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SYNTHESIS AND INVESTIGATION OF BIOLOGICALLY ACTIVE DERIVATIVES OF ALKALOIDS CYTISINE, ANABASINE AND QUININE

monograph

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The monograph summarizes data on the preparation, structure and properties of the main representatives of alkaloids, namely cytisine, anabasine and quinine. The modification and functionalization of new alkaloids derivatives have been considered.
The monograph is indented for researchers, teachers, doctoral students, master students and undergraduates.

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INTRODUCTION

More than sixteen millions of individual compounds were created over nearly two centuries of history of organic chemistry. Synthesis of new organic compounds is becoming more and more widespread, which is dictated by the need to solve fundamental problems, for example, to reveal the interrelation between the chemical structure of compounds and their reactivity. But no less rapid development of organic chemistry is provided by the practical needs of society. The availability of an arsenal of affordable, reliable and effective medicines for the prevention and treatment of human diseases is one of such needs.

Alkaloids are known to be of great importance for pharmaceutical production, biochemistry, technology, clinical and experimental medicine. Cytisine and its derivatives have a wide range of biological activity, namely antispasmodic, insecticidal, cholinergic, analgesic, etc. The poisonous and medicinal properties of anabasine have long been known. The quinolone alkaloid quinine has antipyretic, analgesic properties and a pronounced effect against malarial plasmodia. Currently, the alkaloid quinine is used as the main treatment for malaria.

An in-depth study of alkaloids cytisine, anabasine and quinine and their derivatives to expand the boundaries of their use and create new approaches to the production of practically important heterocyclic compounds remains one of the most important tasks of the current stage in the development of the chemistry of nitrogen-containing heterocycles. The purpose of this monograph is to study alkaloids cytisine, anabasine and quinine in chemical modification reactions, which is of considerable interest in solving important theoretical and practical issues. The monograph deals with the synthesis of hybrid molecules based on alkaloids cytisine, anabasine and quinine aimed at obtaining compounds with a wide range of practically useful properties.

The monograph is intended for a wide range of readers interested in the problems of modern organic chemistry in general and alkaloids chemistry in particular.

Chapter 1

ISOLATION, PRODUCTION AND DETERMINATION OF THE ANABASINE, CYTISINE AND QUININE STRUCTURE

Searching methods for the rational use of natural plant materials and the creation of new biologically active compounds on its basis is one of the urgent problems of modern chemical science. In this regard, plant alkaloids are of particular interest, occupying a “privileged” position among natural compounds, which is explained both by the peculiarity of the alkaloids structure and their high practical value.

Synthetic transformations of plant metabolites are promising areas of medicinal chemistry. It is difficult to imagine modern medical therapy without medicines and preparations isolated or further obtained from plant materials. Of course, this is due to the unique structure of natural biologically active compounds synthesized as a result of complex biochemical processes of the vital activity of a plant organism. Plant bases, namely alkaloids of the quinolizidine (cytisine), quinoline (quinine) and pyridine series (anabasine), the molecular structure of which contributes to the introduction of various functional groups into their structure occupy a special place among the numerous organic compounds in nature.

Cytisine is close to nicotine by pharmacological action and belongs to the group of “ganglionic poisons”. It has analeptic and anti-smoking activity, which is due to its high affinity for nicotinic acetylcholine neuroreceptors. Small doses of cytisine greatly stimulate respiration and increase blood pressure. In the form of a 0.15% aqueous solution (“Cititon”), the alkaloid is used in medicine in cases of asphyxia and intoxication. Cytisine is also important as the main component of anti-smoking drugs (Tabex, Lobesil), which are used to facilitate smoking cessation.

For many decades, the alkaloid cytisine has been one of the most promising synthons in terms of possible modification and the creation of new biologically active substances on its basis. It should be noted that among the various derivatives of cytisine, compounds with other types of biological activity that are not characteristic of itself (antispasmodic, antiarrhythmic, hepatoprotective, analgesic, cholinergic, insecticidal, antioxidant, etc.) are constantly found. It attracts the attention of many researchers and encourages the synthesis and the study of its new derivatives.

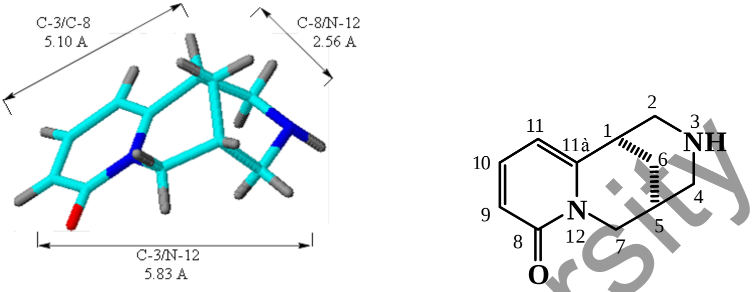
The alkaloid anabasine is one of the promising synthons for modification and creation of new biologically active substances. The poisonous and medicinal properties of anabasin have long been known. So, for example, powder obtained from the stem was sprinkled on wounds; tuberculosis was treated with a decoction of the roots.

The quinolone alkaloid quinine has antipyretic, analgesic properties and a pronounced effect against malarial plasmodia. Currently, the alkaloid quinine is used as the main treatment for malaria.

Thus, taking into account valuable biological properties of natural alkaloids and their synthetic derivatives, it can be assumed that the combination of pharmacophore residues of these natural compounds in one molecule opens up new possibilities for both further chemical modification of the obtained derivatives and a new diverse biological activity.

1.1 Isolation, production and determination of the alkaloid cytisine structure

Cytisine is a quinolizidine alkaloid found in the seeds and parts of plants in the legume family. The alkaloid cytisine was isolated from the plant Fmmodendronargenteum O., belonging to the Leguminosae family. Cytisine is a secondary-tertiary monoacid base. It belongs to the substances of “ganglionic” action and is considered as a respiratory analeptic in connection with the excitatory effect on respiration [1]. Currently, cytisine is included in the composition of anti-tobacco drugs due to the fact that its molecule has a high affinity for nicotinic and acetylcholine receptors in the CNS and peripheral ganglia [2]. In addition, cytisine and its derivatives, according to numerous studies, can also bind to adrenaline, dopamine, serotonin, histamine and muscarinic receptors. Therefore, the possible anti-inflammatory, antispasmodic, antiarrhythmic, antiviral and neurotropic properties of these molecules are now being widely studied. The hypothesis about the activity of cytisine against human respiratory viruses, namely influenza and parainfluenza viruses has not been expressed in scientific publications to date.



(1)

Figure 1. Structural formula of cytisine (1)

Cytisine (1) is a white crystalline substance that crystallizes from acetone in rhombic prisms. Melting point is 153°C, boiling point is 218^{0}C / 2 mm Hg Art., [α] = -1190 (in aqueous solution). It is soluble in water, alcohol, acetone and chloroform, sparingly soluble in benzene and ether [1].

Methods for extracting cytisine from plant materials are considered in the works of a number of authors [3-6]. Chloroform, liquefied ammonia, ethanol, methanol, and aqueous solutions of mineral acids of various concentrations were used as solvents. Scientists [7] present data on a comparative study of the influence of the solvent nature on the process of extracting cytisine and other alkaloids from cheap and cost-effective raw materials, namely the aerial part of Thermopsis alterniflora. It has been established that water is the best extractant; the yield of cytisine in this case reaches 55%.

In [5], there was developed the method for isolating cytisine from the seeds of lanceolate thermopsis, herb thermopsis alternately flowering, and sophora shoots using liquefied ammonia as an extractant, which, during the extraction process, makes it possible to simplify the isolation of cytisine and increase its yield. According to this method, alkaloids were first extracted by infusion at $-33 \div 35^{o}$C. The extraction degree of cytisine from the raw material reached 91-

95% with a fourfold infusion for 1-1.5 h. After evaporation of the ammonia extracts the obtained residue contained 4-5 times more alkaloids than raw material. Ammonia extracts, in contrast to alcohol, dichloroethane and chloroform, contained a significant amount of extractive substances, especially of a protein nature. Therefore, the obtained concentrates were purified before isolation of the alkaloids.

Cytisine was isolated and purified through nitrate followed by recrystallization of the base from acetone. To obtain pharmacopoeial cytisine, 60 g of nitrate was dissolved in 220 ml of water while heating, 0.35 g of sodium hydrosulfite and 0.35 g of activated carbon were added. Cytisine was exhaustively extracted with chloroform at pH 10. The extracts were dried over 20 g of anhydrous sodium sulfate and purified with 3.5 g of alumina. After distillation of chloroform, cytisine was recrystallized from a 10-fold amount of acetone. The yield of cytisine from the mother solutions was 75%.

We developed a laboratory method for obtaining cytisine from plant materials. Thus, the aerial part of the thermopsis was moistened with a 15% ammonia solution and filled with dichloroethane. The next day, the solution was decanted as completely as possible. The seeds were poured with a new portion of the solvent. The extraction was repeated several times until the complete alkaloids extraction. The extraction completeness was checked as follows: 3-4 ml of the next portion of the extract was shaken in a test tube with 0.5 ml of dilute sulfuric acid. After delamination, a 2% solution of silicic-tungstic acid was added dropwise to the sample. The absence of sediment indicated the extraction completeness. The combined dichloroethane extract was transferred to a separating funnel and treated 3-4 times with a small amount of 5% sulfuric acid. Sulfuric acid solution of alkaloids was filtered through a pleated filter and alkalinized with 25% aqueous ammonia (until the smell). The solution was transferred back to a separating funnel and the alkaloids were repeatedly extracted with chloroform. The extraction completeness was controlled with silicotungstic acid. The chloroform extract was dried with calcined sodium sulfate. The solvent was completely distilled off in a water bath from a round-bottom flask (capacity 100 ml) equipped with an addition funnel. The flask was connected by a curved tube to a refrigerator. Residual chloroform was removed by blowing air. The yield of the amount of alkaloids was 3.5 g. 50 ml of acetone was added to the resulting amount and boiled for 10-15 minutes in a water bath to separate cytisine from the bases accompanying it. Acetone was distilled off. The residue was extracted with ether six times. The volume of one portion of ether was 25 ml. The undissolved part (technical cytisine) was recrystallized from as little hot acetone as possible.

Cytisine is needle-shaped crystals. Yield is 0.5-0.55 g; m.p. is 153-155°C. Thus, we isolated and produced 20 g of the alkaloid cytisine. The structure of the resulting cytisine (1) was confirmed by ^{1}H NMR spectroscopy, as well as the data of two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra.

According to the ^{1}H NMR spectrum of the alkaloid (1) recorded on a JNN-ECA Jeol 400 spectrometer with a frequency of 400 MHz three groups of low-field signals correspond to the protons of the pyridine nucleus (Figure 2). Signal in the form of two doublets at 7.25-7.29 ppm correspond to the H-4 proton. Doublet at 6.15-6.17 ppm refers to the H-3 proton and the doublet at 5.99-6.01 ppm to H-5 proton. In the area of 3.63-3.80 ppm resonation of the axial and equatorial protons at the C-10 atom is noted, with the Ha signal shifted to a stronger field. Four protons of the methylene groups associated with the NH group and the methine group at the C-7 atom give signals in the range from 2.73 to 2.90 ppm, splitting under the influence of neighboring atoms into triplets and a multiplet, respectively. Wide signal centered at 2.20 ppm corresponds to the tertiary H-9 proton. Methylene protons H-8 appear in the strongest region of the spectrum centered at 1.77 ppm as two extended doublets.

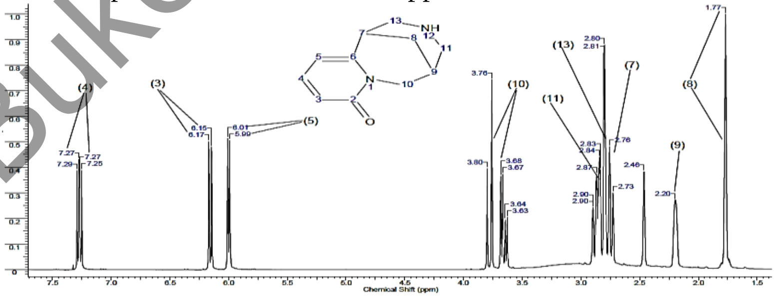


Figure 2. ^{1}H NMR spectrum of cytisine (1)

Analysis of the DEPT spectrum of cytisine (1) indicated the presence of four CH_2- and five CH-group signals in the carbon spectrum (Figure 3).

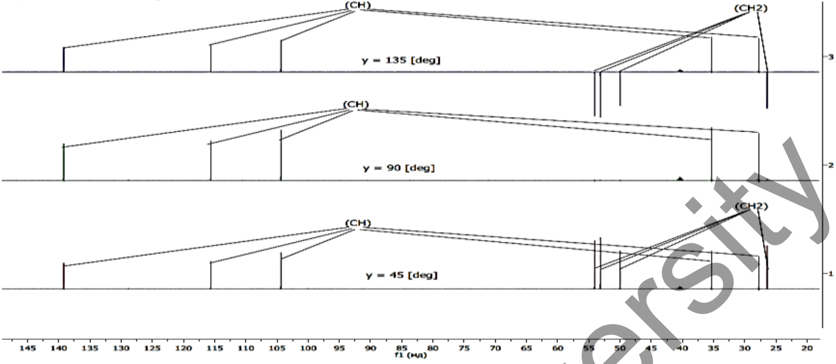


Figure 3. DEPT spectrum of cytisine (1)

Detailed interpretation made it possible to correlate the signals at 139.19 ppm, 115.60 ppm and 104.36 ppm with C-4, C-3 and C-5 methine groups of the α-pyridine system. Two other tertiary carbon signals appear at 35.33 and 27.75 ppm and correspond to C-7 and C-9, respectively. The signals correlation of the methylene groups was carried out as follows: δ(C-8)=26.41 ppm; δ(C-10)=49.98 ppm; δ(C-11)=53.16 ppm; δ(C-13)=54.07 ppm. In the weakest field (152.95 ppm, 162.85 ppm), low-intensity signals of quaternary C-6 and C-2 atoms appear.

The results of the analysis of two-dimensional spectra, namely COSY (^1H-^1H), TOCSY (^1H-^1H) and HMQC (^1H-^{13}C), indicating homo- and heteronuclear interactions in the molecule, are presented in Figures 4-7.

