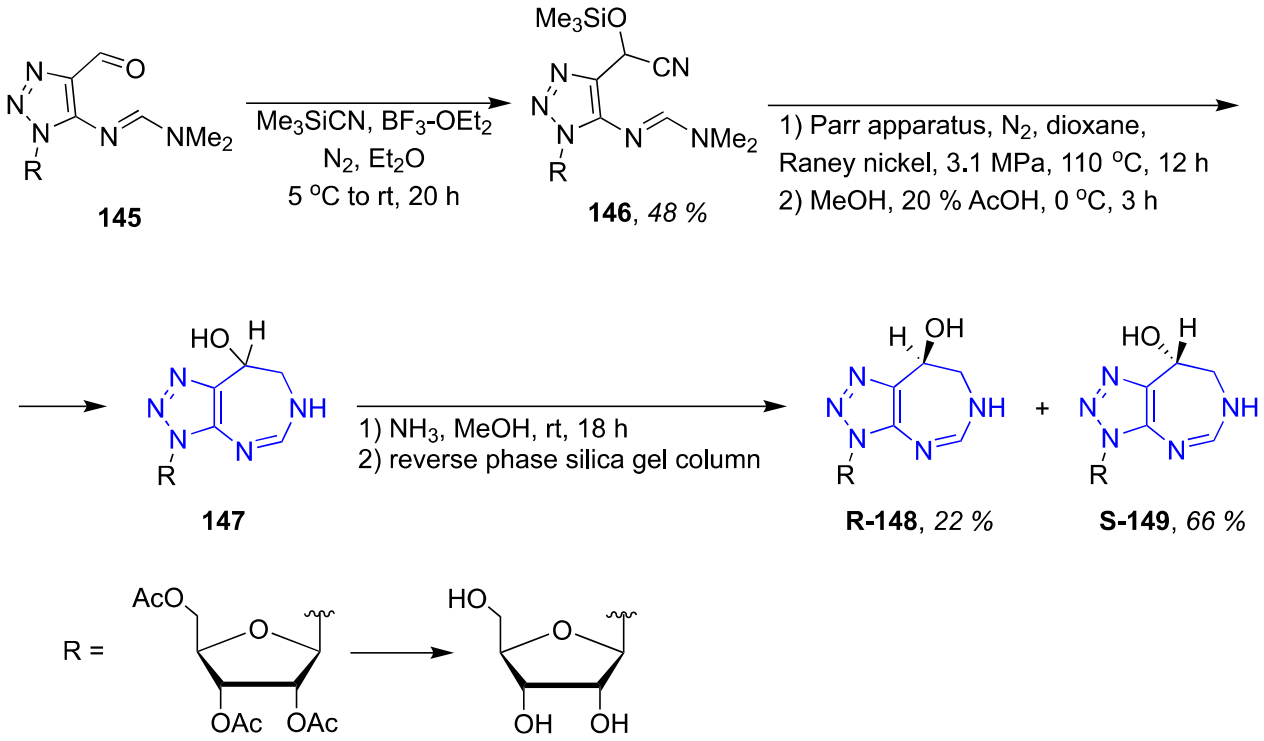
Scheme 43. 4,5-Diaminotriazoles **130** in the synthesis of triazolo[4,5-*b*][1,4]diazepines



Scheme 44. The synthesis of triazolo[4,5-*b*][1,5]benzodiazepines **144**

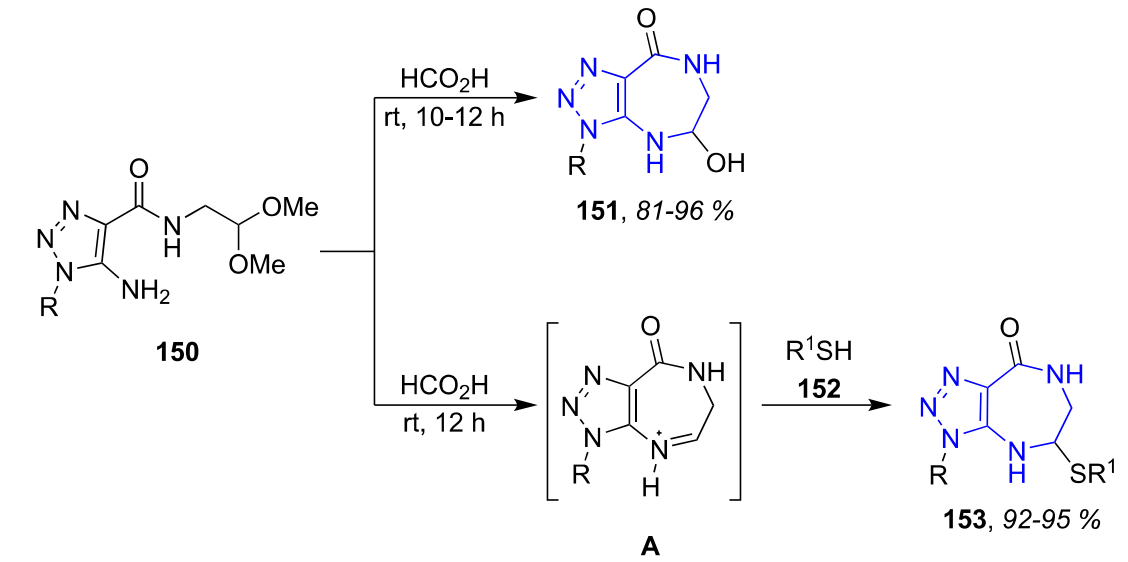
based on the intramolecular cyclization of *N*-functionally substituted aminotriazolocarboxamides **150** (Scheme 46). It was found that the latter were easily cyclized in formic acid at room temperature to 5-hydroxysubstituted triazolo[4,5-*e*][1,4]-diazepines **151** in almost quantitative yields. Under similar conditions, the action of *S*-nucleophiles **152** led to 5-sulfanylsubstituted triazolodiazepines **153**. It was most likely that in this reaction, the acid-catalyzed formation of the cyclic iminium intermediate **A** took place, to which the reagents containing the thiol group were then added [76].

N-Boc-4-amino-1,2,3-triazole-5-carboxylic acids **154** are a new type of bifunctional reagents. They were transformed into the corresponding amides **155** by the action of ethyl glycinate hydrochloride in the presence of a 2-fold excess of CDI (Scheme 47). Removal of the Boc-protection from their amino group by the action of an



145 **146**, *48 %* **147** **R-148**, *22 %* **S-149**, *66 %*

Scheme 45. The synthesis of triazolo[4,5-*d*][1,3]diazepin-8-oles **148**-**149** with optical purity



150 **151**, *81-96 %* **A** **153**, *92-95 %*

R = *i*Bu, Bn, 4-MeO-Bn, 3-Cl-Bn, Ph, 4-Cl-C_6H_4, 4-Me-C_6H_4, 4-MeO-C_6H_4, 4-NO_2-C_6H_4, 2,4-*di*-F-C_6H_3, 2,4-*di*-Me-C_6H_3, 1-methylpyrazol-3-yl

R^1 = CH_2CO_2H, CH_2CO_2Me, Ac, 4-Me-C_6H_4

Scheme 46. The synthesis of 5-hydroxy- та 5-sulfanylsubstituted triazolo[4,5-*e*][1,4]diazepines **151, 153**

equivalent amount of hydrogen chloride in dioxane at room temperature and the subsequent cyclocondensation by the action of NaOEt in the ethanol solution were optimal conditions for obtaining target triazolo[4,5-*e*][1,4]diazepine-5,8-diones **156** [77].

The authors of [78] used the reduction of amino ketones **157** using $NaBH_4$ to alcohols **158**; they proved to be convenient substrates for further transformations (Scheme 48). In particular, triazolooxazepinones **160** were obtained by the reaction of amino alcohols **158** with bromoacetyl bromide **159** followed by the cyclization under basic conditions. In turn, triazolothiazepinone **162** was synthesized by the cyclocondensation of aminoalcohol **158** with thioglycolic acid (**161**).

A selective *S*-alkylation of 4-(*N*-Boc-amino)-1,2,3-triazole-5-carbothioamides **163** with ethyl

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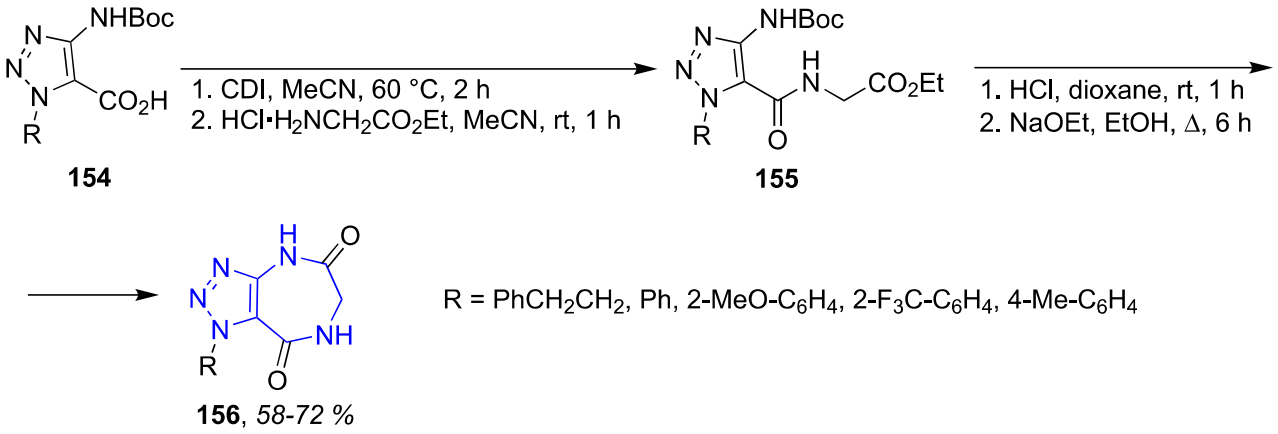
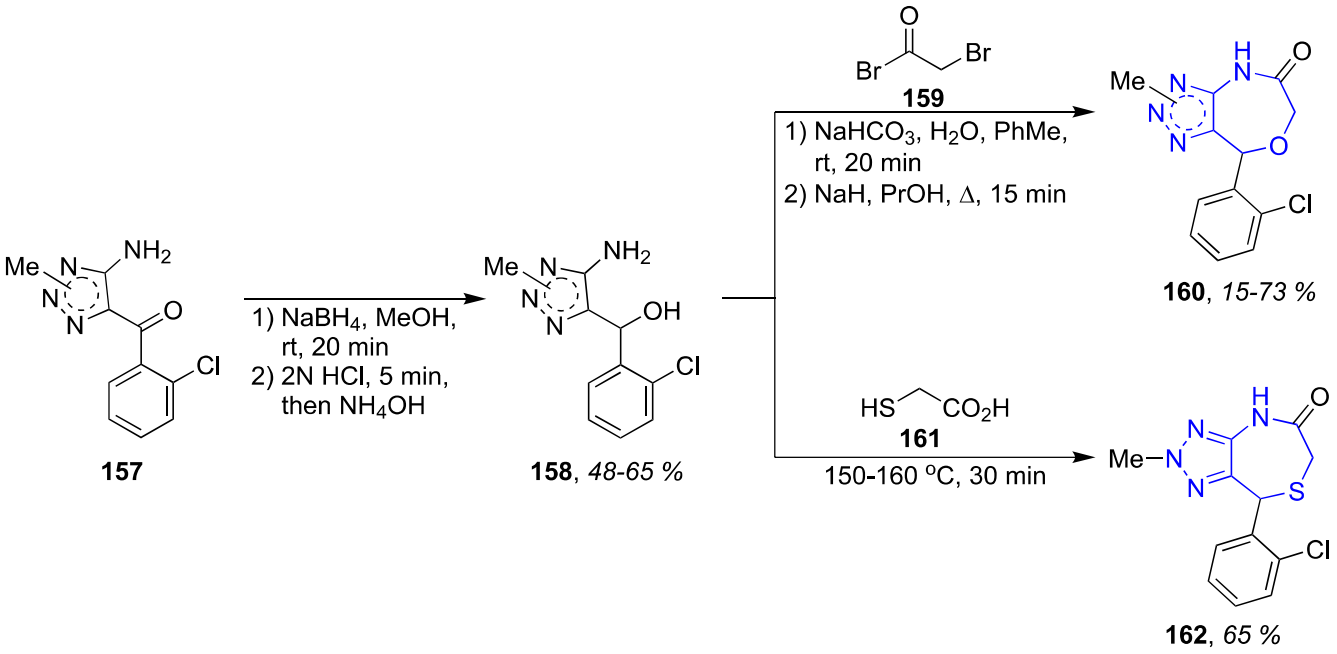
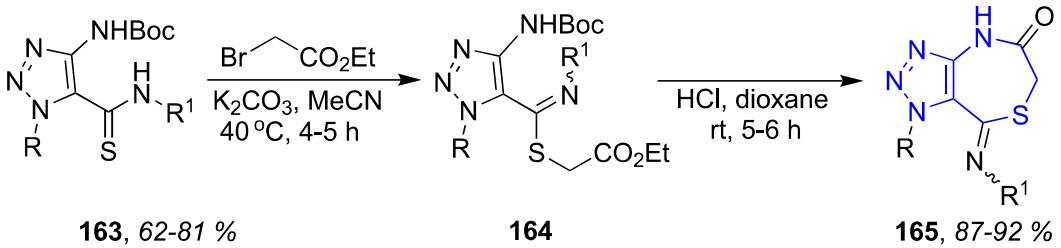


Scheme 47. The synthesis of triazolo[4,5-*e*][1,4]diazepine-5,8-diones **156**



Scheme 48. Preparation of triazolooxo- and triazolothiazepinones **160** and **162**



R = Me, R^1 = Et, *i*Pr, *t*Bu, cyclohexyl
R = CH_2CH_2Ph, R^1 = Et, *t*Bu
R = Ph, R^1 = Et, *t*Bu
R = 2-MeO-C_6H_4, R^1 = Et, *i*Pr

Scheme 49. The synthesis of [1,2,3]triazolo[4,5-*e*][1,4]thiazepin-5(6*H*)-ones **165**

bromoacetate under mild conditions led to the formation of 4-(*N*-Boc-amino)-5-thioimidates **164** (Scheme 49). The latter, when the protective Boc-group was removed by the action of hydrogen chloride in dioxane, underwent the intramolecular cyclocondensation with the formation of target [1,2,3]triazolo[4,5-*e*][1,4]thiazepin-5(6*H*)-ones **165** in high yields [79].

■ Conclusions

The analysis, systematization and generalization of literature sources related to the synthetic potential of 1,2,3-triazole-4(5)-amines convincingly indicate that structures of this type are easily accessible and convenient building blocks for the construction of triazoloannulated pyridine, azine and azepine systems that are important for synthetic and biomedical research.