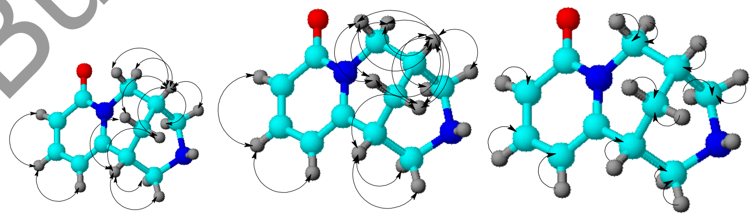

a b c

Figure 4. Scheme of correlations of COSY (^1H-^1H) (a), TOCSY (^1H-^1H) (b), HMQC (^1H-^{13}C) (c) in the cytisine molecule (1)

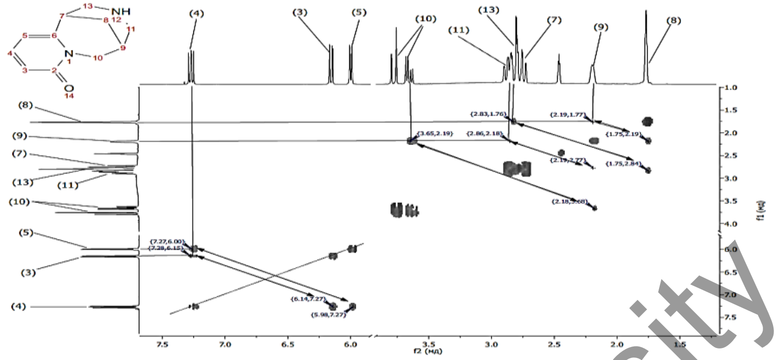


Figure 5. COSY spectrum of cytisine (1)

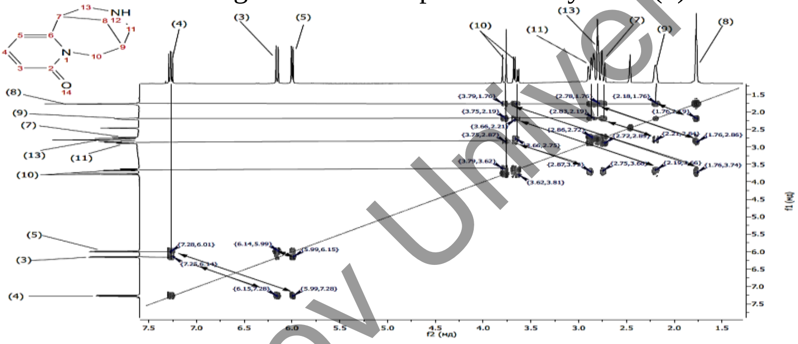


Figure 6. TOCSY spectrum of cytisine (1)

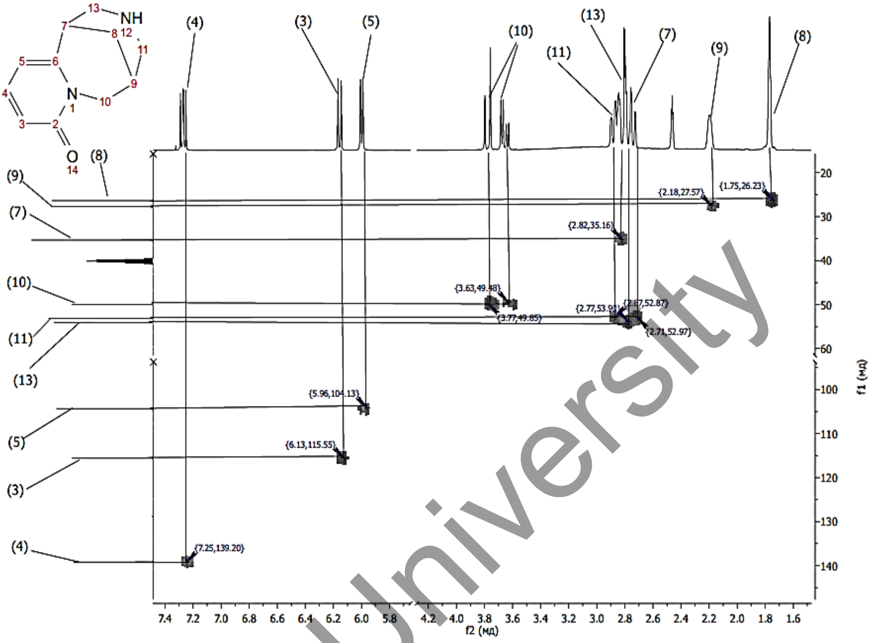


Figure 7. HMQC spectrum of cytisine (1)

As a result of the research, we developed a laboratory method for obtaining cytisine from plant raw materials from the herb Thermopsis alteflorum and Sophora shoots, and we produced 20 g of cytisine alkaloid. The structure of the resulting cytisine was confirmed by ^{1}H NMR spectroscopy, as well as data from two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra.

1.2 Isolation, production and determination of the alkaloid anabasine structure

Anabasine (α-piperidyl-β-pyridine) (2) belongs to the group of pyridine alkaloids. It was discovered in 1929 by Academician A.P. Orekhov in the Central Asian leafless blackberry (anabasis) plant Anabasis aphilla L. (of the haze family Chenopodiaceae) and in tobacco (Nicotiana tabacum) [1]. Anabasis leafless is an eastern Mediterranean species penetrating east to the Mongolian Altai [8-10].

The main areas of industrial harvesting of this plant are Kyzylorda, Zhambul and South Kazakhstan regions.

Leafless blackberry (anabasis) is a perennial plant. It is a shrub 30-70 cm high. It grows as a flattened-spherical bush 55-60 cm (up to 140 cm) in diameter.

All existing industrial and laboratory methods for the isolation of anabasine from Anabasis aphilla are aimed at obtaining a mixture of alkaloids either in the form of anabasine sulfate or as a sum of bases.

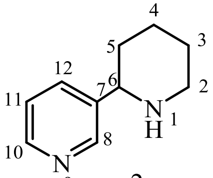
One of the important methods for the separation of alkaloids is the derivatives formation method. It is used in cases where alkaloids in the mixture differ from each other in chemical properties. One of the alkaloids of the mixture reacts with some reagent, while the other remains unchanged. After that, separation is carried out either by vacuum distillation or fractional crystallization. In this case, the original alkaloid from the derivative should be easily obtained back. The Anabasis aphilla plant contains a mixture of at least five alkaloids. Separation of this mixture is partially achieved by fractional distillation in vacuum [10].

The separation of anabasine and lupinine has been the subject of many works. Attempts to separate them by careful fractionation were made by many authors, but they failed to obtain completely pure anabasine, even in a minimal amount.

We carried out the separation of a mixture of alkaloids anabasine and lupinine (60 g) by column chromatography on aluminum oxide of the II degree of activity. The column was eluted with petroleum ether. There were collected 22 factions. The solvent was distilled off. At the same time, lupinine was obtained from the first 4-15 fractions. Next, the column was eluted with a mixture of solvents (petroleum ether - methanol, with a gradually increasing concentration of the latter). There were collected 30 fractions. Next, the column was eluted with methanol, while the fractions, characterized by a refractive index of 1.542-1.543, contain the alkaloid anabasine. They were combined and distilled in vacuum. Thus, we obtained 30 g of anabasine by separating a mixture of alkaloids with the help of column chromatography. This alkaloid was stored in sealed ampoules.

Alkaloid anabasine ($C_{10}H_{14}N_{12}$) is a colorless thick liquid, bp is 276° (760 mm), 104-105° (2 mm), d_{20} is 1.0455, n_D is 1.5430, $[\alpha]_D$ is 82°. It is easily soluble in water and common organic solvents.

The structure of the resulting anabasine (2) was confirmed by ^{1}H and ^{13}C NMR spectroscopy, as well as data from two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra.



2

In the ^{1}H NMR spectrum of anabasine (2) in the range from 1.25 to 1.52 ppm there is an accumulation of 4 protons signals, which can be correlated with CH_2 groups at the C-4 and C-5 atoms of the piperidine system (Figure 8). The protons of the two adjacent methylene groups of the heterocycle resonate at 1.76 (H-3), 2.61 (H-2 axial) and 2.99 ppm (H-2 equatorial). The hydrogen atom of tertiary C-6 resonates at 3.54 ppm. The highest frequency signals belong to the methine proton of the pyridine ring: δ(H-11) = 7.26 ppm; δ(H-12) = 7.68 ppm; δ(H-10) = 8.38 ppm; δ(H-8)= 8.50 ppm.

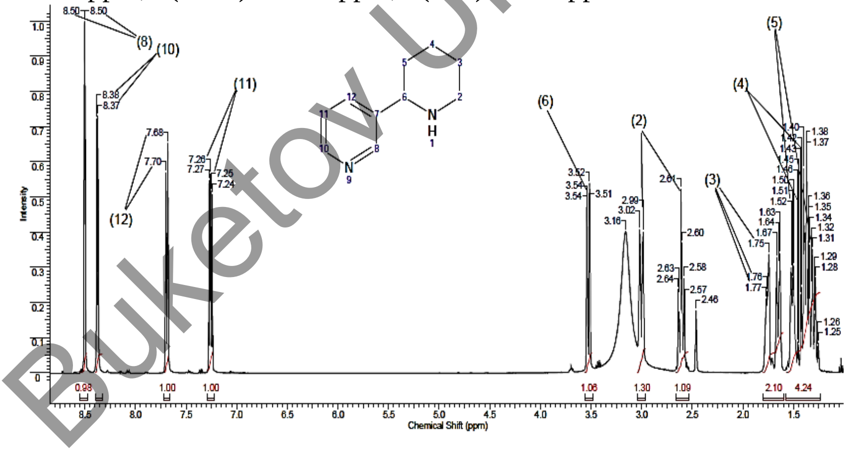


Figure 8. ^{1}H NMR spectrum of anabasine (2)

A similar picture is observed in the case of the carbon spectrum. Signals of the ^{13}C nuclei of the piperidine fragment are found in the high field region, while the pyridine ring give signals in the downfield part (Fig. 9). The methylene atoms C-2, C-3, C-4, and C-5 of the saturated heterocycle correspond to signals with chemical shifts of 47.20, 25.97, 25.81, and 35.38 ppm, respectively. The C-6 methine atom resonates at a frequency of 59.37 ppm. Carbon atoms in the *o*-position of the pyridine ring give signals in the region of 148.26-148.86 ppm. The *m*-atoms C-7 and C-11 corespond to signals at 141.60 and 123.66 ppm, respectively. Signal with δ=134.52 ppm can be attributed to the p-atom of C-12.

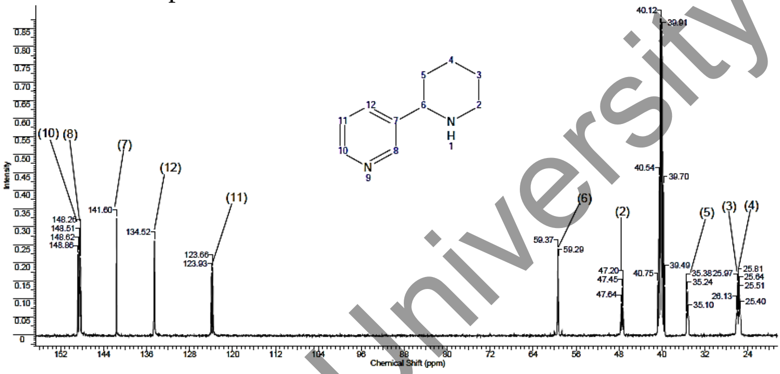
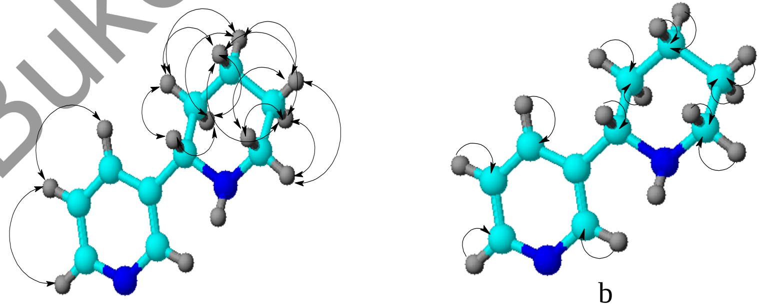


Figure 9. ^{13}C NMR spectrum of anabasine (2)

Homo- and heteronuclear correlations in the anabasine molecule were established using two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra (Figure 10).



b

a

Figure 10. Correlations of COSY (^{1}H-^{1}H) (a) and HMQC (^{1}H-^{13}C) (b) in anabasine molecule (2)

Thus, a laboratory method for obtaining anabasine from the anabasine-lupinine mixture was developed and 30 g of anabasine alkaloid was produced. The structure of the resulting anabasine was confirmed by ^{1}H NMR spectroscopy, as well as data from two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra.

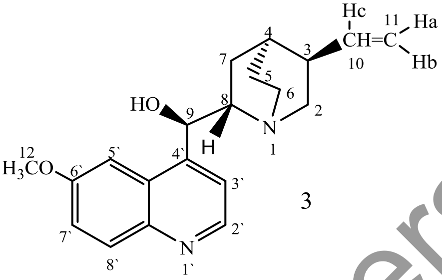
1.3 Isolation, production and determination of the alkaloid quinine structure

Alkaloid quinine (3) contained in the cinchona tree and plants of the genus Remijia of the madder family is a derivative of quinolone. It inhibits the centers of thermoregulation, lowers the excitability of the heart muscle, lengthens the refractory period and somewhat reduces it [11-13]. The presence of several reactive fragments in the structure of quinine makes it possible to modify it.

For further study of biological activity of the alkaloid quinine (3), the extraction of cinchona bark (Cinchona) with 96% ethanol was carried out, followed by chromatographic separation of the resins on columns with Al_2O_3. At the same time, a crystalline substance was isolated, which was identified as an alkaloid of the quinoline type, namely quinine (3) according to physico-chemical constants and spectral data.

Isolation of the quinoline alkaloid quinine: 1.0 kg of crushed air-dry raw materials were moistened with a 5% Na_2CO_3 solution and dried in air. The finished raw material was loaded into the extractor, poured into 7.5 kg of ethanol and infused for 2 hours. The raw material was exhaustively (3 times) extracted with ethanol (at 75-80°C), cooled and drained, filtered through a layer of medical gauze. The total extract of alkaloids obtained from 3 extracts was concentrated in a vacuum evaporator. There were obtained the extractives in the amount of 0.11 kg with a yield of 11.0% (by weight of air-dry raw materials), with a content of quinine (3) 2.8% according to HPLC. Further the amount of extractive substances was chromatographed on a column with Al_2O_3 (in a ratio of 1:20). Elution of the column with ethyl acetate precipitated a colorless crystalline solid. The resulting technical crystals of quinine (3) were collected and purified by decantation with ethyl acetate and ethanol. The purified precipitate was recrystallized from ethanol. There was obtained quinine (3) with mp 176-178°C, yield was 19 g (1.9% by weight of air-dry raw materials). Purity was 98.0% according to HPLC analysis, retention time was t_R=14.13±2 min.

IR spectrum (ν, cm^{-1}, KBr): 2956, 2919 (OCH_3), 2850, 1652 (C=O), 1559, 1540 (aromatic rings), 1471, 1463, 1377, 890.



3

The composition of the quinine molecule includes quinoline and quinuclidine cycles linked by a group of atoms -CH-OH. Protons of the quinuclidine ring appear in the strong region in the ^1H NMR spectrum of quinine alkaloid (Figure 11). So, the methylene protons H5 and H7, as well as the methine proton H4, resonate in the region at 1.49-1.78 ppm. The methine proton H3 appears in the region of 2.36 ppm. H2 methylene protons resonate as a doublet at 2.62 ppm, H6 methylene protons are recorded as a complex signal at 3.02-3.12 ppm, and H8 methine proton is found at 3.42 ppm. Vinyl group protons ($CH=CH_2$) resonate as doublets for Ha (δ = 4.89 ppm), Hb (δ = 4.93 ppm) and Hc multiplet (δ = 4.71 ppm). The H9 proton at the asymmetric carbon gives a wide singlet at 3.42 ppm. The protons of the quinoline ring appear as doublets in the weak region of the spectrum: methine protons δ (H3`) = 7.44 ppm, δ (H5`) = 7.25 ppm, δ (H7') = 7.20 ppm, δ (H8') = 7.90 ppm and δ (H2`) = 8.50 ppm. The methoxy protons appear as an intense singlet at 3.96 ppm.