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Selection of "Green" Conditions for Identifying Components in a Combined Medicine by TLC/HPTLC Methods

Abstract

Aim. To select "green conditions" for identifying components in a combined medicine for the treatment of alcohol intoxication.
Materials and methods. Thin-layer chromatography and high-performance thin-layer chromatography methods were used. An analytical GREEnness calculator was applied to assess the environmental friendliness of the analytical procedure.
Results and discussion. The choice of mobile and stationary phases that comply with the principles of "green chemistry" and can be used to detect glutamic acid and glycine in the composition of a combined medicine has been substantiated. It has been determined that by the indicators R_f, R_s, ΔR_f, α, N, H the most effective for the division is the *ethanol (96 %) – water* (70:30) mobile phase (the length of the solvent front is 10 cm, the application volume is 5 µL), which allows, in addition to amino acids, to determine another prescription component – ascorbic acid. The conditions for identification of substances by the high-performance thin-layer chromatography method (the length of the solvent front is 7 cm, the application volume is 2 µL) have been selected. It has been found that to detect chromatographic zones, it is optimal to use *ninhydrin solution R1* with further heating of the plate at a temperature of 100–105 °C for 5 min. The specificity of determination of glutamic acid, glycine and ascorbic acid in comparison with solutions of standard substances has been proven. While studying the robustness of the method the influence of chromatographic conditions on the final result (influence of plate materials of different manufacturers, chamber saturation, application volume, distance from the "start line" to "finish line", influence of the detection solution, the stability of analyte in solution and on the plate) has been researched. The precision of the method on one and three plates of the same type has been studied; the intermediate precision has been researched. The calculated assessment of greenness of the analytical procedure is 0.66.
Conclusions. As a result of the studies conducted, "green conditions" for identifying amino acids (glutamic acid, glycine), as well as ascorbic acid in a combined medicine by thin-layer chromatography and high-performance thin-layer chromatography methods have been selected. The validation characteristics of the method (specificity, robustness and precision) have been studied.
Keywords: "green" chemistry; thin-layer chromatography; high-performance thin-layer chromatography; glycine; glutamic acid; ascorbic acid

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Добір «зелених» умов для ідентифікації компонентів у комбінованому лікарському засобі методами ТШХ/ВЕТШХ

Анотація

Мета. Добрати «зелені» умови для ідентифікації компонентів у складі комбінованого лікарського засобу, призначеного для лікування алкогольної інтоксикації.
Матеріали та методи. Під час дослідження використано методи тонкошарової та високоефективної тонкошарової хроматографії. Для оцінювання екологічності аналітичної методики було застосовано аналітичний калькулятор «GREEnness».
Результати та їх обговорення. Обґрунтовано вибір рухомих фаз, які відповідають принципам «зеленої хімії» та можуть бути використані для виявлення глутамінової кислоти та гліцину у складі комбінованого лікарського засобу. Визначено, що за показниками R_f, R_s, ΔR_f, α, N, H найефективнішою для розділення є рухома фаза *етанол (96 %) – вода* (70:30) (довжина фронту розчинника – 10 см, об'єм для нанесення – 5 мкл), яка дозволяє, окрім амінокислот, також визначити ще один компонент пропису – кислоту аскорбінову. Дібрано умови для визначення речовин методом

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високоефективної тонкошарової хроматографії (довжина фронту розчинника – 7 см, об'єм для нанесення – 2 мкл). З'ясовано, що для виявлення хроматографічних зон оптимальним є використання *нінгідрину розчину Р1* із подальшим нагріванням пластини за температури 100–105 °С протягом 5 хвилин. Доведено специфічність визначення глутамінової кислоти, гліцину та аскорбінової кислоти проти розчинів стандартних речовин. Під час вивчення робасності методики досліджено вплив умов проведення хроматографування на кінцевий результат (тип нерухомої фази, насиченість камери, об'єм нанесення, відстань від лінії старту до фінішу, розчин для виявлення, стабільність розчинів для нанесення). Вивчено прецизійність методики на одній та на 3-х пластинах одного типу, досліджено внутрішньолабораторну прецизійність. Розрахункова оцінка «зеленості» аналітичної методики становить 0,66.

Висновки. У результаті проведених досліджень було дібрано «зелені» умови для ідентифікації амінокислот (глутамінової кислоти, гліцину) та аскорбінової кислоти в комбінованому лікарському засобі методами тонкошарової і високоефективної тонкошарової хроматографії, а також вивчено валідаційні характеристики методики (специфічність, робасність та прецизійність).

Ключові слова: «зелена хімія»; тонкошарова хроматографія; високоефективна тонкошарова хроматографія; гліцин; глутамінова кислота; аскорбінова кислота

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■ Introduction

Nowadays pharmaceutical analysis of medicines presupposes the use of chemical substances, solvents and reagents, which, of course, have a negative impact on both the laboratory personnel and the environment. Quite often, a significant volume of waste, including toxic organic solvents, which require further processing and disposal, can also appear after carrying out the analysis.

Taking into consideration the increasing concern of the society for the environment, modern approaches to the pharmaceutical development and research foresee the implementation of the concept "*Quality by design*", which means not the use of the correct methods of drug quality control, but also taking into account modern demands to these methods from the point of view of "green chemistry". The "green chemistry" principles are aimed at diminishing the resources, energy costs, time minimization, actions and operations during the analysis, waste minimization and, where possible, replacement of toxic chemicals with less harmful and safer for people [1–5].

Amino acids used in medicine for treating metabolic disorders, diseases of the gastrointestinal tract, nervous system, etc., are common active pharmaceutical ingredients of both mono- and combined medicines. A considerable arsenal of analytical methods is used to control the quality of such medicines in modern pharmaceutical analysis. However, chromatographic methods of research, which are known to allow solving several issues of drug quality control at the same time, play the main role [6, 7].

For example, thin-layer chromatography method (TLC) is an important pharmacopoeial express method for the identification and semi-quantitative determination of amino acids [8–13]. Due to differences in the chemical structure of the compounds analyzed, their solubility and polarity, they enter into a specific interaction with the stationary and mobile phases, which causes different speed of the substance transport [12]. This allows the simultaneous separation of both amino acid mixtures, including enantiomers and medicines, which are combinations of amino acids with other active pharmaceutical ingredients. In addition, the method is convenient and economical.

High-performance thin-layer chromatography method (HPTLC) is becoming more and more common nowadays as an alternative and more "green" method for studying amino acids. Due to the use of plates with a smaller particle size of the sorbent (from 2 to 10 μm), the effective chromatographic separation is achieved using a smaller plate size, application volume, and, accordingly, spending less mobile phase volume [12, 13].

However, the procedure of carrying out the research by TLC/HPTLC methods quite often requires the use of toxic solvents both for the

moving phase, the samples under study, and for the identification of the chromatography zones obtained [1, 3, 4]. The amino acid analysis is most often performed in the system of the normal-phase chromatography where combinations of silica gel with two- or three-component mobile phases are used for the research. One of the solvents is often acetone, methanol, acetic and formic acid, sometimes with the addition of ammonia, pyridine, chloroform, etc. [7, 12, 13].

One of the twelve principles of "green chemistry" supported by *P. T. Anastas* and *J. C. Warner* in 1998 is the use of safer solvents and excipients. As a result, there has been a growing trend in recent decades to use more environmentally friendly alternative solvents in analytical research [14].

An important issue of choosing a solvent is the assessment of its environmental friendliness. To date, there are numerous guidelines in the scientific literature on the choice of solvents, which provide recommendations for the choice of solvents, taking into account their impact on the environment, stability, flammability and explosiveness [3–5, 14, 15]. However, the use of environmental assessment criteria requires special tools that will allow obtaining an easily interpreted and informative result, especially for comparing analysis methods. For this purpose, a GREEnness analytical calculator is used; its evaluation criteria are taken from the twelve principles of "green chemistry", transformed into a unified scale 0–1, and the final score is calculated based on the principles of importance. The result is an icon indicating the final score, the effectiveness of the analytical procedure for each criterion and the user-assigned weights that are convenient to use both when developing a new "green" method of analysis and comparing it with the existing methods [16].

In the modern scientific literature, scientific publications that offer more "green" mixtures of mobile phases for the amino acid analysis, including pure water, aqueous solutions of surfactants, ethyl acetate, *n*-butanol, *n*-butyl acetate, ethylene glycol, have been described. They may become the dominant "green eluents" in the chromatographic analysis [7, 14].

However, taking into account the significant variety of medicines with amino acids, the possible effects of active substances and excipients, this issue still remains open.

Therefore, the selection of conditions for the efficient separation of amino acids included into the composition of combined medicines, which will allow us to identify specifically the substances studied and which will meet the principles of "green chemistry", is an urgent task of the research.

Materials and methods

The study object was a combined original medicine intended for the pharmacotherapy of alcohol intoxication. By its chemical composition it is a mixture of amino acids with other substances in the form of an effervescent powder (two sachets) for the preparation of oral solution (*sachet bag 1*: glutamic, acetylsalicylic, ascorbic acids and anhydrous citric acid, sorbitol; *sachet bag 2*: glycine, sodium bicarbonate, sorbitol) [17, 18].

The research was carried out according to the requirements of the European Pharmacopoeia (EP)/the State Pharmacopoeia of Ukraine (SPhU), 2.2.27 "Thin-layer chromatography" [19, 20]. The identification procedure was performed simultaneously with the determination of its validation characteristics [19, 20].

A GREEnness analytical calculator for the assessment of the analytical procedure's greenness was also used [16].

Stationary phase

TLC-plates: TLC Silica gel (Supelco), aluminum plates; TLC Silica gel 60 (Merck), aluminum plates; Silica gel 60 (Merck), glass plates; *HPTLC-plates:* HPTLC Silica gel 60 (Merck), glass plates.

Verification of the separation ability of the stationary phase for the identification was performed as required by the EP/SPhU, 4.1.1 [19, 20].

Before using the plates were activated by means of heating in an electric drying cabinet (model "2 ІІІ-0-01") at a temperature of 120°C for 20 min [19, 20] to remove residual moisture, which could reduce the sorbent activity.

Solvents and reagents

Water for chromatography and solvents of the appropriate quality and purity meeting the requirements of the EP/SPhU were used for the study.

Reference standards (RS)

The substances of glutamic acid (c. SLBS0553V, manufactured by Sigma Aldrich), glycine (c. LRAA8813, manufactured by Sigma Aldrich) and ascorbic acid (c. DYD2622000008, manufactured by Northeast Pharmaceutical Group Co., Ltd) were used as reference standards.

Test solutions (TS)

Test solution 1 (TS-1). To 200 mg of powder from sachet bag 1, equivalent to 25 mg of glutamic

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acid and 5 mg of ascorbic acid, add 15 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min and cool. Dilute to 25.0 mL with *water R* and mix.

Test solution 2 (TS-2). To 130 mg of powder from sachet bag 2, equivalent to 10 mg of glycine, add 15 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min and cool. Dilute to 25.0 mL with *water R* and mix.

Reference solutions (RS)

Glutamic acid reference solution (RS-1). To 25 mg of *glutamic acid RS*, add 15.0 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min, cool. Dilute to 25.0 mL with *water R* and mix.

Glycine reference solution (RS-2). Dissolve 10 mg of *glycine RS* in *water R* and dilute to 25.0 mL with the same solvent.

The mixture of glutamic acid and glycine reference solutions (RS-3). To 25 mg of *glutamic acid RS* and 10 mg of *glycine RS,* add 15.0 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min, cool. Dilute to 25.0 mL with *water R* and mix.

Ascorbic acid reference solution (RS-4). Dissolve 5 mg of *ascorbic acid RS* in *water R* and dilute to 25.0 mL with the same solvent.

The type and configuration of the chromatographic chambers

To conduct the research, the chambers with the split-performance of the firms "Sorbfill" (19×19.5×6.5 cm) and "Camag" (27×7×26 cm) were used.

Drying the plates after the elution and detection of chromatographic zones (derivatization)

Drying the plates after the elution was performed in the air. The detection of chromatographic zones was carried out by sprinkling with a solution for detection and further heating in a drying electric cabinet (model "2III-0-01"). The results were evaluated in the daylight.

For derivatization of chromatographic zones, *ninhydrin solution R* and *ninhydrin solution R1* prepared according to the requirements of the EP/SPhU were used [19, 20].

The sample application

A Hamilton Bonaduz AG microsyringe (Via Crusch 8, CH-7402 Bonaduz, Switzerland) with the volume of 10.0 µL was used for the sample application.

Documentation

After detection, chromatographs were documented using a Camag® TLC Visualizer 2 and a winCATS® software.

Temperature and humidity

To get the results, the experiment was conducted at the temperature of 25°C and the air relative humidity of not more than 75% [19, 20].

Calculation of criteria

The retardation factor (retention factor) (R_f), ΔR_f, resolution (R_s), selectivity (α) was calculated using the following formulas (1–4) [9, 19–22]:

$$R_f = \frac{l}{L}, \quad (1)$$

$$\Delta R_f = R_{f1} - R_{f2}, \quad (2)$$

$$R_s = \Delta X / [\frac{(W_1 + W_2)}{2}], \quad (3)$$

$$\alpha = \frac{\left(\frac{1}{R_{f2}} - 1\right)}{\left(\frac{1}{R_{f1}} - 1\right)} \quad (4)$$

where, l is the distance from the centre of the chromatographic zone to the "start line", mm;
L is the distance of the solvent front, mm;
ΔX is the distance between the centers of chromatographic zones, mm;
W is the height of a chromatographic zone, mm.

The separation efficiency was calculated by the quantity of theoretical plates (N) and their height (H) using the following formulas (5–6) [9, 19–22]:

$$N = 16\left(\frac{L \times R_f}{W}\right)^2, \quad (5)$$

$$H = \frac{L}{N}. \quad (6)$$

The study of validation characteristics

Specificity. It was determined on one plate by comparing the chromatographic zones of *Test* and *Reference solutions*. The method is specific if the chromatographic zones obtained with *Test solutions* are similar to the chromatographic zones of *Reference solutions* with respect to position, color, and intensity of bands.

Robustness. The influence of chromatographic conditions on the final result (the impact of the stationary phase type, chamber saturation, application volume, distance from the "start line" to the "finish line", the effect of the detection solution, stability of solutions for application) was assessed.

The influence of the chamber saturation. The study was conducted in a chromatographic chamber pre-saturated (for 1 h) and unsaturated by an eluent [11, 23].

The influence of the distance that the mobile phase should overcome. Such distances of solvents front as 10, 12 and 15 cm were compared [11].

The influence of plate materials of different manufacturers. The impact of the plate materials of different manufacturers on the result of the separation capacity of the mixture components and the fluctuation of R_f values for the corresponding zones was assessed.

The influence of the volume of application solutions. The results obtained during application of *Test solutions* and *Reference solutions* of different volumes were compared.

The stability of the analyte in the solution and on the plate. The stability of the solutions studied was assessed simultaneously on one TLC plate. *Test solutions* and *Reference solutions* were applied 3 hours earlier and immediately before chromatography.

The stability of derivatization results. The impact of heating conditions of the plate after the elution at a temperature from 100°C to 105°C for 5–15 min was studied.

The stability of chromatographic results. The stability results were assessed in 5, 15, 30 and 60 min after chromatography.

Precision. R_f values for chromatographic zones with testing on one TLC plate, on 3 different plates of the same type were calculated. The analysis was performed on different days by different analysts (the intermediate precision) [7, 12, 19, 20, 23].

■ Results and discussion

1. The TLC-method development

To select conditions for the identification of amino acids (glutamic acid and glycine) in a combined medicine for the treatment of alcohol intoxication by TLC/HPTLC methods, the primary task of the research was the choice of the mobile phase, which would meet the principles of "green chemistry" and was suitable for separation of amino acids in a combined medicine.