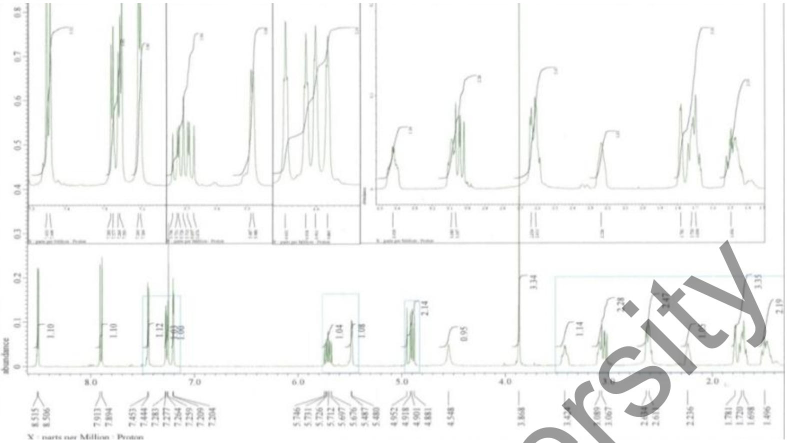


Figure 11. ^{1}H NMR spectrum of quinine (3)

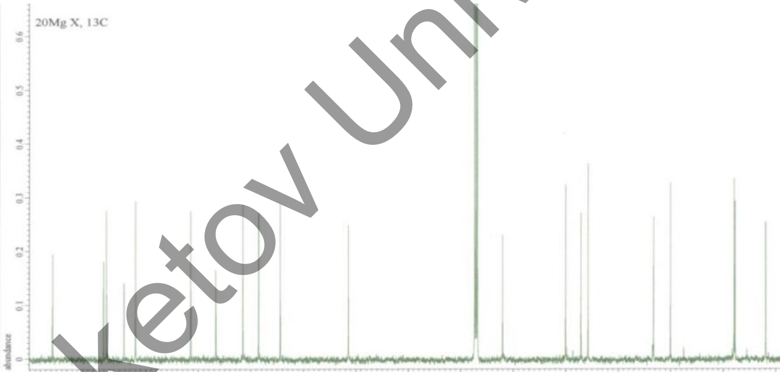


Figure 12. ^{13}C NMR spectrum of quinine (3)

Chapter 2

COMBINED COMPOUNDS BASED ON VARIOUS ALKALOIDS

2.1 Chemical transformation of the alkaloid cytisine with coumarin derivatives

A targeted synthesis based on modern data on the structure-activity relationship is one of the main ways to search for promising biologically active compounds. Due to their structural diversity, natural compounds, namely alkaloids, flavonoids, and terpenoids, make it possible to create on their basis a number of new medicinal substances with a wide spectrum of pharmacological action. The synthesis of compounds combining various functional fragments in one molecule is of interest in terms of studying their mutual influence on biological activity and opens up new possibilities for subsequent targeted chemical modification.

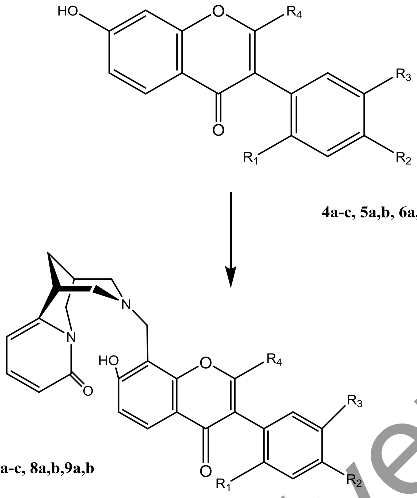
At present, there is a growing interest in the synthesis of combined compounds based on various alkaloids by introducing fragments of lactones, flavonoids, and other biologically active compounds into their molecule.

For many decades, the alkaloid cytisine has been one of the most promising synthons in terms of possible modification and the creation of new biologically active substances on its basis. Cytisine is a secondary-tertiary monoacid base, although monochlorohydrate and dihydrochloride have been derived from it. In other cases, the tertiary nitrogen atom does not show basic properties. Oxygen in cytisine is in an inactive form, which is incapable of reactions. Therefore, one of the nitrogen atoms is in the lactam rearrangement, the other nitrogen atom in cytisine is secondary and it is also in the cycle. Cytisine has several functional groups and can be successfully used as a chiral template for the synthesis of more complex semisynthetic molecules. Modification of the cytisine molecule structure can lead to the emergence of new types of biological activity and the establishment of regularities in the structure-activity relationship.

Cytisine and its derivatives have a wide spectrum of biological activity, namely antispasmodic, insecticidal, cholinergic, analgesic, etc. Therefore, possibilities of studying the cytisine modification are far from being exhausted and have great prospects for the development and replenishment of this interesting natural compound with new data.

The various fragments combination of natural compounds in one molecule is of interest in terms of studying their mutual influence on biological activity. Therefore, authors of [14] studied possibilities of studying cytisine in the aminomethylation reaction of natural 7-hydroxyisoflavones and their analogues methoxylated at ring B, which are known to be natural antioxidants; they are also used to prevent cardiovascular diseases, have a hypolipidemic effect. The authors studied the aminomethylation of natural isoflavones and their analogs using cytisine and formalin, since the presence of electron-donating substituents in their molecules prevents the occurrence of electrophilic substitution reactions. The search for optimal conditions for aminomethylation in a series of natural flavonoids and their analogues using cytisine consisted in selecting a suitable solvent and catalyst for the reaction. When introducing cytisine into the aminomethylation reaction of natural isoflavones and their analogs, as in the case of 7-hydroxy-3-arylcoumarins [15], the most satisfactory results were obtained using 4-N,N-dimethylaminopyridine (DMAP) as a catalyst.

Heating of compounds (4a-c, 5a, b, 6a, b), cytisine and formalin in propanol-2 in the presence of a catalytic amount of DMAP led to aminomethylation of the chromone ring with the formation of 8-(cytisin-12-yl)methyl-7- hydroxyisoflavones (7a-c, 8a, b, 9a, b).

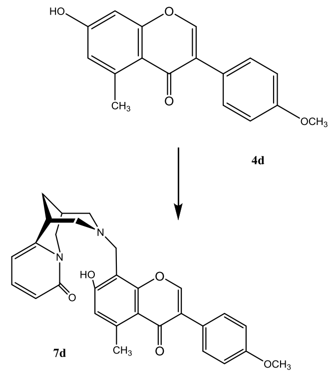


4a-c, 5a,b, 6a,b

7a-c, 8a,b,9a,b

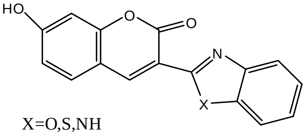
4a,7a: R_1=R_3=R_4=H, R_2=OCH_3; 4b,7b: R_1=R_4=H,R_2=R_3=OCH_3
4c,7c:R_1=OCH_3, R_2=R_3=R_4=H; 4a,8a: R_1=R_2=R_3=H, R_4=CH_3
5b,8b: R_1=H, R_2=R_3=OCH_3, R_4=CH_3
6a,9a: R_1=R_3=H, R_2=OCH_3, R_4=CF_3
6b,9b: R_1=OCH_3, R_2=R_3=H, R_4=CF_3

At the same time, the aryl substituent at C-3 with electron-donating methoxy groups significantly increased the reactivity of the chromone ring to electrophilic attack. The interaction of 7-hydroxyisoflavones (4a-c, 5a, b, 6a, b) with formalin and cytisine proceeded for 3-7 hours, which indicated a significant reactivity of the benzopyran cycle in the Mannich reaction. 5-Methylformonentine (4d) was used to study the effect of substituents in ring A on the course of the aminomethylation reaction of natural isoflavones and their analogs. The presence of an electron-donating methyl group in compound (4d) accelerated its interaction with cytisine and formalin, and 8-cytisinylmethyl derivative (7d) was synthesized.



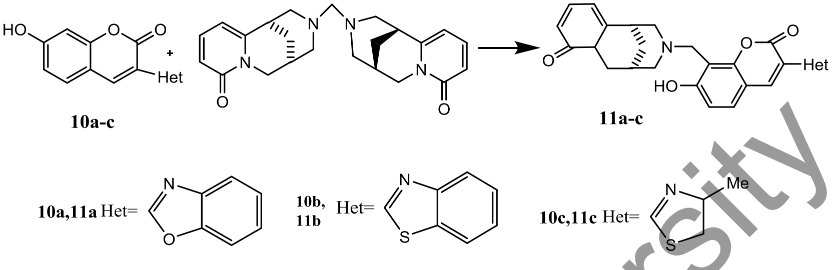
Optimal conditions for aminomethylation of natural isoflavones and their analogues with the alkaloid cytisine participation have been developed. This allows synthesizing compounds containing pharmacophore residues of these natural compounds in one molecule. It indicates their diastereotopy due to the presence of optical centers in the cytisine fragment, and also opens up new chemical possibilities for modifications of the alkaloid cytisine.

One of the interesting ways to modify the cytisine structure is the coumarin fragment introduction into its molecule. The coumarin derivatives have various types of biological activity and their pharmacological value can hardly be overestimated. Derivatives of coumarin containing a nitrogen atom are very rare in the plant world. The presence of electron-withdrawing heterocycles fragments in the C-3 position of coumarin affects their physical and chemical properties. In particular, anomalously large bathochromic shifts are observed in their UV spectra, as a result of which the absorption maxima of coumarins are shifted to the visible region, especially in the presence of 7 electron donor hydroxyl or dialkylamino groups. 3-Hetarylcoumarins also have valuable fluorescent properties, which allow the use of similar compounds, namely 7-hydroxy-3-benzazolylcoumarins (10).



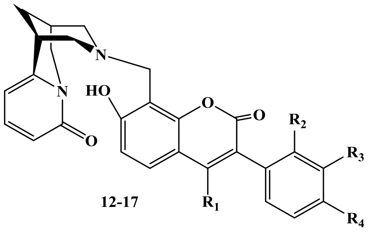
(**10**)

In [16], 8-aminomethyl derivatives of 7-hydroxycoumarins (11a-c) were obtained by the reaction of methylene-bis-cytisine with substituted 7-hydroxycoumarins.



Taking into account the reduced reactivity of 3-hetarylcoumarins to electrophilic substitution reactions and the possible mechanism of the Mannich reaction through the formation of aminals, the authors managed to carry out aminomethylation of 7-hydroxy-3-hetarylcoumarins using methylene-bis-cytisine obtained from cytisine and diiodomethane or formalin.

In [17], the authors studied the possibility of using cytisine as an amine and formalin to introduce an aminomethyl group into the benzopyrone ring. The interaction of cytisine and aqueous formalin in dioxane in the presence of a catalytic amount of 4 N,N-dimethylaminopyridine results in aminomethylation of the coumarin fragment with the formation of 8-(cytisin-12-yl)methyl-7-hydroxycoumarins (12-17).

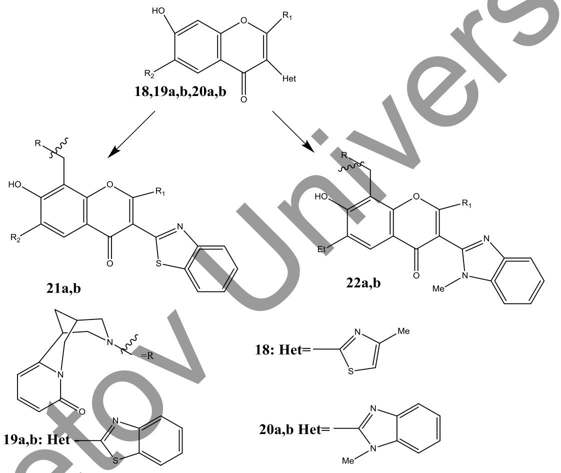


12-17

12: R_1=R_2=R_3=R_4=H; 13:R_1=R_2=R_3=H, R_4=OMe;
14: R_1=R_2=H, R_3=R_4=OMe; 15: R_1=R_3=H, R_2=R_4=OMe
16: R_1=Me; R_2=R_3=R_4=H; 17: R_1=Me; R_1=Me; R_2=R_2=R_4=OMe

It has been established that the aryl substituent at C-3 with electron-donating methoxy groups significantly increases the reactivity of the coumarin ring to electrophilic attack. In the case of 3-hetaryl-coumarins, the introduction of an aminomethyl group requires heating of the starting compounds with aminals for 8-10 h [18], the interaction of 7-hydroxy-3-arylcoumarins with formalin and cytisine takes 2-8 hours, which indicates a significant reactivity of the coumarin cycle in the Mannich reaction.

In [19], the reaction of methylene-bis-cytisine with substituted 7-hydroxychromones (18, 19a,b, 20a,b) yielded 8-aminomethyl derivatives of 7-hydroxychromones (21a,b, 22a,b). Structural features of the synthesized compounds were studied using NMR spectroscopy.



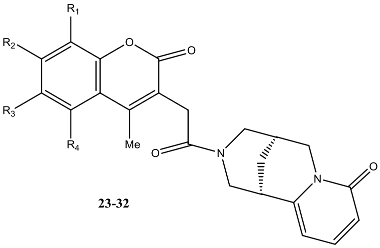
18, 19a, 21a: R_1=H; 19b, 20a, 21b, 22a: R_1=Me; 20b, 22b: R_1=$CHMe_2$

18, 19a, 20a, 20b, 21a, 22a, 22b: R_2=Et; 19b, 20b: R_2=H

At the same time, the developed aminomethylation methods can be used for the synthesis of compounds containing the cytisine fragment (1), which opens up new chemical possibilities for modifying the cytisine and expands the understanding of its reactivity.

New modified derivatives were obtained by acylation of the alkaloid cytisine with 3-coumarinacetic acids [20]. Condensation of the obtained activated esters with cytisine in dioxane at room temperature leads to the formation of N-acyl derivatives of cytisine

(23-32) in high (62-91%) yields, the molecules of which contain coumarin residues.



23-32

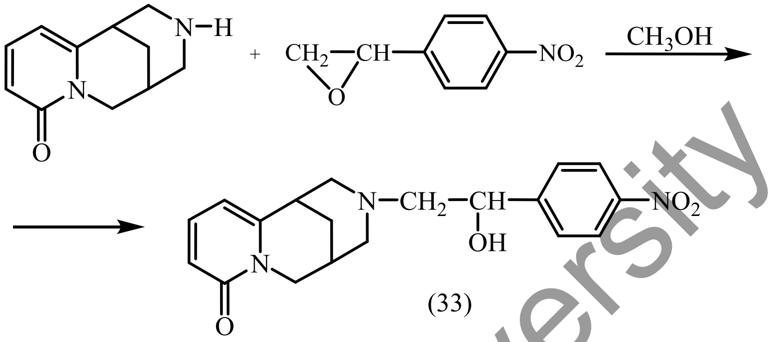
23,28: $R_1=R_3=R_4=H$: **24,29**: $R_1=Me$, $R_3=R_4=H$; **23-26:** $R_2=OH$
25,30: $R_1=R_4=H$, $R_3=Cl$; **26**:$R_1=R_3=H$, $R_4=OH$; **31:** $R_1=R_3=H$, $R_4=OMe$
27: $R_1=R_3=H$, $R_2=Me$, $R_4=OH$; **28-31**:$R_2=OMe$; **32**:$R_1=R_3=H$, $R_2=Me$, $R_4=OMe$

A double set of proton signals is observed in the ^1H NMR spectra for all compounds obtained. Obviously, due to the formation of amide conjugation in such systems, invertomers with hindered rotation along the N-C bond appear which can be considered as Z-, E-isomers.

An extensive array of information on the synthesis and study of the biological activity of combined derivatives based on the molecules of natural compounds indicates the exceptional possibilities of a targeted search for new biologically active compounds and medicinal substances.

2.2 Synthesis and investigation of 1-N-nitrophenyl-2-cytisinylethanol

Aminoalkanols, which have at least two reaction centers, are of considerable interest, both chemically and pharmacologically. This section presents the investigation results of the interaction of the alkaloid cytisine with N-nitrophenyloxirane, which proceeds with the formation of the corresponding secondary β-amino alcohol (33). The latter is close to a certain extent to analogues of ephedrine alkaloids and choline $[HOCH_2CH_2N^+(CH_3)_3]OH^-$ [21]. The chemical structure of the synthesized compound (33) is somewhat reminiscent of the acetylcholine molecule, with the only difference. They have a tertiary nitrogen atom instead of a quaternary nitrogen atom, and the α,β-hydroxyethyl part is “heavy” with various groups. It is known [21] that the presence of a tertiary amino group in an anticholinergic gives its molecule lipid solubility and thus facilitates the substance penetration into the central nervous system.



(33)

The main regularities of the saturated α-oxides reactions with ammonia and amines were established in the classical works of K.A. Krasusskii [22]. Depending on the reagents ratio and temperature conditions, the reaction products can be different. According to the spectral analysis results, the interaction of cytisine with N-nitrophenylethylene oxide proceeds from the side of the least substituted carbon atom of the epoxy ring, since this position is most accessible for nucleophilic attack, resulting in the formation of the corresponding secondary hydroxy compound (33).