A great number of mobile phases for the identification of amino acids by the TLC method have been described in the scientific literature. For example, in the EP/SPhU, glycine and glutamic acid are determined in the mobile phase of *glacial acetic acid – water – butanol* (20:20:60) [10, 24]; among other mobile phases *isopropyl alcohol – concentrated ammonia* (70:30), *acetonitrile – water* (10:90), *chloroform – methanol* (1:1), etc., are the most common [7, 12, 13]. However, based on the work of scientists in defining the criteria of solvents according to their safety, the impact on human health and environment [1–5, 24], it was found that not all combinations of solvents used met the principles of "green chemistry".

Thus, in the theoretical evaluation of these mobile phases using a GREEnness analytical calculator [16] (Figure 1) it was found that under the same conditions of determination (the number of test samples, type and amount of a derivatizing reagent), but with different types and volumes of solvents and the total amount of waste, the methods analyzed had similar values of the degree of environmental friendliness.

In accordance with the principles of "green chemistry" in the development of "green" methods of research the choice of solvents should avoid the formation of large volumes of waste and give preference to reagents derived from renewable sources. Commonly recommended analytical solvents (after water) are alcohols (ethanol, isopropyl alcohol, butanol, etc.) [1, 4, 5].

When evaluating the existing mobile phases for the amino acid analysis using a GREEnness analytical calculator it was found that the combinations "alcohol – water" had values close to 1, indicating that the evaluated procedure was

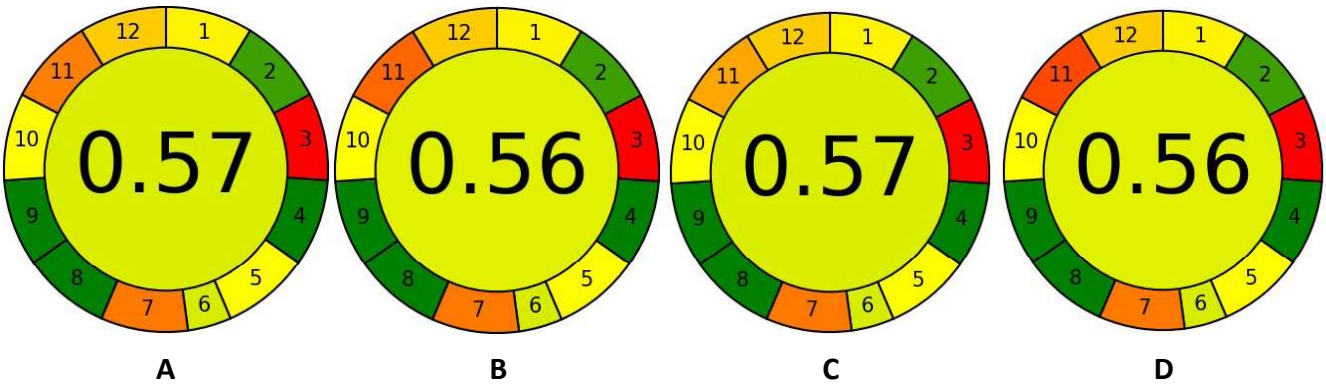

Figure 1. The results of the assessment of analytical procedures of greenness using different mobile phases: **A** *glacial acetic acid – water – butanol* (20:20:60); **B** *isopropyl alcohol – concentrated ammonia* (70:30); **C** *acetonitrile – water* (10:90); **D** *chloroform – methanol* (1:1)

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Table 1. Chromatographic parameters R_f, ΔR_f, R_s, α, *N, H* on the TLC plate Supelco, aluminum plate

Mobile phase/analyte		R_f	ΔR_f	R_s	α	*N*	*H*
Mobile phase 2: *isopropyl alcohol – water* (70:30)	Glutamic acid	0.40 ± 0.01	0.04	1.00	1.19	1024	0.098
	Glycine	0.36 ± 0.01				829	0.121
Mobile phase 3: *ethanol (96 %) – water* (70:30)	Glutamic acid	0.58 ± 0.01	0.10	2.50	1.50	3364	0.030
	Glycine	0.48 ± 0.01				2304	0.043

"greener" [16]. Therefore, the following mobile phases were selected for further study:

- mobile phase 1: *isopropyl alcohol – water* (10:90) [7, 13];
- mobile phase 2: *isopropyl alcohol – water* (70:30) [7, 13];
- mobile phase 3: *ethanol (96 %) – water* (70:30) [7, 13, 25].

To select the most optimal mobile phase, the mixture of glutamic acid and glycine, *Reference solution* (RS-3), was used. To standardize the analysis conditions, the distance travelled by the solvent front from the point of application to the "finish line" was 10 cm.

According to the results of the study it was found that mobile phase 1 was not suitable for the separation of glutamic acid and glycine mixture, and unseparated chromatographic zones concentrated near the "finish line". Mobile phases 2 and 3 seemed to be more effective for the implementation of the task set. According to the classification of solvents by *L. Snaider*, isopropyl alcohol and ethanol have close polarity values. In mobile phase 1 the quantity of even more polar solvent – water – three times exceeds its content compared to mobile phases 2 and 3. From literary sources, it is known that with a decrease of the organic solvent concentration, the stronger hydrogen bonds in the amino group with alcohol molecules, which are on the surface of a sorbent, can be formed, and thus, we can see the predominance of the process of sorption on the surface of sorbent over association in the solution [26].

In this regard, further research was conducted using mobile phases 2 and 3; their suitability was assessed with the values R_f, R_s, ΔR_f, *a, N, H* (Table 1).

The chromatographic system must be selective for the separation of substances, which means that the compounds must be retained in different ways. The separation of two substances is practically possible if $R_{f1} > R_{f2}$ and $\Delta R_f \geq 0.1$ [22]. According to the results of the analysis (Table 1), R_f of the first component (glutamic acid) exceeds R_f of the second component (glycine) in each of the given mobile phases. However, when comparing ΔR_f of chromatographic zones it was found that mobile phase 3 had the optimal value. According to the resolution (R_s) and selectivity (α), mobile phase 3 was characterized by a more complete separation of amino acid chromatographic zones. While assessing separation efficiency parameters (*H*, *N*) it was concluded that in mobile phase 3 the balance between the phases was more often achieved, and the separation of the components of the mixture analyzed went on more effectively.

Therefore, mobile phase 3 among tested ones is the most optimal for the separation of glutamic acid and glycine.

It was also concluded that a mixture of glutamic acid and glycine (RS-3) should be used to test the suitability of the chromatographic system for analysis: two clearly separated chromatographic zones should be identified.

The next stage of the research was to select the optimal reagent for detecting chromatographic zones of amino acids. It is known that the effectiveness of the derivatizing reagent is determined by the ability to color the compounds analyzed in a specific way [9]. *Ninhydrin solution R* and *ninhydrin solution R1* are used to identify amino acids according to the EP/SPhU.

When evaluating them according to the principles of "green chemistry" it was found that, by the amount of waste, *ninhydrin solution R* had a higher value of the indicator approaching 1, i.e. was "greener" than *ninhydrin solution R1.*

However, while performing chromatography of the mixture of glycine and glutamic acid (RS-3) in the *ethanol (96 %) – water* (70:30) mobile phase it was found that *ninhydrin solution R1* providing a clearer and more intense coloring of the chromatographic zones was the most optimal one (Figure 2).