TLC and ^{1}H NMR spectroscopy data show that the process proceeds regio- and stereoselectively with the formation of only one product. The need for the presence of proton-donor components in the reaction medium, which have a catalytic effect on the course of the process, has been established. The reaction proceeds through the formation of an intermediate oxonium compound, which is more reactive than the initial oxide [22]. It has been found that the highest yield of the target product is formed in an alcoholic medium, i.e., apparently, the mechanism of general acid catalysis is realized for N-nitrophenyl-ethylene oxide. Naturally, the second factor affecting the yield of end products is the basicity of amines. An increase in basicity facilitates the ring-opening reaction.

The resulting amino alcohol (33) is a crystalline substance soluble in alcohol, acetone, and benzene (when heated). The structure of β-amino alcohol (33) was determined by IR and ^{1}H NMR spectroscopy data.

In the IR spectrum of compound (33) there is no absorption band of the epoxy ring in the region of 840-940 cm^{-1}, but there is a wide band in the region of 3350-3480 cm^{-1}, which is characteristic of the hydroxyl group. The stretching vibrations of the NO_2 group appear in the region of 1370 -1340 cm^{-1}.

Analysis of the ^{1}H NMR spectrum of 1-N-nitrophenyl-2-cytisinylethanol (33) shows that four groups of low-field signals correspond to the protons of the N-nitrophenyl fragment and α-pyridine core. Two doublets at 7.89 and 7.14 ppm correspond to the ortho and meta protons of the *p*-nitrophenyl fragment. Signal at 7.18 ppm corresponds to H2 protons with the spin-spin coupling constant J_{HH}=7.8 Hz. Split doublet at 6.22 ppm corresponds to H1 protons with J_{HH}=9.8 Hz, and the doublet at 5.97 ppm corresponds to H3 proton. Complex multiplet in the region of 4.61 ppm corresponds to the methine proton of the CH-OH fragment. The next group of lines is a complex signal in the region of 3.28-3.70 ppm corresponds to the axial and equatorial H7 proton. Doublet at 2.40 ppm refers to the methylene protons of the CH_2-CH group. A group of lines in the region of 2.72-2.79 ppm represents signals from five protons: protons of NCH_2 groups (4H) and tertiary proton H4. The tertiary proton H6 resonates in the region of 2.35 ppm. Methylene protons at H5 appear as a complex multiplet in the area with the center at 1.76 ppm.

The spatial structure of the amino alcohol was determined by X-ray diffraction analysis (33). The structure of the 1-N-nitrophenyl-2-cytisinylethanol molecule (33) is shown in Figure 13. The bond lengths and bond angles in the compound (33) molecule are close to the corresponding values in (-)-cytisine and (-)-methylcytisine [23].

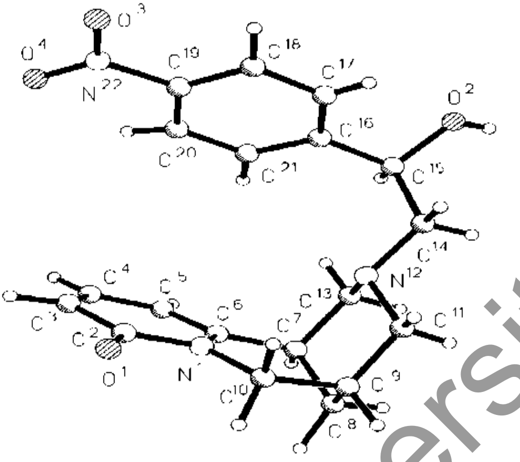


Figure 13. General view of the 1-N-nitrophenyl-2-cytisinylethanol molecule (33)

The dihydropyridine ring is flat with an accuracy of ±0.01 Ǻ. The carbonyl oxygen atom O1 deviates slightly from this plane (by 0.06 Ǻ). The N1C6C7C8C9C10 tetrahydropyridine ring adopts a somewhat distorted sofa conformation ($\Delta C_S^8 - 5.9^o$) with the bridging C8 atom moving out of the middle plane of the others (±0.03 A) by 0.75 Ǻ. The piperidine ring C7C8C9C11N12C13 takes the form of an almost perfect chair ($\Delta C_S^8 - 1.3^o$). The C7, C8, C11, and C13 atoms are coplanar with an accuracy of ±0.01 Ǻ, while the C8 and N12 atoms go out of their plane by 0.73 and 0.65 A in opposite directions. The nitrophenyl group lies in one plane; the escape of atoms from it does not exceed 0.01 Ǻ on average.

Method for obtaining 1-p-nitrophenyl-2-cytisinylethanol (33): An equimolecular amount of 4.73 g (0.023 mol) of cytisine was added with stirring to 4.76 g (0.023 mol) of N-nitrophenyloxirane dissolved in 30 ml of methanol. The reaction mixture temperature was brought to 55-60^{0}C and stirred for 5 hours. Methanol was distilled off. The residue was recrystallized from benzene. There was obtained 5.51 g (66.4% from the theoretically possible yield). Product (33) has melting point 202-204^{o}C.

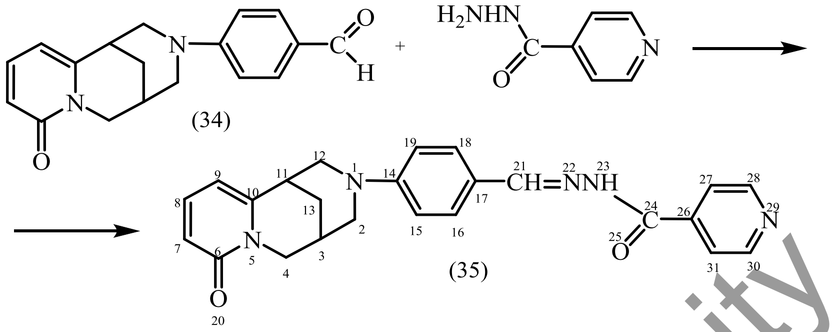
Thus, the interaction of the alkaloid cytisine with N-nitrophenyloxirane resulted in the synthesis of a pharmacologically active β-amino alcohol. It is shown that the process proceeds regio- and stereoselectively with the formation of only one product.

2.3 Synthesis and structure of N-(4-(cytisino)benzylidene)isonicotinohydrazide

The strategy of the modern search for chemical compounds with anti-tuberculosis activity is aimed at chemical modifications of known anti-tuberculosis drugs, using the most effective tuberculostatic drugs widely used in clinical practice as a “mother” model, in particular, isonicotinic acid hydrazide (INH) and its derivatives. To date, many different derivatives with a wide variation in anti-tuberculosis activity and toxicity of the compounds have been synthesized based on INH [24]. However, the problem of drug resistance of many pathogenic bacteria and viruses to drugs used for treatment requires a constant search and expansion of the arsenal of new highly effective and low-toxic drugs. Studies related to the synthesis of isonicotinic acid hydrazones, which are included in the structure of many anti-tuberculosis drugs are of considerable scientific and practical interest.

In this regard, we were interested in the synthesis of a new hydrazone based on the widely used anti-tuberculosis preparation INH and 4-cytisinylbenzaldehyde (34) with the introduction of pharmacophore fragments into their structure. 4-(N-Cytisinyl)benzaldehyde (34) was previously obtained in [25].

Condensation of 4-(N-cytisinyl)benzaldehyde (34) with isonicotinic acid hydrazide in an alcoholic solution at an equimolar ratio of the reagents used was used to synthesize cytisine-containing isonicotinic acid hydrazone (35), potentially possessing biological activity.


(34)

(35)

The structure of the compound N-(4-(cytisino)benzylidene)isonicotinohydrazide (35) was confirmed by ^{1}H and ^{13}C NMR spectroscopy, as well as by the data of two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra.

In the ^{1}H NMR spectrum of compound (35), the protons H-13 and neighboring H-3 and H-11 of the diazabicyclic fragment of the cytisine part of the molecule appeared as broadened singlets at 1.88 (2H), 2.55 (1H), and 3.20 (1H) ppm, respectively. Axial protons H-2ax and H-12ax resonated as doublets at 2.92 and 2.99 ppm, respectively with identical spin-spin splittings (3J 11.0 Hz). The remaining diazabicyclic protons appeared as two-proton multiplets at 3.71 (H-4ax, 12eq) and 3.89-3.92 (H-2eq, 4eq). In the downfield part of the spectrum, there were multiplets at 6.14-6.16 and 7.29-7.32 ppm corresponding to the cytisine protons H-7, 9, and H-8, respectively. The equivalent benzylidene protons H-15, 19 and H-16, 18 appeared in the aromatic band of the spectrum as a two-proton multiplet at 6.83-6.85 and a doublet at 7.48 ppm with 3J 7.3 Hz, respectively. The pyridine protons H-27, 31 and H-28, 30 resonated as broadened two-proton singlets at 7.76 and 8.72 ppm, respectively. The unsaturated H-21 proton appeared as a broadened singlet between the signals of pyridine protons. Amide proton H-23 resonated as a broadened singlet in the most downfield region at 11.81 ppm.

In the ^{13}C NMR spectrum of compound (35), signals of the cytisine fragment were observed at 25.11 (C-13), 27.62 (C-3), 34.61 (C-11), 49.77 (C-4), 54.64 (C-2), C-12), 104.81 (C-9), 116.22 (C-7), 139.49 (C-8), 149.68 (C-10) and 161.72 (C-6) ppm. The carbon nuclei of the benzylidene ring resonated at 115.47 (C-15, 19), 124.51 (C-17), 128.94 (C-16, 18) and 153.09 (C-14) ppm. The carbon atoms of the pyridine fragment were observed at 122.02 (C-27, 31), 141.24 (C-26) and 150.81 (C-28, 30) ppm. Chemical shift signal at 151.50 ppm corresponded to the C-21 carbon atom linked by a double bond to the nitrogen atom. The signal of the C-24 carbonyl atom was found in the downfield part of the spectrum at 162.61 ppm.

The structure of hydrazone (35) was also confirmed by two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) NMR spectroscopy, which makes it possible to establish spin-spin interactions of a homo- and heteronuclear nature. The observed correlations in the molecule are shown in Figures 14-16. In the ^{1}H-^{1}H COSY spectra of the compound, spin-spin correlations were observed through three protons bonds of neighboring methylene and methine groups of the cytisine fragment H13-H3 (1.91, 2.54 and 2.54, 1.91), H13-H11 (1.91, 3.21 and 3.21, 1.91), H3 -H4.12 (2.53, 3.70 and 3.68, 2.53), H3-H4 (3.00, 3.73 and 3.68, 3.01), H12-H11 (3.00, 3.73 and 3.73, 3.01), H7.9-H8 (6.17, 7.31 and 7.31, 6.17), the benzylidene ring H15,19-H16,18 (6.84, 7.49 and 7.48, 6.84) and the pyridine ring H27,31-H28.30 (7.75, 8.73 and 8.73, 7.73). Heteronuclear interactions of protons with carbon atoms through one bond were established using ^{1}H-^{13}C HMQC spectroscopy for all pairs present in the compound: H13-C13 (1.87, 25.49), H3-C3 (2.53, 28.18), H12ax-C12 (3.03, 56.07), H4ax-C4 (3.69, 50.43), H4eq-C4 (3.92, 50.43), H12eq-C12 (3.73, 55.95), H2-C2 (3.95, 55.33), H9-C9 (6.18, 105.26), H7-C7 (6.16, 116.71), H15.19-C15.19 (6.85, 115.95), H8-C8 (7.31, 139.88), H16.18-C16.18 (7.47, 129.36), H27.31-C27.31 (7.75 , 122.47), H28.30-C28.30 (8.25, 150.16), H21-C21 (8.72, 151.24).

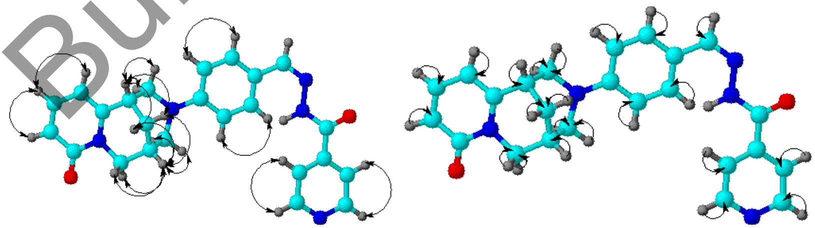

a b

Figure 14. Scheme of correlations in the COSY (a) and HMQC (b) spectra of compound (35)

The ratio of the integral intensities of protons in the considered molecule (35) corresponded to the presented structure. The assignment of the ^{1}H and ^{13}C NMR spectra was confirmed by the results of DEPT and ^{1}H-^{13}C HMBC correlations.

Method for the preparation of N-(4-(cytisino)benzylidene)isonicotinohydrazide (35): A mixture of 0.2 g (0.0007 mol) of 4-(cytisinyl)benzaldehyde and 0.1 g (0.0007 mol) of isonicotinic acid hydrazide in 10 ml of ethanol in the presence of 2 drops of acetic acid at a reaction mixture temperature of 55-60^{0}C was stirred for 3 hours. After that a yellow powder was obtained. The powder was filtered off, washed with cold ethanol. It was obtained 0.15 g (50% from the theoretically possible yield). Melting point of product (35) is 291-292°C.

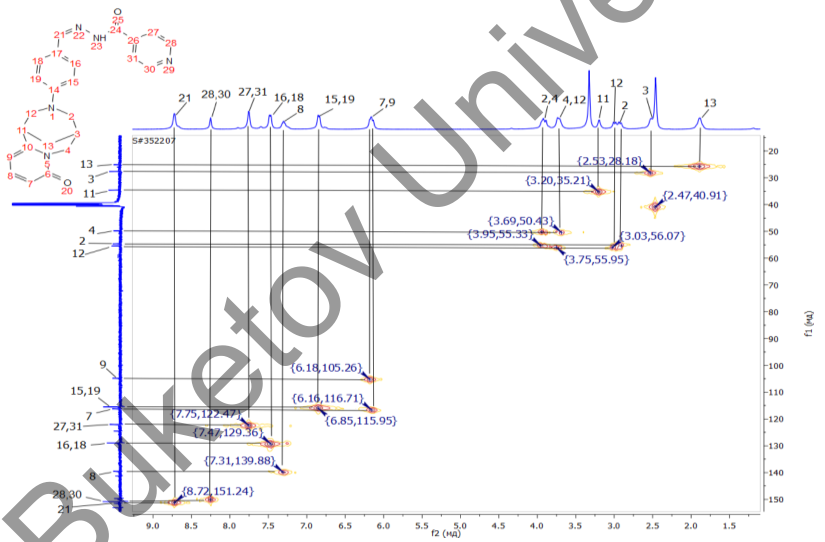


Figure 15. HMQC spectrum of compound (35)

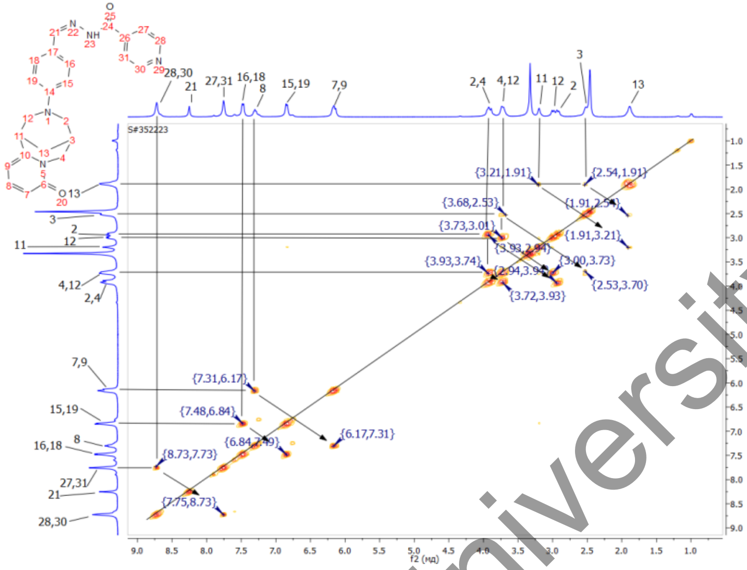


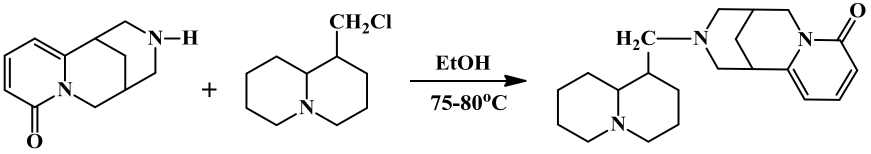
Figure 16. COSY spectrum of compound (35)

Thus, the interaction of isonicotinic acid hydrazide with 4-(N-cytisinyl)benzaldehyde has been used to synthesize and characterize a new biologically very promising N-(4-(cytisino)benzylidene)isonicotinohydrazide.