The research also showed that the processing of chromatograms consumed less volume of the derivative reagent *ninhydrin solution R1* and reduced the duration of heating (chromatographic zones had intense color after 5 minutes of heating at 100–105°C). Therefore, from the point of view of the efficiency of detection of analytical zones and the most optimal one principles of "green

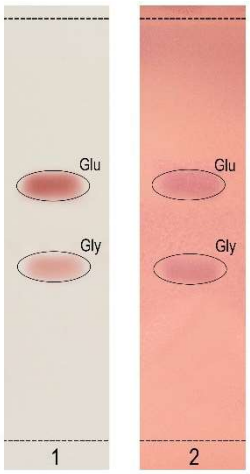

Figure 2. The chromatogram of the mixture of *Reference solution* of glutamic acid (Glu) and glycine (Gly), RS-3, in the *ethanol (96%) – water* (70:30) mobile phase*:* **1** – using *ninhydrin solution R1*; **2** – using *ninhydrin solution R* on the TLC Silica gel (Supelco), aluminum plate

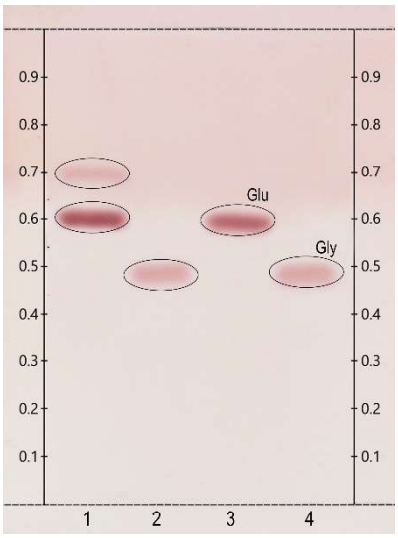

Figure 3. The chromatogram of *Test solution* 1, TS-1 (1); *Test solution* 2, TS-2 (2); *Reference solution* of glutamic acid (Glu), RS-1 (3); *Reference solution* of glycine (Gly), RS-2 (4) on the TLC Silica gel (Supelco), aluminum plate

chemistry", the use of *ninhydrin solution R1* is the optimal for analysis.

In the given conditions the chromatographic study of sachet bag 1 (TS-1) and sachet bag 2 (TS-2) compared to glutamic acid *RS* (RS-1) and glycine *RS* (RS-2) was carried out (Figure 3).

In the above mobile phase, *ethanol (96%) – water* (70:30), for TS-1 and TS-2, chromatographic zones were detected at the level of the zones of glutamic acid *RS* (RS-1) and glycine *RS* (RS-2). The zones of *Reference solutions* and *Test solutions* have a complete separation, coincided in color and location, indicating the specificity of the separation of the mixture components.

After considering the results of chromatography it was found (Figure 3) that in addition to glutamic acid zones in TS-1 there was another unidentified zone. This indicates that another component of sachet bag 1 entered the derivatization reaction with *ninhydrin solution R1*. Taking into account the composition of the sachet bag studied we can assume that ascorbic acid reacts with *ninhydrin* since it has the strong reducing properties (E_o = +0.18 V) [27, 28]. After conducting the study for the second time with ascorbic acid *RS* (RS-4), it was proven that the unidentified zone corresponded to ascorbic acid (Figure 4).

Thus, under the given conditions, it is possible to simultaneously identify three components of the dosage form – glycine, glutamic acid and ascorbic acid. This approach is optimal in terms of the principles of "green chemistry" as it allows reducing the number of analytical operations during the medicine quality control.

2. The TLC-method validation

2.1. Specificity

The method is specific, which is confirmed by the chromatogram (Figure 4). The chromatographic zones obtained with *Test solutions* are similar to the chromatographic zones of *Reference solutions* with respect to the position, color, and intensity of bands.

2.2. Robustness

The next stage of the research was to study the procedure robustness.

The influence of the distance that the mobile phase should overcome

Such distances of the solvent front as 10, 12 and 15 cm were compared (Figure 5).

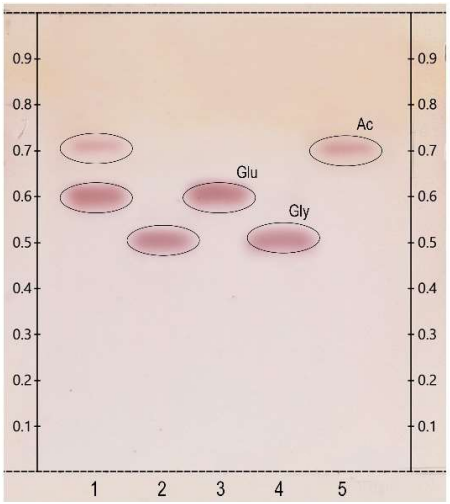

Figure 4. The chromatogram of *Test solution* 1, TS-1 (**1**); *Test solution* 2, TS-2 (**2**); *Reference solution* of glutamic acid (Glu), RS-1 (**3**); *Reference solution* of glycine (Gly), RS-2 (**4**); ascorbic acid RS (Ac), RS-4 (**5**) on the TLC Silica gel (Supelco), aluminum plate

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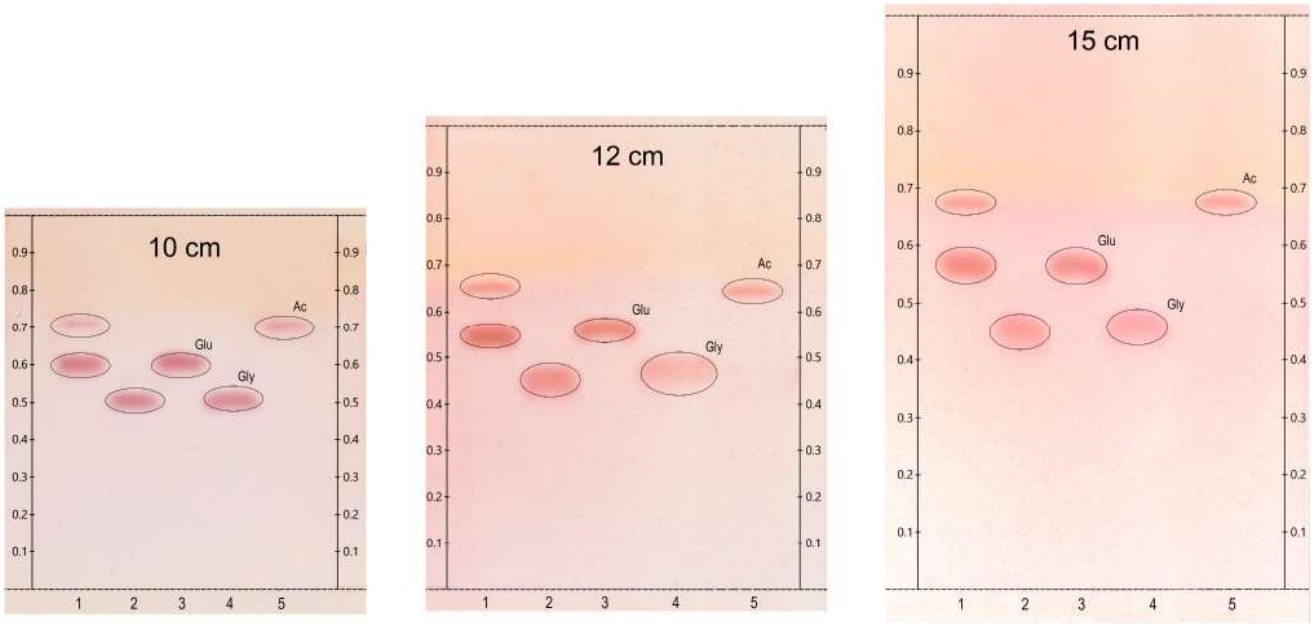

Figure 5. The influence of the distance for the mobile phase to overcome: Test solution 1, TS-1 (**1**); Test solution 2, TS-2 (**2**); glutamic acid RS (Glu), RS-1 (**3**); glycine RS (Gly), RS-2 (**4**); ascorbic acid RS (Ac), RS-4 (**5**) on the TLC Silica gel (Supelco), aluminum plate

A value of 10 cm is optimal as increasing the distance from the "start line" to the "finish line" leads to blurring, lengthening of the zones obtained and, in general, increases the duration of the analysis and reduces the "greenness" of the method [16].