2.4 Synthesis and investigation of N-lupinylcytisine

Compounds combining various functional fragments in one molecule are interesting for subsequent targeted chemical modification. At present, researchers focus on the synthesis of combined compounds based on various alkaloids by introducing fragments of lactones, flavonoids, and other biologically active compounds into their molecules. Therefore, the introduction of a lupinyl fragment into the composition of the alkaloid molecule makes it possible to obtain new original compounds with specific fragments.

We performed the synthesis of N-lupinylcytisine for the first time for the dimeric compounds preparation.



(1) (36) (37)

0.71 g (3.7 mmol) of chlorolupinine was added to a 25 ml suspension solution of 0.71 g (3.7 mmol) of cytisine in ethanol. The reaction mixture was stirred with heating at 75-80°C for 27 hours, and then it was cooled. After settling for 10 hours, the reaction mixture was dissolved in 250 ml of ice water and extracted with chloroform (3x100 ml). The combined chloroform extracts were washed with water (3x50) and dried over Mg_2SO_4. The solvent was evaporated in vacuo, the residue was chromatographed on alumina column (eluent was chloroform, chloroform-ethanol, 100:1). In this case, 0.34 g (47.88%) of N-lupinylcytisine (37) was isolated in the form of a yellow-green thick oil.

2.5 Preparation of glycosylthiourea derivatives based on alkaloids cytisine and anabasine

The molecules blending concept is one of the modern widely used approaches in the search for new and improvement of known drugs with a high level of selectivity and low toxicity [26-29]. The combination of heterocyclic systems of natural alkaloids and pharmacophore groups in one structure is one of the promising directions of this strategy. Numerous data on the manifestation of a wide spectrum of bioactivity by these compounds serve as good prerequisites for this [30-32]. Therefore, the introduction of fragments with biological and physiological activity into the composition of the alkaloid molecules of cytisine, anabasine, lupinine, as well as the synthesis of new representatives of these heterocyclic compounds, is an urgent task and is of scientific and practical interest.

The natural compounds chemistry, including alkaloids, is one of the rapidly developing areas of organic chemistry. The systematic study of new substances requires not only the creation of effective methods for the synthesis of these compounds, but also the preparation of their various derivatives. Given the valuable biological properties of alkaloids and their derivatives, the search for new ways of chemical modification of alkaloids is certainly relevant, and the attention of researchers is attracted by the production of increasingly complex heterocyclic systems.

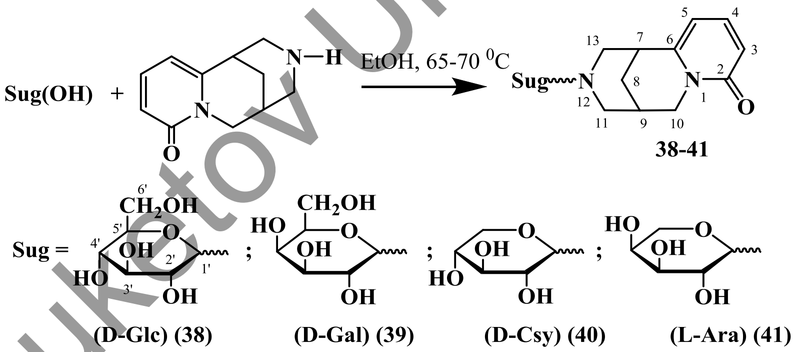
The relevance of carrying out chemical transformations of plant alkaloids is determined by the need to obtain new substances characterized by improved physico-chemical properties, more pronounced biological activity, reduced toxicity, and prolonged action compared to the original natural analogues [32]. To date, there is a growing interest in the synthesis of combined compounds based on various alkaloids by introducing fragments of carbohydrates, flavonoids, and other biologically active compounds into their molecule. They are widely used in the treatment of cardiovascular diseases. They are used as antitumor, antimicrobial, anticholinesterase agents. The presence in the molecules of alkaloids of one or more nitrogen atoms with unpaired pairs of electrons determines their chemical properties. The chemical synthesis of alkaloid derivatives allows expanding the possible ways of searching for drugs.

It is known that carbohydrates in the form of various derivatives are part of the cells of any living organism, acting here as a structural material, energy supplier, substrates and regulators of specific and biochemical processes. Carbohydrates, combined with nucleophilic acids, proteins and lipids, form complex high-molecular complexes that underlie subcellular structures and are the basis of living matter [33]. They are the primary products of photosynthesis, i.e. the first organic substances in the carbon cycle in nature and serve as a bridge between mineral compounds and the world of organic substances. A significant number of substances have been found in the series of carbohydrates derivatives that are used in medicine for various purposes. Therefore, the chemical modification of known drugs based on carbohydrates is one of the promising directions in the search for new biologically active substances [34].

It is known that N-glycosylation of many amino compounds, including natural physiologically active ones, is considered as a new approach to the development of promising and effective drugs with targeted action due to the active transport of carbohydrate fragments [35-

41]. The introduction of carbohydrate fragments into the structure of biologically active substances not only increases their water solubility, but also significantly reduces toxicity. The latter makes it possible to recommend the glycosylation method of a physiologically active compound at the glycosidic center of sugars as one of the possible ways to obtain low-toxic drugs [42].

In this regard, it was of interest to us to obtain N-glycosylamines based on the alkaloid cytisine and some monosaccharides for the subsequent study of their biological properties. The synthesis of N-glycosylamines was carried out using the well-known classical method proposed by V. Sorokin in [43]. The reaction consisted in the direct condensation of amines with a monosaccharide in an alcoholic solution, sometimes in the presence of catalytic amounts of weak acids. Thus, the condensation of D-glucose, D-galactose, D-xylose, and L-arabinose with the alkaloid cytisine in a small amount of absolute ethanol (without the addition of a catalyst) resulted in the corresponding 1-glycopyranosylamines (38-41):

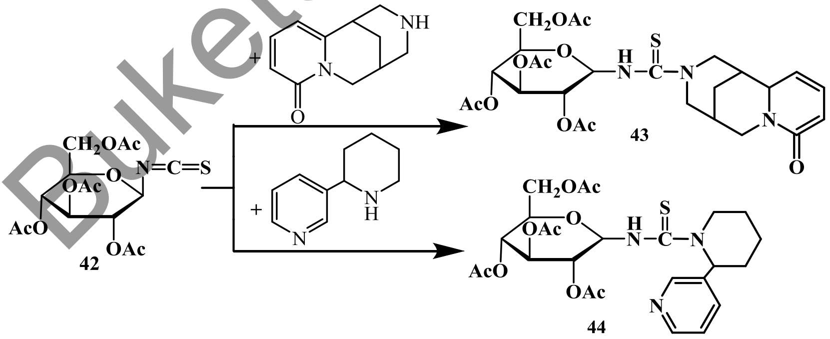


Condensation, and most importantly, the subsequent isolation of the target products, is significantly improved when absolute ethanol is used, since the synthesized glycosides are highly soluble in water, and even small amounts of it hinder the crystallization of the products. It has also been established that the use of catalytic amounts of acetic acid initially in the reaction significantly affects the rate of aminoglycosides formation, but significantly reduces the yields and isolation of the final products.

The resulting N-glycosylcytisines (38-41) can be very promising substitutes for already used pharmaceutical preparations based on the alkaloid cytisine (respiratory analeptic “Cytiton”, smoking cessation drugs “Lobesil”, “Tabex”). Undoubtedly, they will have a much lower toxicity and prolongation of action due to their gradual hydrolysis in the body.

Glycosyl isothiocyanates play an important role in the chemistry of carbohydrates, being synthons in the synthesis of various biologically active compounds [44]. The isothiocyanate method makes it possible to introduce a thioamide group into the structure of amines (alkaloids) and hydrazides to form thioureas and thiosemicarbazides, which not only expands the boundaries of modification of these compounds, but can also lead to the emergence of new types of bioactivity.

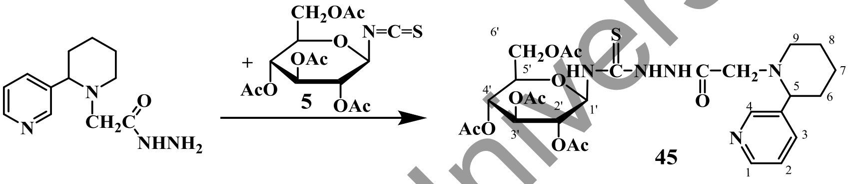
It is known from the literature review that many glycosylthioureas, which are the initial synthons for the synthesis of many nucleosides, are obtained with the help of the Fischer reaction by the interaction of the corresponding amino compounds with glycosylisothiocyanate [44-46]. In order to obtain glycosylthiourea derivatives based on alkaloids, 1-isothiocyano-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (42) was reacted with cytisine and anabasine. It has been found that glycosylisothiocyanate (42) reacts quite easily with these alkaloids in *o*-xylene solution at room temperature. Synthesized compounds (43, 44) after evaporation of the solvent are obtained with a yield of 70-80%.



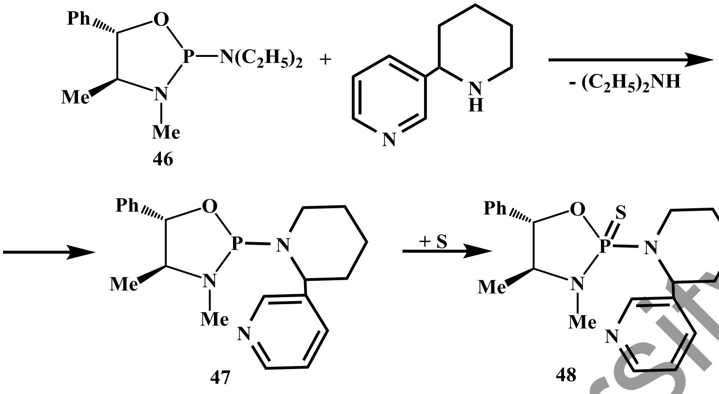
The addition of hydrazides to isothiocyanates is one of the convenient methods for thiosemicarbazides synthesis.

Thiosemicarbazide derivatives are known to have a wide range of biological effects, namely anticonvulsant, glypoglycemic, anti-inflammatory, and antibacterial ones [47-49].

In this regard, it was interesting to carry out the synthesis of a new thiosemicarbazide derivative (45) based on N-anabasinylacetic acid hydrazide. Thus, a thiosemicarbazide derivative (45), potentially having biological activity, was synthesized based on N-anabasinylacetic acid hydrazide by condensation of N-anabasinylacetic acid hydrazide with 1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylisothiocyanate (42) in an alcoholic solution at an equimolar ratio of the reagents used.



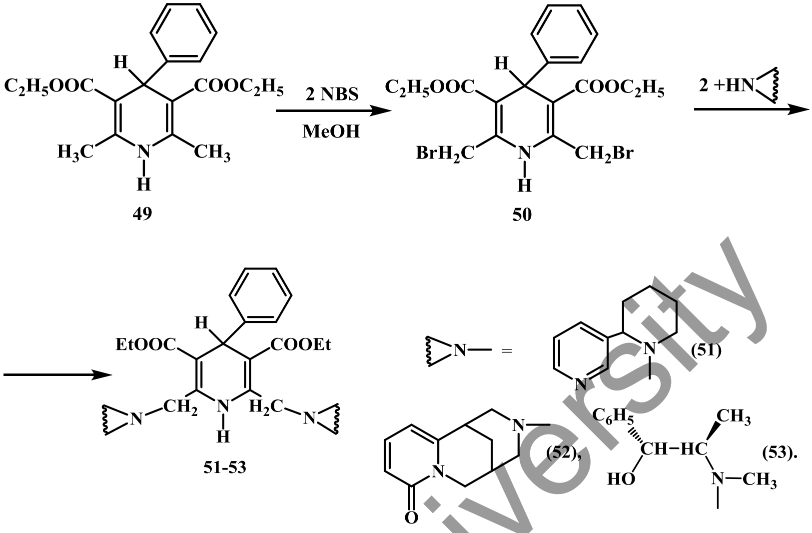
The authors of [50] studied the transamidation of d-pseudoephedrine cycloamidophosphite (46) with anabasine alkaloid. It has been established that cycloamidophosphite (46) is an effective phosphorylating agent, the use of which makes it possible to introduce the oxazaphospholane cycle into the backbone of the alkaloid anabasine. Further, the resulting cycloamidophosphite (47) was modified by interaction with sulfur to give the 2-thione derivative of amidophosphate (48).



Recently, a new class of heterocyclic compounds with 1,4-dihydropyridine structural fragment, which posess high antihypertensive and nootropic activity, has been widely used in medical practice [51].

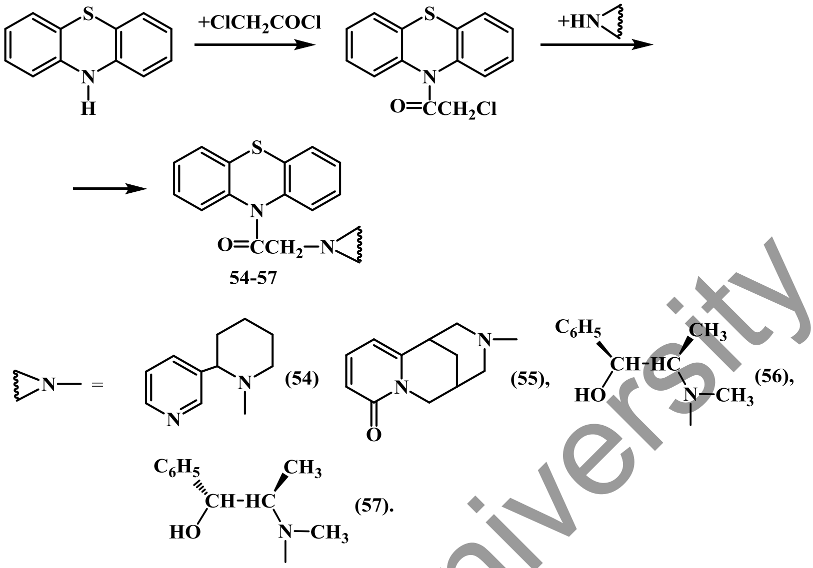
The Hantzsch method used for the synthesis of symmetrical 1,4-dihydropyridines has a wide variety of used, practically available aliphatic, aromatic or heterocyclic aldehydes, various derivatives of acetoacetic ester and ammonia (or primary amines). The latter makes it very promising for further search for new biologically active compounds and their chemical modification.

In [52], diethyl-4-(4-phenyl)-2,6-dimethyl-1,4-dihydro-pyridino-3,5-dicarboxylate (49) was synthesized in 60% yield by the Hantzsch method, namely three-component condensation of 2 moles of acetoacetic ester, benzaldehyde, and 25% aqueous ammonia solution. The obtained compound (49) was used further for the subsequent halogenation and substitution reaction. Bromination of (49) was carried out using a mild brominating agent, bromosuccinimide, at room temperature in methanol according to the procedure described in [53]. Using a double excess of bromosuccinimide, the corresponding dibromomethyl derivative (50) was obtained. The obtained dibromomethyl derivative of 1,4-dihydropyridine (50) turned out to be quite reactive in the nucleophilic substitution reaction. Thus, in the interaction of a benzene solution (47) with a double amount of anabasine, cytisine, and d-pseudoephedrine alkaloids, in the presence of an excess of triethylamine at room temperature and intensive stirring for a day, we isolated alkylation products (51-53).



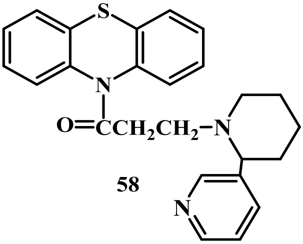
The combination of two or more pharmacophore fragments in a molecule is one of the main approaches to the chemical design of a new biologically active substance, including natural alkaloids. It is known that substances containing heterocyclic fragments in their structure occupy the first place in the general arsenal of all drugs [54]. S, N-containing heterocyclic compounds with a wide spectrum of biological activity occupy a special place among the numerous derivatives of heterocycles. For example, phenothiazine with a condensed tricyclic system is of great importance as an insecticide and antihelminthic drug. In addition, phenothiazine itself, like many sulfur-containing derivatives, has very low toxicity for warm-blooded animals [55, 56].

However, despite the huge number of synthesized phenothiazine derivatives, compounds that combine in their structure the tricyclic phenothiazine core and some physiologically active alkaloids as substituents have not been described in the literature. Thus, in [57], the authors synthesized previously unknown phenothiazine derivatives of the alkaloids cytisine, anabasine, l-ephedrine, and d-pseudoephedrine (54-57):



Alkylation of the alkaloids cytisine, anabasine, l-ephedrine, and d-pseudoephedrine with 10-(2-chloroacetyl)phenothiazine was carried out in boiling toluene in the triethylamine presence. Column chromatography and reprecipitation of hydrochlorides into a base were used to purify the target products.

N-alkaloid propionyl derivatives of phenothiazine (58-61) were also obtained in order to further study the structure-activity relationship. 10-Aminopropionyl derivatives of phenothiazine possess high anticholinergic and adrenolytic activity, antianginal and antiarrhythmic action [58].



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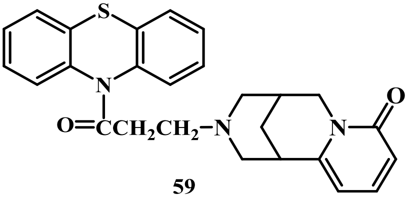

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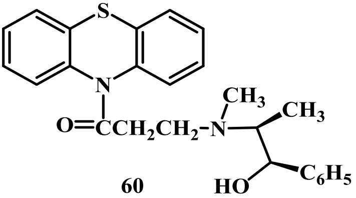

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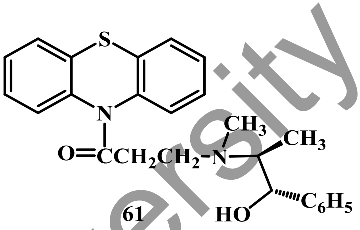


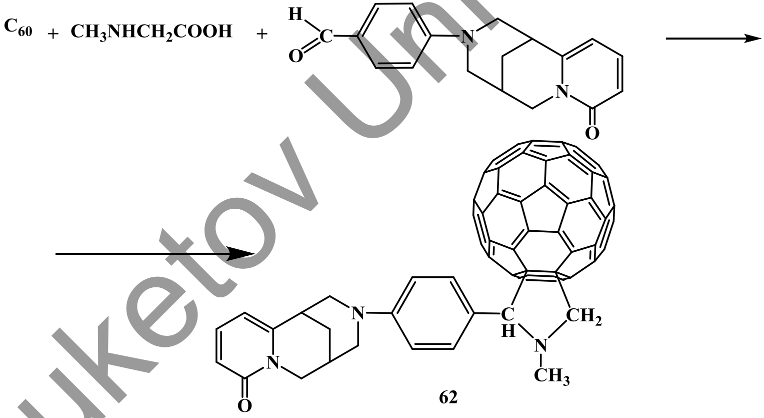
61

In the ^{1}H NMR spectra of compounds (58-61), protons signals of the alkaloid frameworks are recorded in their characteristic regions in addition to the protons of the phenothiazine fragment, which appear as duplicate groups of lines of multiplets and doublets in the region of 7.20-7.70 ppm. It should be noted that the methylene protons CH_2 of the carbonyl group of propionyl derivatives, as well as for acetyl derivatives, turn out to be nonequivalent and therefore they are recorded in complex multiplets.

2.6 Synthesis of new fullerene- and quercetin-containing cytisine alkaloid derivatives

Fullerenes attract the attention of researchers with their practical applications in science, biology and medicine, semiconductor technology and nanoelectronics [59-61]. The main directions for obtaining new materials and biologically active compounds based on fullerenes are associated with their functionalization using various reagents. An analysis of the literature data shows that the synthesis of C60 fullerene derivatives containing "pharmacophore" groups in their composition is of greatest interest [62-65]. The pyrrolidine ring is an interesting "pharmacophore" group. It is known that compounds containing a pyrrolidine cycle in ordinary organic compounds have a wide spectrum of biological activity and are included in many drugs of both natural and synthetic origin, such as proline, atropine. However, compounds containing both the pyrrolidine ring and the fullerene sphere have been little studied so far. In fullerene chemistry, fulleropyrrolidines obtained by the Prato reaction are most widely studied. One of the interesting ways to modify the C60 molecule is the introduction of a natural compound into its molecule, in particular, the alkaloid cytisine, whose derivatives exhibit various types of biological activity [66]. Cytisine, like nicotine, belongs to the group of ganglionic poisons that excite the central nervous system and ganglia of the autonomic nervous system, reflexively enhances respiration [28] and exhibits lipid-lowering activity [29].

Synthesis of a new fullerene-containing derivative of the alkaloid cytisine (62) was carried out by the interaction of C60 fullerene with sarcosine and 4-cytisinobenzaldehyde (obtained for the first time by us) in boiling toluene for 4 h according to the following scheme:



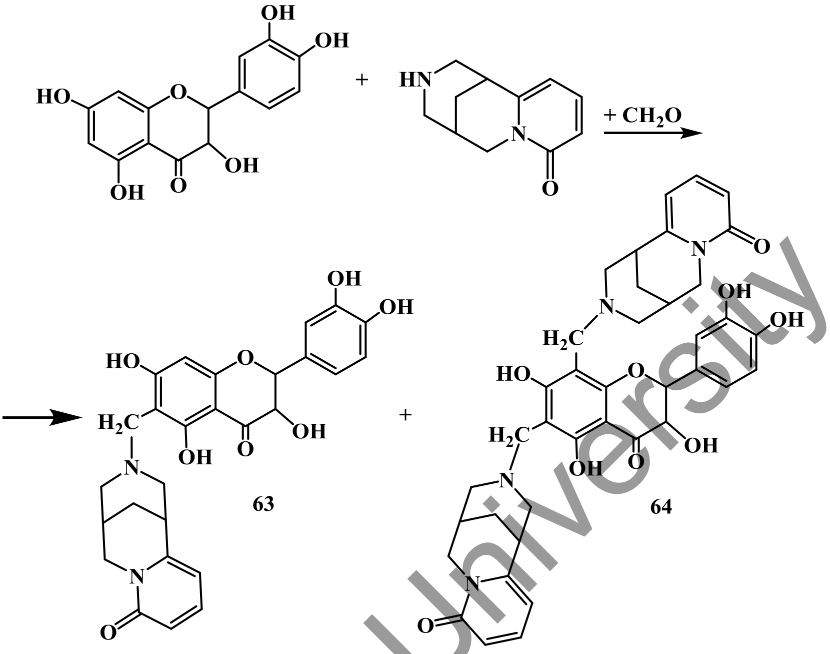
62

The unreacted starting materials and reaction product (62) after the reaction were separated by column chromatography on SiO_2, eluting with toluene and then with pyridine. In this case, the initial C60 fullerene, which did not enter into the reaction, was isolated at the beginning, and then the target fulleropyrrolidine (62) was isolated in 38% yield.

A probable scheme for the formation of fulleropyrrolidine (62) was proposed as a 1,3-dipolar addition to C60 fullerene through the intermediate formation of active azomethine ylides. At the first stage of the reaction, the condensation of an aromatic aldehyde with sarcosine occurred as a result of the nucleophilic addition of the amino group of sarcosine to the carbonyl group of the aldehyde. Further, in the resulting adduct, water was first eliminated, and then decarboxylated with the azoylide formation, which nucleophilically attacked the fullerene core at the (6-6) bond. As a result of the azoylide addition to the bond (6-6) of the fullerene core, a pyrrolidine ring was formed.

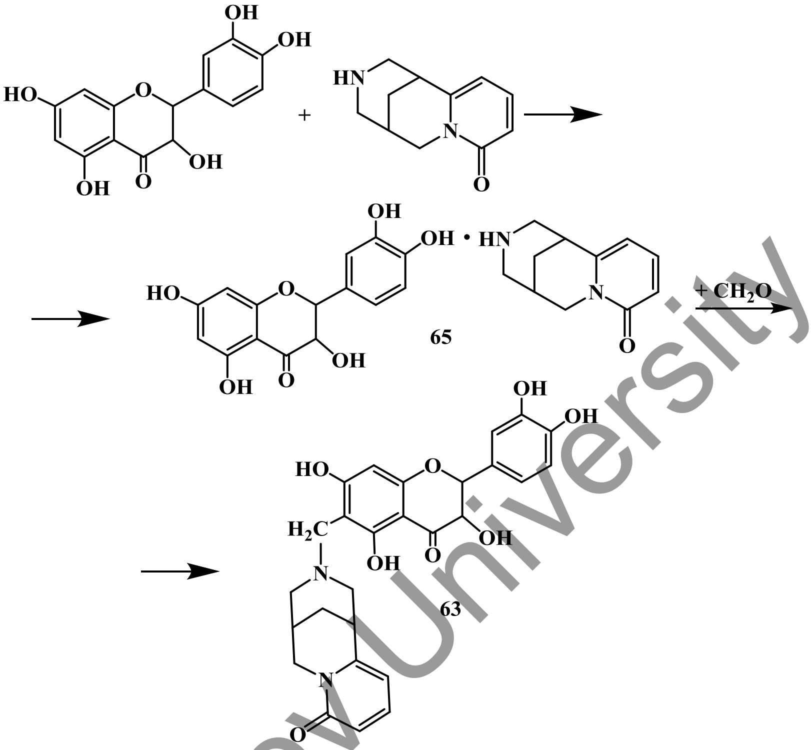
The synthesis of hybrid molecules containing fragments of natural compounds, in particular flavonoids and alkaloids, opens the way to a wide range of new compounds with potential biological activity. Flavonoids are natural biologically active compounds, among which dihydroquercetin (DHQ) occupies a special place. The latter has powerful antioxidant, hepatoprotective, antitumor, immunomodulating, and other properties [27]. The high biological activity and low toxicity of dihydroquercetin make it possible to attribute it to the group of leading compounds for chemical transformation in order to synthesize new hybrid polyfunctional pharmacologically active substances.

Kosheleva et.al obtained a mixture of mono- and disubstituted derivatives (63, 64) in a ratio of 2:1 (according to the HPLC method) by the interaction of DHQ, cytisine and formaldehyde in a molar ratio of 1:1.4:1.4 by adding a mixture of reagents to the substrate. With a twofold excess of reagents and back addition, the authors obtained a mixture of mono- and disubstituted derivatives of DHQ in a ratio of 2:1. It was shown that the formation of disubstituted derivative 64 was associated with a higher basicity of the alkaloid cytisine.



To simplify the direction of the reaction under study, we introduced a significant change. Dihydroquercetin was replaced by its complex with cytisine. Complex 65 was obtained upon short-term contact of equimolecular amounts of the initial reagents and was spontaneously isolated from the reaction mixture. According to its properties, adduct was not a salt, like ammonia derivatives, formed due to the interaction of one of the phenolic hydroxyls of dihydroquercetin with the nitrogen atom of the alkaloid cytisine.

Complex 65 was reacted with formaldehyde in 2-propanol at room temperature. The expected reaction product was isolated in the form of an individual yellowish powder (63).



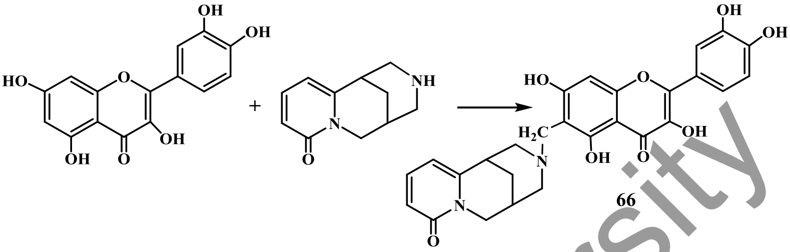
65

63

Interest in dihydroquercetin and quercetin is due to the fact that these flavonoids are actively used in the food industry and medicine. Both flavonoids belong to the group of phenolic compounds with antioxidant activity. In medical practice, dihydroquercetin and quercetin are used to treat radiation sickness, septic endocarditis, to prevent capillary lesions, etc. [29]. In recent years, the activity of quercetin against reverse transcriptase and HIV-1 integrase, as well as an inhibitory effect against the herpes virus, has been discovered [37, 38].

The presence of several hydroxyl groups, two aromatic rings and a pyrone ring in the quercetin molecule makes it possible to carry out its chemical modification in order to obtain a number of new biologically active derivatives for medicinal use. Thus, we were interested in the synthesis of the quercetin aminomethyl derivative

(66) based on the physiologically active alkaloid cytisine. Synthesis was carried out in a dioxane medium by adding an equimolar amount of a mixture of paraform and cytisine in dioxane to quercetin.



66

When analyzing the ^1H NMR spectrum of the compound 6-(cytisinemethyl)quercetin (66), it was found that the signal in the form of two doublets at 7.16 ppm corresponded to the H-28 proton. Doublet at 6.42 ppm referred to the H-27 proton and the doublet at 5.86 ppm referred to H-29 proton. The next group of lines, which was a complex signal in the region of 3.60-3.75 ppm corresponded to the methylene protons H-24. A group of lines in the region of 3.00-3.35 ppm represented signals from five protons H-32, H-22 and the tertiary proton H-23. Wide signal centered at 2.70 ppm corresponded to the tertiary proton H-31. The protons of the NCH_2 group resonated as a singlet at 3.77 ppm. The methine proton at C9 was recorded as a singlet at 6.49 ppm. The methine proton at C15 of ring B appearred as a doublet at 6.89 ppm, and the neighboring methine proton at C16 also resonated as a doublet at 7.58 ppm, and the methine proton at C12 appearred as a singlet in the region of 7.71 ppm.

2.7 Preparation of an inclusion complex based on β-CD and cytisine

At present, the interest of many scientists in the chemical modification of the molecules of heterocyclic compounds, namely natural alkaloids, does not weaken, due to the pronounced biological activity. In addition, it is widely known that the chemical transformation of natural compounds by introducing specific pharmacophore groups into their structure, including nitrogen- and sulfur-containing groups and heterocyclic structures that are part of the arsenal of many drugs, is considered as one of the promising methods for obtaining new classes of compounds with unique biological properties. The combination of two or more pharmacophore fragments in one molecule structure is one of the main approaches to the chemical design of a new biologically active compound, including natural alkaloids.