The influence of plate materials of different manufactures

The separation of the mixture components was achieved on all the plates analyzed (Figure 6, Table 2).

However, evaluating the results obtained on the plates of different manufacturers with aluminum and glass plates (Table 2) it was found that a more complete and efficient separation of chromatographic zones was achieved on the plates produced by "Merck".

The influence of the chamber saturation

As a result of the study, it was concluded that in the pre-saturated chromatographic chamber, due to the formation of the gas phase of the eluent, there was a better separation of chromatographic zones. The elution process in these conditions was faster, so the chromatographic time was reduced.

The influence of the volume of the application solution

While conducting the research, the results obtained when applying *Test solutions* and *Reference solutions*, which sample volumes were 5, 10, 15 µL, were compared (Figure 7).

As we can see, the optimal application volume is 5 µL, including 5 µg of glutamic acid, 3.25 µg of glycine and 1.95 µg of ascorbic acid.

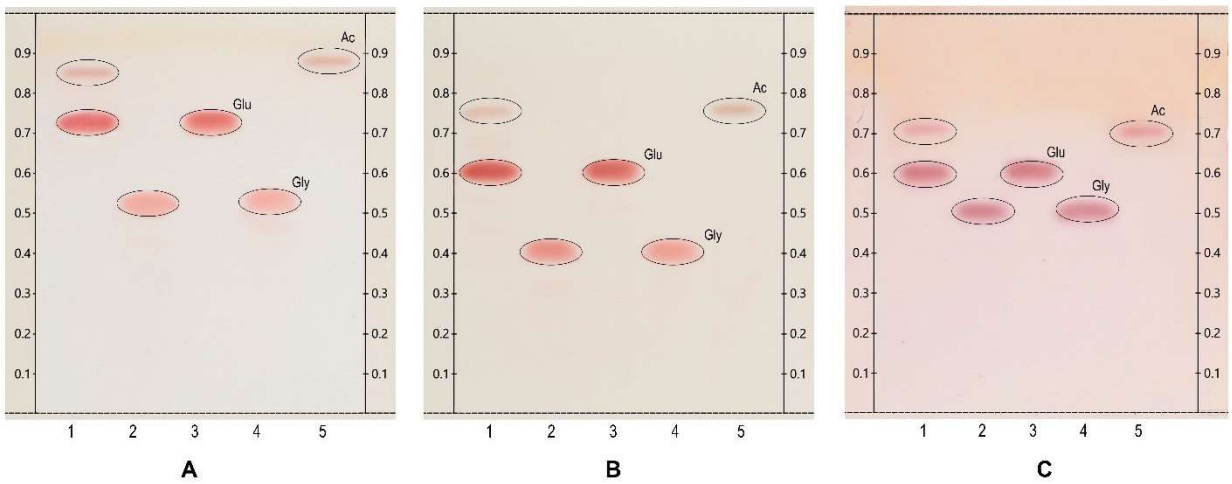

Figure 6. The influence of plate materials of different manufactures: **A** – TLC Silica gel 60 (Merck), glass plate; **B** – TLC Silica gel 60 (Merck), aluminum plate; **C** – TLC Silica gel (Supelco), aluminum plate; **1** – *Test solution* 1, TS-1; **2** – *Test solution* 2, TS-2; **3** – RS-1; **4** – RS-2; **5** – RS-4

Table 2. Chromatographic parameters *Rf*, ΔR_f, R_s, α, *N*, *H* on different TLC plates

Mobile phase/analyte		R_f	ΔR_f	R_s	α	*N*	*H*
TLC Silica gel 60 (Merck), glass plate	Glutamic acid	0.73 ± 0.01	0.21	5.25	2.50	5329	0.019
	Glycine	0.52 ± 0.01				2704	0.037
TLC Silica gel 60 (Merck), aluminum plate	Glutamic acid	0.61 ± 0.01	0.21	5.25	2.35	3721	0.027
	Glycine	0.40 ± 0.01				1600	0.063
TLC Silica gel (Supelco), aluminum plate	Glutamic acid	0.58 ± 0.01	0.10	2.50	1.50	3364	0.030
	Glutamic acid	0.48 ± 0.01				2304	0.043

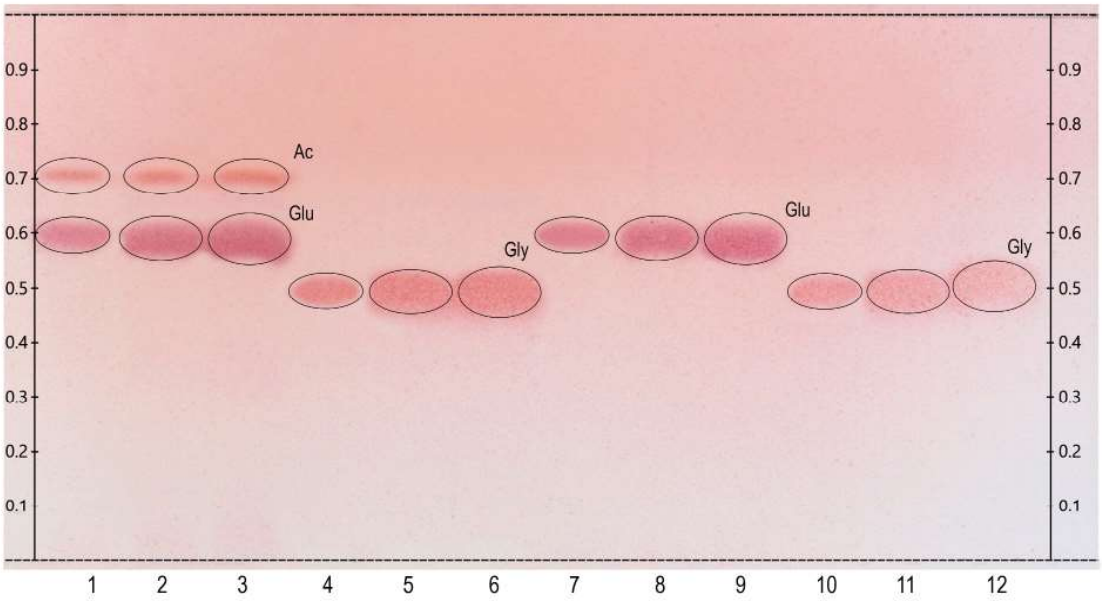

Figure 7. The influence of the volume of the application solution: **1**–**3** – Test solution 1, TS-1; **4**–**6** – Test solution 2, TS-2; **7**—**9** – glutamic acid RS (Glu), RS-1; **10**–**12** – glycine RS (Gly), RS-2: **1**, **4**, **7**, **10** – 5 μL; **2**, **5**, **8**, **11** – 10 μL; **3**, **6**, **9**, **12** – 15 μL on the TLC Silica gel (Supelco), aluminum plate

While increasing the application volume the blurring of chromatographic zones and changing their form can be seen. The size of the chromatographic zone samples allowing to achieve reproducible results is 10×2 mm.

The stability of an analyte in the solution and on the plate

The results are shown in Figure 8. It was found that the individual zones obtained did not differ in location, color, intensity and shape, indicating the stability of the solutions analyzed within the specified period of time.

The stability of derivatization results

As mentioned earlier (Figure 3, 4), during the research it was concluded that heating the plate at a temperature of 100–105°C for 5 min was sufficient for the derivatization process.

The stability of chromatographic results

The stability of the results was evaluated at 5, 15, 30 and 60 min after chromatography. Within the given time, no difference was found between the areas studied.