For many decades, the cytisine alkaloid has been one of the most promising synthons in terms of possible modification and the creation of new biologically active substances on its basis [67-69]. Cytisine is close to nicotine in pharmacological action and belongs to the group of “ganglionic poisons”. It has analeptic and anti-smoking activity, which is due to its high affinity for nicotinic acetylcholine neuroreceptors [67, 70]. It is promising to use this alkaloid as a component of a supramolecular system (substrate) with oligosaccharides of a cyclic structure, namely β-cyclodextrins (β-CDs) [71, 72] (receptors), which have a molecule in the form of a truncated cone with internal protons H-3 and H-5 and external H-2 and H-4. The possibility of including the active substance in the β-cyclodextrin capsule is due to hydrophobic interactions between the biologically active substance and the complexing agent.

The detection of complex formation by NMR spectroscopy is based on determining the difference in the values of the chemical shifts of β-CD protons in the free state and in the composition of complexes. It makes possible to draw conclusions about the formation of internal or external clathrates. It should be noted that the change in the chemical shifts of ^{1}H and ^{13}C in the spectra of the substrate can determine the direction in which the latter enters the receptor cavity.

As noted earlier, the study of the proton spectra of the objects of study will make it possible to reveal the difference in the values of the chemical shifts of the ^{1}H nuclei, and thereby draw conclusions about the existence of the complex and the interacting atoms of its constituent parts. The ^{1}H NMR spectrum of β-CD (Figure 17, a; Table 1) is characterized by the manifestation of six groups of signals in the region of 3.23-3.32; 3.45-3.60; 4.47-4.49; 4.77-4.78; 5.66; 5.71-5.73 ppm. The lowest-field doublet signal in the range of 5.71-5.73 ppm with a splitting of 4 Hz belongs to the proton of the hydroxyl group at the C-2 atom. The proton of the OH group of the neighboring atom

(OH-3), located in the inner cavity of the β-CD molecule, also resonates in the weak field region (δ 5.66 ppm, doublet). Doublet signal in the region of 4.77-4.78 ppm responds to the H-1 proton. The location of this proton in a weaker field compared to the protons of other CH groups is due to the oxygen atom influence. The OH-6 hydroxyl group resonates, splitting into a triplet centered at 4.48 ppm. In the high field region (3.49-3.60 ppm), accumulation of H-6 a, b signals of the methylene group is observed. High intensity signal at 3.45 ppm corresponds to the H-3 and H-5 protons of the glucopyranose unit. In the range from 3.23 to 3.32 ppm methine protons H-2 and H-4 appear. In the downfield part of the proton spectrum of cytisine (Figure 17, b), two doublet and one triplet signals of the 1H α-pyridine nucleus are observed: δ(H-5) 5.99-6.01 ppm; δ(H-3) 6.15-6.17 ppm; δ(H-4) 7.25-7.29 ppm. In the area of 3.63-3.80 ppm resonation of the axial and equatorial protons at the C-10 atom has been noted, with the Ha signal shifted to a stronger field. Four protons of the methylene groups associated with the NH group and the methine group at the C-7 atom give signals in the range from 2.73 to 2.90 ppm, splitting under the influence of neighboring atoms into triplets and a multiplet, respectively. The signals of the high-field part of the spectrum (δ 1.77 ppm, δ 2.20 ppm) correspond to the H-8 and H-9 protons.

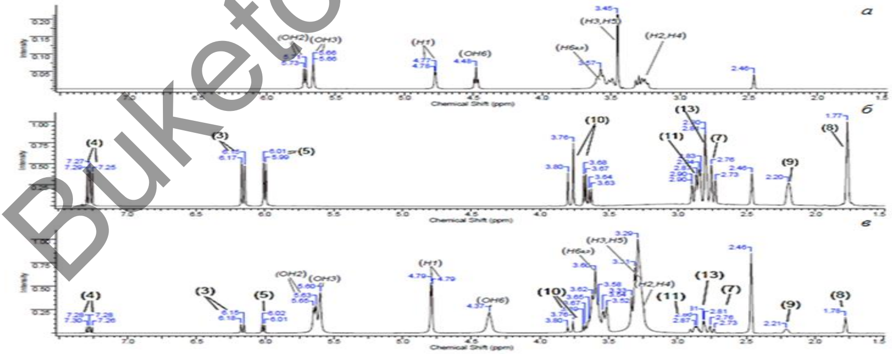


Figure 17. ^{1}H NMR spectra of β-CD (a), cytisine (b) and their inclusion complex (c)

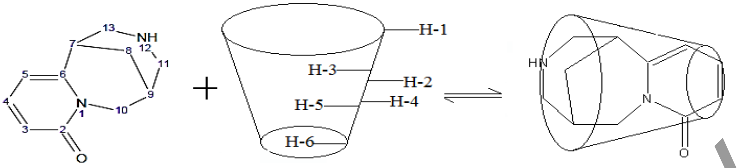
Table 1

Chemical shifts of the ^1H and ^{13}C NMR spectra of cytisine and β-CD outside the complex and within it

Group	In the free state (δ_0)		As part of the complex (δ)		$\Delta\delta(\delta - \delta_0)$	
CH_x	$\delta(^1H)$	$\delta(^{13}C)$	$\delta(^1H)$	$\delta(^{13}C)$	$\Delta\delta(^1H)$	$\Delta\delta(^{13}C)$
>C=O	–	162.85	–	162.92	–	0.07
=CH-	6.16	115.60	6.17	115.62	0.01	0.02
=CH-	7.27	139.19	7.28	139.27	0.01	0.08
=CH-	6.00	104.36	6.01	104.51	0.01	0.15
>C=	–	152.95	–	152.89	–	-0.06
>CH-	2.76	35.33	2.76	35.27	0	-0.06
$-CH_2-$	1.77	26.41	1.78	26.35	0.01	-0.06
>CH-	2.20	27.75	2.21	27.69	0.01	-0.06
$-CH_2-$	3.76	49.98	3.76	49.98	0	0
$-CH_2-$	2.84	53.16	2.86	53.08	0.02	-0.08
$-CH_2-$	2.80	54.07	2.81	54.01	0.01	-0.06
>CH-	4.77	102.43	4.79	102.49	0.02	0.06
>CH-	3.27	72.87	3.29	72.97	0.02	0.10
>CH-	3.45	73.54	3.60	73.60	015	0.06
>CH-	3.30	82.00	3.31	82.12	0.01	0.12
>CH-	3.45	72.52	3.60	72.59	0.15	0.07
$-CH_2-$	3.57	60.40	3.62	60.49	0.05	0.09

Based on the data presented in Table 1, it can be noted that all protons of the glucopyranose unit of β-CD in the complex are shifted towards a weaker field compared to similar protons of an individual complexing agent. The greatest difference in the values of the chemical shift (Δδ(1H) 0.15 ppm) is characteristic of the protons of the internal cavity of the toroidal molecule H-3 and H-5, which is caused by the interaction of these protons with the polar ligand and the formation of an internal nanocomplex.

Taking into account the results of the NMR spectroscopic study, as well as the data on the sizes of the molecules of cytisine and β-CD (Figure 18), it was concluded that the substrate completely entered the receptor cavity and the formation of an inclusion complex with a composition of 1:1.



(67)

Figure 18. Schematic representation of the complexation of cytisine with β-CD (67)

The inclusion complex of cytisine with β-cyclodextrin (67) was obtained by the interaction of 0.19 g (0.001 mol) of cytisine in 10 ml of ethanol and 1.135 g (0.001 mol) of β-cyclodextrin in 30 ml of water. The solution was stirred on a magnetic stirrer at 50°C for 5 hours. The precipitate was filtered off, washed with acetone and dried at room temperature. There was obtained a β-CD with cytisine complex in the form of a white powder, melting with decomposition at 280°C.

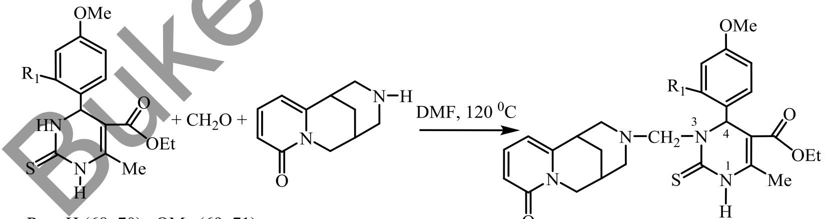
2.8 The Mannich aminomethylation of 3,4-dihydropyrimidine-(1H)-2-thiones with the alkaloid cytisine

Recently, the number of publications on the chemistry of 4-aryl-3,4-dihydropyrimidine-2-thiones and 4-aryl-3,4-dihydropyrimidine-2-ones obtained by three-component condensation has significantly increased in the scientific literature, which is associated with the manifestation of a wide range of spectrum of biological activity [73-75]. The attention of many chemists is also attracted by the presence of several reactive nucleophilic centers in 4-aryl-3,4-dihydropyrimidine-2-thiones, which allow not only to carry out various mono- and dialkylation and acylation [76-78], but also to carry out very promising cyclization reactions on their basis.

The Mannich reaction (aminomethylation according to Mannich) is widely used in organic practice for the synthesis of various practically important compounds. The Mannich reaction is based on the interaction between the "C-H"-acid component, a primary or secondary amine, and a carbonyl component. Secondary amines are most often used as the amine component (to obtain Mannich monobases), although primary amines and even ammonia can be used. In this case, the main condition for the possibility of the Mannich reaction to occur is the use of an amine with a higher nucleophilicity than that of the initial C-H acid compound.

Since the starting 3,4-dihydropyrimidine-(1H)-2-thiones have two reaction centers with nucleophilic N atoms (in the ring) and an S atom, which also has a certain nucleophilicity and is involved in possible thione-thiol tautomerism, it was of interest to us to study the possibility the participation of these thiones in the Mannich synthesis as an N-H or S-H acidic component, as well as the possibility of obtaining mono- or bis-Mannich bases on their basis, including pharmacologically important alkaloids.

In order to obtain combined compounds (70, 71), for the first time we performed Mannich aminomethylation of 3,4-dihydropyrimidine-(1H)-2-thiones (68, 69) with a 40% aqueous solution of formaldehyde and cytisine. The reaction was carried out by boiling the initial reagents in a DMF solution at a temperature of 120^{0}C for 15-20 hours with an excess of formalin and with different ratios of thione to taken cytisine (1:1; 1:1.5; 1:2). At the same time, in each case, the formation of one reaction product was observed by TLC analysis. The acceptable yields of products, when isolated from the reaction medium, were maximum at a thione-cytisine ratio (1:1.5; 1:2).



R$_1$ = H (68, 70), OMe (69, 71)

(70, 71)

During the reaction, the formation of both N- and S-aminomethyl derivatives of Mannich bases was assumed. In addition, the formation of possible bis-Mannich bases with an aminomethyl group bonded to the N(1)-, N(3)-atoms or N-, S- of the dihydropyrimidine ring was not excluded. It was also possible that 3,4-dihydropyrimidine-(1H)-2-thione would not enter into the aminomethylation reaction, but a simple crosslinking of two cytisine molecules would occur to form dicytisinomethane. Analysis of the mass spectrum of compound (70) showed the presence of a molecular ion with a low intensity: [M^+] 508 (2%), corresponding to the molecular weight of the proposed structure (70), as well as fragments of the decomposition of the molecule 306 (22), 277 (32), 233 (39), 203 (76), 58 (100), 42 (59), among which the fragment with m/z 203 (76%) corresponds to the $\rangle N\text{-}CH_2^+$ fragment of the cytisine framework.

^1H NMR spectroscopic study of the isolated reaction compounds also showed the presence of protons of the starting 3,4-dihydropyrimidine(1H)-2-thione and the alkaloid cytisine (Figure 19).

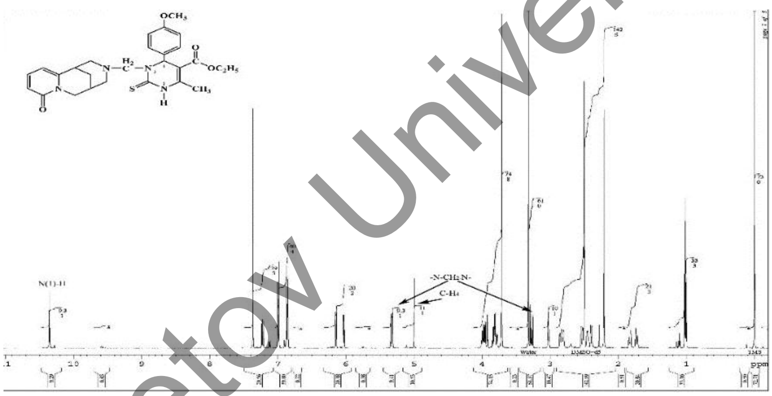


Figure 19. ^1H NMR spectrum of 4-(4-methoxyphenyl)-6-methyl-3-(N-cytisinomethyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (70)

In addition, analysis of the ^1H NMR spectrum of compound (70) showed the presence of a characteristic singlet of the pyrimidine ring C(4)-H proton at 5.00 ppm, indicating the absence of interaction with the neighboring N(3)-H proton, in the presence of which, for example, in the initial 3,4-dihydropyrimidine-(1H)-2-thiones, the C(4)-H proton signal is split into a doublet. The free proton N(1)-H was recorded as a singlet at 10.35 ppm. At the same time, the aminomethylene protons of the >NCH_2N< fragment were nonequivalent and appearred as two characteristic doublets in different spectral regions at 5.33 ppm and 3.27 ppm with a difference of 2.06 ppm and SSCC J = 11.7 Hz. This correlation of aminomethylene protons was also confirmed by the recorded two-dimensional NOESY spectrum, which confirmed the responses of interacting unequal aminomethylene protons, as well as the response of the N(1)-H proton with the neighboring methyl C(6)-CH_3 group (Figure 20).

Therefore, according to the ^{1}H and NOESY NMR spectra, N(3)-aminomethylation of the starting 3,4-dihydropyrimidine-(1H)-2-thiones occurs.

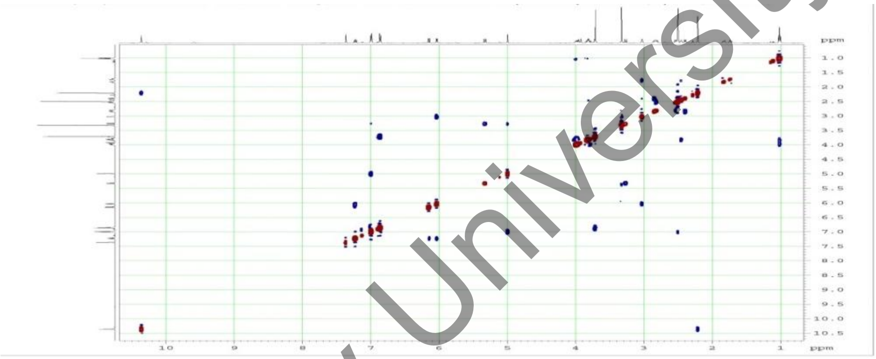


Figure 20. ^{1}H–^{1}H NOESY spectrum of 4-(4-methoxyphenyl)-6-methyl-3-(N-cytisinomethyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (70)

Method for the synthesis of compound (70): 1.23 g (4 mmol) of 3,4-dihydropyrimidine (1H)-2-thione (68) and 10 ml of DMF were added to 2.5 g of 40% formalin solution. The reaction mixture was heated to reflux with stirring on a magnetic stirrer and after 2 minutes, 1.52 g (8 mmol) was added. The reaction mixture was further heated for 25-30 hours until the disappearance of the original cytisine by TLC. After cooling, the solution was poured into a beaker with 100 ml of ice water. The precipitated gray precipitate was filtered off and washed. There was obtained 1.85 g (90%) of product (70). After 2 recrystallizations from benzene and a mixture of benzene-hexane, white needles were obtained with mp 214-215^{0}C. Compound (71) was obtained by a similar procedure in 70% yield, mp was 227-228^{0}C.