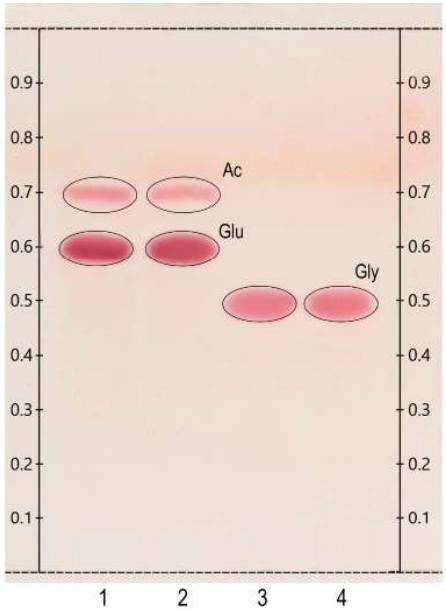

Figure 8. The stability of *Test solution* 1, TS-1 (**1**, **2**) and *Test solution* 2, TS-2 (**3**, **4**): **1**, **3** – samples applied 3 hours before chromatography; **2**, **4** – samples applied just before chromatography on the TLC Silica gel (Supelco), aluminum plate

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2.3. Precision

When studying the procedure precision the values of R_f for the zones of glutamic and ascorbic acids (TS-1) and glycine (TS-2) were calculated:

- on one TLC plate;
- on 3 plates of the same type;
- in the analysis on different days by different analysts (the intermediate precision).

The analysis was performed on the TLC (Supelco) aluminum plate [29].

It was summed up that the chromatographic zones obtained were clearly separated, identical in their location and color. Metrological characteristics of R_f values for chromatographic zones corresponding to glutamic acid, glycine and ascorbic acid are as follows:

- on one plate:

$R_f(Ac) = 0.71$; RSD,% = 0.81%; $R_{f\,max} - R_{f\,min} = 0.01$
$R_f(Glu) = 0.58$; RSD,% = 1.00%; $R_{f\,max} - R_{f\,min} = 0.01$
$R_f(Gly) = 0.48$; RSD,% = 1.21%; $R_{f\,max} - R_{f\,min} = 0.01$

- on 3 plates of the same type:

$R_f(Ac) = 0.72$; RSD,% = 1.61%; $R_{f\,max} - R_{f\,min} = 0.02$
$R_f(Glu) = 0.58$; RSD,% = 1.72%; $R_{f\,max} - R_{f\,min} = 0.02$
$R_f(Gly) = 0.48$; RSD,% = 2.08%; $R_{f\,max} - R_{f\,min} = 0.02$

- the intermediate precision:

$R_f(Ac) = 0.72$; RSD,% = 2.90%; $R_{f\,max} - R_{f\,min} = 0.04$
$R_f(Glu) = 0.58$; RSD,% = 3.45%; $R_{f\,max} - R_{f\,min} = 0.04$
$R_f(Gly) = 0.48$; RSD,% = 4.31%; $R_{f\,max} - R_{f\,min} = 0.04$

For the TLC method developed for the analysis of amino acids and ascorbic acid using a "GREEnness" analytical calculator the greenness of the analytical procedure was calculated (Figure 9). As we see, it exceeds the results when using other mobile phases (Figure 1). Thus, we can conclude that the method developed is greener.

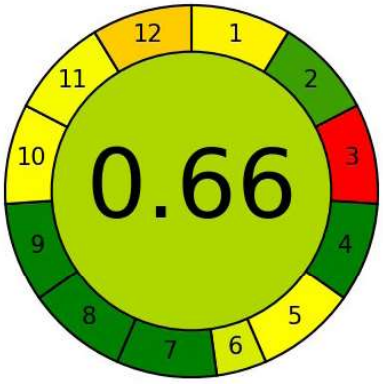

Figure 9. The results of assessing the greenness of the analytical procedure for the method developed

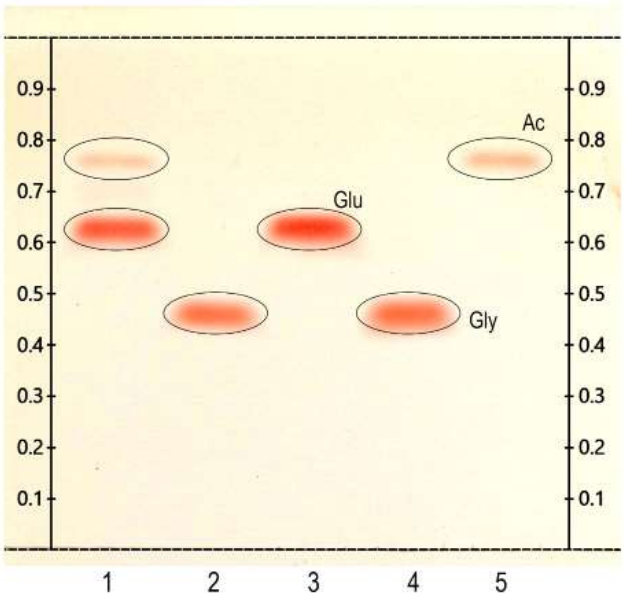

Figure 10. The chromatogram of *Test solution* 1, TS-1 (**1**); *Test solution* 2, TS-2 (**2**); glutamic acid (Glu) *RS*, RS-1 (**3**); glycine *RS* (Gly), RS-2 (**4**); ascorbic acid *RS* (Ac), RS-4 (**5**) on the HPTLC Silica gel (Merck), glass plate

3. Development of the HPTLC method

At the same time, a procedure was developed for its use by the HPTLC method. The research was conducted at the stage of studying robustness. The optimal sample volume for plate application was 2 µL; the solvent front distance was 7 cm. The chromatographic results are shown in Figure 10.

4. Method of analysis

The validated TLC/HPTLC method for analyzing glutamic acid, glycine, ascorbic acid is given below.

Thin-layer chromatography (EP/SPhU, 2.2.27).

Test solution (a): To 200 mg of powder from sachet bag 1, equivalent to 25 mg of glutamic acid and 5 mg of ascorbic acid, add 15 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min, cool. Dilute to 25.0 mL with *water R* and mix.

Test solution (b): To 130 mg of powder from sachet bag 2, equivalent to 10 mg of glycine, add 15 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min, cool. Dilute to 25.0 mL with *water R* and mix.

Reference solution (a): To 25 mg of *glutamic acid RS*, add 15 mL of *water R*. Sonicate in an ultrasonic bath at 50°C for 12 min, cool. Dilute to 25.0 mL with *water R* and mix.

Reference solution (b): Dissolve 10 mg of *glycine RS* in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (c): To 25 mg of *glutamic acid RS* and 10 mg of *glycine RS,* add 15 mL of *water R*. Sonicate in an ultrasonic bath at 50°C

for 12 min, cool. Dilute to 25.0 mL with *water R* and mix.

Reference solution (d): Dissolve 5 mg of *ascorbic acid RS* in *water R* and dilute to 25.0 mL with the same solvent.

Plate: TLC silica gel plate R (HPTLC silica gel plate R).

Mobile phase: ethanol (96%) R – water R (70:30).

Application: 5 µL (2 µL).

Development: 10 cm (7 cm).

Drying: in air.

Detection: spray with *ninhydrin solution R1* and heat at 100–105°C for 5 min.

System suitability: *Reference solution (c)*:

– the chromatogram shows 2 clearly separated spots.

Results: the principal spots in the chromatogram obtained with *Test solutions* (*a, b*) are similar in position, color and size to the principal spots on the chromatogram obtained with *Reference solutions* (*a, b, c, d*).

Conclusions

As a result of the studies conducted, "green conditions" for identifying amino acids (glutamic acid, glycine), as well as ascorbic acid in a combined medicine by thin-layer chromatography and high-performance thin-layer chromatography methods have been selected. The validation characteristics of the method (specificity, robustness and precision) have been studied.

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