Chapter 3

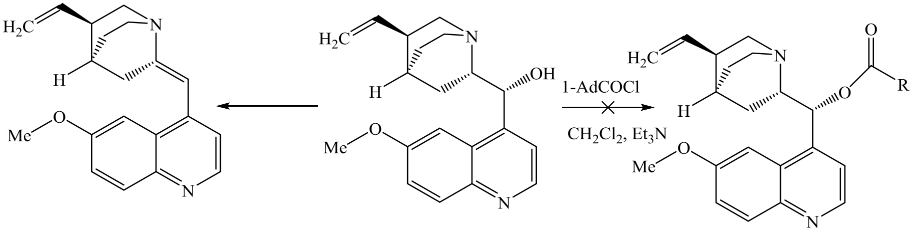
METHODS FOR THE SYNTHESIS OF COMBINED DERIVATIVES OF SOME ALKALOIDS

The IR spectra of the compounds were recorded on a Nikolet Protege-460 Fourier spectrophotometer with preparation of samples in the form of tablets with KBr or in a thin layer. The UV spectra of the compounds were recorded on a Varian Cary 300 spectrophotometer using quartz cuvettes with *l* = 1 cm. Methanol was used as a solvent. The concentration of the studied compounds was $c = 10^{-5}$ mol/l. ^{1}H and ^{13}C NMR spectra were recorded on an Avance-500 Bruker spectrometer in $CDCl_3$ or DMSO-d_6 relative to residual solvent signals. [$CDCl_3$, δH 7.26, δC 77.2 ppm; DMSO-d_6, δH 2.5, δC 40.1 ppm]. HPLC-MS studies were performed using an Agilent 1200 liquid chromatograph with an Agilent 6410 Triple Quad mass selective detector in Positive ESI MS2 Scan mode. There was used the ZORBAX Eclipse XDB-C18 column (4.6 x 50 mm; 1.8 μm). Mobile phase was water containing 0.05% (v/v) formic acid - acetonitrile (40 to 90% in 10 minutes). Elution rate was 0.5 ml/min. Elemental analysis was performed on a Vario MICRO cube V1.9.7 CHNS analyzer.

Method for the synthesis of quinine esters (KN-1, 8, 11). Quinine (2.6 g, 0.008 mol) was dissolved in 100 ml of dry dichloromethane, which was preliminarily kept for 1 day over $CaCl_2$ (to remove 0.5% of ethanol used to stabilize dichloromethane). 1.0 g (0.01 mol) of triethylamine and 0.009 mol of 4,5-dichloroisothiazole-3-carboxylic acid chloride (KN-1), 5-(*p*-tolyl)isoxazole-3-carboxylic acid (KN-11) or 1-adamantanecarboxylic acid chloride (KN-8) were added successively with stirring to the resulting solution. The mixture was stirred for 1 hour and left for 15 hours at 20-23°C. The mixture was washed with water (2 x 200 ml, 1 hour each with stirring) and 5% sodium bicarbonate solution (2 x 200 ml, 1 hour each with stirring). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed; the residue was crystallized from a mixture of ether and hexane.

(1*R*)-6-Methoxyquinolin-4-yl[(2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl]methyl 4,5-dichloroisothiazole-3-carboxylate (KN-1). Yield is 88%, m.p. is 131-132°C. IR spectrum (KBr), ν, cm^{-1}: 3075, 3010, 2996, 2964, 2946, 2906, 2889, 2863, 1732 (C=O), 1623, 1589, 1560, 1512, 1464, 1455, 1403, 1354, 1263, 1241, 1213, 1174, 1082, 1036, 1018, 986, 875, 954, 917, 906, 859, 843, 822, 790, 740, 710, 690, 645, 630, 605. UV spectrum (MeOH, $c = 4 \cdot 10^{-5}$ mol/l), λ_{max}, nm (ε): 232 (37000), 274 (8000), 317 (5000), 333 (5000). ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.40-1.49 m (1H, CH_2), 1.51-1.60 m (1H, CH_2), 1.70-1.77 m (1H, CH_2), 1.77-1.82 m (1H, CH), 1.93-2.03 m (1H, CH_2), 2.17-2.26 m (1H, C$\underline{H}$–CH=CH_2), 2.39-2.49 m (2H, CH_2 + CH_2), 2.77-2.89 m (1H, CH_2), 3.13-3.22 m (1H, CH_2), 3.50 k (1H, CH–N, J 8.3 Hz), 3.95 s (3H, OMe), 4.95-5.07 m (2H, CH=C$\underline{H_2}$), 5.89-6.00 m (1H, C$\underline{H}$=CH_2), 6.59 d (1H, CH–O, J 8.6 Hz), 7.44 dd ($1H_{quin}$, J 9.2, 2.6 Hz), 7.56 d ($1H_{quin}$, J 4.5 Hz), 7.63 d ($1H_{chin}$, J 2.6 Hz), 7.96 d ($1H_{chin}$, J 9.2 Hz), 8.71 d ($1H_{chin}$, J 4.5 Hz). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 25.76 (CH_2), 27.62 (CH), 27.74 (CH_2), 40.36 ($\underline{C}$H–CH=CH_2), 42.21 (CH_2), 56.10 (OMe), 56.24 (CH_2), 60.05 (CH–N), 76.34 (CH–O), 102.75 ($1CH_{quin}$), 114.91 (=CH_2), 119.89 ($1CH_{quin}$), 122.13 ($1CH_{quin}$), 131.91 ($1CH_{quin}$), 142.88 ($\underline{C}$H=CH_2), 148.13 ($1CH_{quin}$), 125.16, 127.40, 144.19, 144.55, 151.26, 153.49, 157.90, 158.48 (8 $C_{quaternary}$). It was found, %: C 57.44; H 3.99; Cl 20.41; N 12.11; S 9.24. Mass spectrum, *m/z* (I_{rel}, %): 504.10 $[M+H]^+$ (42.3). $C_{24}H_{23}Cl_2N_3O_3S$. It was calculated, %: C 57.15; H4.60; Cl 14.06; N 8.33; S 6.36. *M* is 504.43.

(1*R*)-6-Methoxyquinolin-4-yl[(2*S*,4*S*,5*R*)-5-vinyl-quinuclidin-2-yl]methyladamantane-1-carboxylate (KN-8). Compound KN-8 could not be obtained due to the elimination of 1-adamatanecarboxylic acid and the formation of (4*S*,5*R*,E)-2-(6-methoxyquinolin-4-yl)methylene-5-vinylquinuclidine.



KN-8

(*R*)-6-Methoxyquinolin-4-yl[(1*S*,2*R*,4*S*,5*R*)-5-vinyl-quinuclidin-2-yl]methyl-5-(*p*-tolyl)isoxazole-3-carboxylate (KN-11). Yield is 86%, m.p. is 148-149^{0}C. IR spectrum (KBr), ν, cm^{-1}: 3137 (CH_{isox}), 3072 3029, 2945, 2923, 2882, 2866, 1737 (C=O), 1622, 1592, 1513, 1460, 1446, 1316, 1295, 1265, 1240, 1206, 1172, 1136, 1112, 1084, 1037, 1020, 999, 947, 928, 851, 823, 812, 800, 781, 715, 687, 677, 567, 501. UV spectrum (MeOH, $c = 6{\cdot}10^{-5}$ mol/l), λ_{max}, nm (ε): 233 (38000), 278 (20000), 317 (5000), 332 (5000). ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.41-1.50 m (1H, CH_2), 1.53-1.62 m (1H, CH_2), 1.70-1.77 m (1H, CH_2), 1.78-1.84 m (1H, CH), 1.98-2.07 m (1H, CH_2), 2.18-2.27 m (1H, $C\underline{H}$–CH=CH_2), 2.37 s (3H, Me), 2.40-2.49 m (2H, CH_2 + CH_2), 2.82-2.90 m (1H, CH_2), 3.15-3.24 m (1H, CH_2), 3.50 q (1H, CH–N, J 8.3 Hz), 3.96 s (3H, OMe), 4.96-5.07 m (2H, =CH_2), 5.92-6.02 m (1H, $C\underline{H}$=CH_2), 6.59 d (1H, CH–O, J 8.4 Hz), 7.37 d ($2H_{arom}$, J 8.1 Hz), 7.45 dd ($1H_{quin}$, J 9.2, 2.6 Hz); 8.72 d ($1H_{quin}$, J 4.5 Hz). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 21.59 (Me), 25.45 (CH_2), 27.68 (CH), 27.76 (CH_2), 39.88 ($\underline{C}$H–CH=CH_2), 42.22 (CH_2), 56.19 (OMe), 56.26 (CH_2), 60.07 (CH–N), 76.30 (CH–O), 100.79 (CH_{isox}), 102.69 ($1CH_{quin}$), 114.92 (=CH_2), 119.82 ($1CH_{quin}$), 122.12 ($1CH_{quin}$), 126.40 ($2CH_{arom}$), 130.44 ($2CH_{arom}$), 131.94 ($1CH_{quin}$), 142.94 ($\underline{C}$H=CH_2), 148.14 ($1CH_{quin}$), 123.88, 127.88, 141.75, 144.17, 144.58, 156.94, 155.90). It was found, %: C 73.44; H 6.36; N 18.99. Mass spectrum, *m/z* (I_{rel}, %): 510.20 $[M+H]^+$ (52.7). $C_{31}H_{31}N_3O_4$. It was calculated, %: C 73.06; H 6.13; N 8.25. *M* is 509.61.

Method for the synthesis of anabasine amides (KN-2, 3, 7, 10). Anabasine (1.6 g, 0.01 mol) was dissolved in 100 ml of dry dichloromethane, which was preliminarily kept for 1 day over $CaCl_2$ (to remove 0.5% of ethanol used to stabilize dichloromethane). 1.2 g (0.012 mol) of triethylamine and 0.011 mol of 4,5-dichloroisothiazole-3-carboxylic acid chloride (KN-2), 5-tolylisoxazole-3-carboxylic acid (KN-3), 5-phenylisoxazole-3-carboxylic (KN-7) or 1-adamantanecarboxylic acid (KN-10) were added successively with stirring to the resulting solution. The mixture was stirred for 1 hour and left for 15 hours at 20-23°C. The mixture was washed with water (2 x 200 ml, 1 hour each with stirring) and 5% sodium bicarbonate solution (2 x 200 ml, 1 hour each with stirring). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed; the residue was crystallized from a mixture of ether and hexane (KN-2) or isolated as a viscous oil (KN-3, 7).

(*S*)-(4,5-Dichloroisothiazole-3-yl)(2-pyridin-3-ylpiperidin-1-yl)methanone (KN-2). Yield is 82%, m.p. is 154-155°C. IR spectrum (KBr), ν, cm^{-1}: 3095, 3085, 3070, 3041, 3010, 2981, 2950, 2935, 2872, 1637 (C=O), 1587, 1572, 1500, 1477, 1448, 1440, 1416, 1381, 1346, 1319, 1289, 1270, 1253, 1245, 1192, 1163, 1129, 1109, 1072, 1040, 1025, 1014, 962, 922, 890, 853, 835, 825, 808, 780, 770, 755, 730, 712, 691, 641, 620, 571, 548, 493. UV spectrum (MeOH, $c = 6 \cdot 10^{-5}$ mol/l), λ_{max}, nm (ε): 257 (9000), 263 (11000), 269 (10000). ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.35-1.72 m (4H, $2CH_2$), 1.81-1.95 m, 2.40-2.56 m (2H, CH_2); 2.61-2.73 m, 2.89-3.01 m (1H, NCH_2); 3.33-3.44 m, 4.39-4.49 m (1H, NCH_2); 4.96-5.04 m, 5.86-5.93 m (1H, CH); 7.38-7.49 m ($1H_{pyr}$); 7.67-7.77 m ($1H_{pyr}$); 8.46-8.61 m (2Hpyr). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 19.58, 19.65 (CH_2); 25.53, 26.08 (CH_2); 27.45, 28.77 (CH_2); 38.65, 43.72 (NCH_2); 50.12, 55.39 (CH); 124.18, 124.33 ($1CH_{pyr}$); 134.92, 135.04 ($1CH_{pyr}$); 148.57, 148.70 ($2CH_{pyr}$); (121.65, 121.78); (134.24, 134.35); (149.59, 149.73); 160.68; (162.07, 162.17) (5 $C_{quaternary}$). It was found, %: C 49.42; H 4.01; Cl 20.41; No. 12.00; S 9.03. Mass spectrum, *m/z* (I_{rel}, %): 342.00 $[M]^+$ (100). $C_{14}H_{13}Cl_2N_3OS$. It was calculated, %: C 49.13; H 3.83; Cl 20.72; N 12.28; S 9.37. *M* is 342.24.

(*S*)-(2-Pyridin-3-ylpiperidin-1-yl)[5-(*p*-tolyl)isoxazol-3-yl]methanone (KN-3). Yield is 78%, thick viscous oil. IR spectrum (thin layer), ν, cm^{-1}: 3128 (CH_{isox}), 3090, 3032, 2925, 2857, 1635 (C=O), 1594, 1573, 1510, 1478, 1442, 1418, 1392, 1355, 1321, 1258, 1235, 1185, 1147, 1124, 1027, 1019, 980, 948, 880, 860, 820, 810, 760, 720, 706, 680, 640, 620, 503. UV spectrum (MeOH $c = 9{\cdot}10^{-5}$ mol/l), λ_{max}, nm (ε): 270 (25000). ^1H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.52-1.82 m (4H, $2CH_2$); 1.98-2.11 m, 2.43-2.51 m (2H, CH_2); 2.31-2.43 m (3H, Me); 2.64-2.77 m, 2.97-3.13 m (1H, NCH_2); 4.29-4.42 m, 4.61-4.73 m (1H, NCH_2); 5.88-5.98 m, 6.08-6.18 m (1H, CH); 6.78 s, 6.80 s (CH_{isox}); 7.19-7.28 m ($2N_{arom}$); 7.28-7.35 m ($2H_{pyr}$); 7.57-7.66 m ($2N_{arom}$); 7.67-7.72 m ($1H_{pyr}$); 8.52 d ($1H_{pyr}$, J 4.5 Hz); 8.58-8.62 m ($1H_{pyr}$). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 19.62 (CH_2); 21.63 (Me); 25.55, 26.41 (CH_2); 27.07, 28.37 (CH_2); 39.02, 43.87 (NCH_2); 50.32, 55.35 (CH); 100.09 (CH_{isox}); 123.75 ($1CH_{pyr}$); 125.81, 125.99 ($2CH_{arom}$); 129.91, 130.18 ($2CH_{arom}$); 134.90 ($1CH_{pyr}$); 148.44, 148.52 ($1CH_{pyr}$); 148.65 ($1CH_{pyr}$); 124.13, (134.15, 134.30); 141.20, 159.15; 161.08; 170.82 (6 $C_{quaternary}$). It was found, %: C 72.98; H 6.22; N 11.95. Mass spectrum, *m/z* (I_{rel}, %348.20 $[M]^+$ (100), 349.20 $[M+H]^+$ (43.2), 695.30 [2*M*] (6.0), 717.30 $[2M+Na]^+$ (12.1). $C_{21}H_{21}N_3O_2$. It was calculated, %: C 72.60; H 6.09; N 12.10. *M* is 347.41.

(S)-(2-Pyridin-3-ylpiperidin-1-yl)(5-phenylisoxazole-3-yl)methanone (KN-7). Yield is 87%, thick viscous oil. IR spectrum (thin layer), ν, cm^{-1}: 3121 (CH_{isox}), 3090, 3032, 2925, 2857, 1635 (C=O), 1594, 1573, 1510, 1478, 1442, 1418, 1392, 1355, 1321, 1258, 1235, 1185, 1147, 1124, 1027, 1019, 980, 948, 880, 860, 820, 810, 760, 720, 706, 680, 640, 620, 503. UV spectrum (MeOH, $c = 1{\cdot}10^{-4}$ mol/l), λ_{max}, nm (ε): 264 (23000). ^1H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.35-1.81 m (4H, $2CH_2$), 2.00-2.11 m, 2.44-2.52 m (2H, CH_2); 2.66-2.76 m, 2.99-3.12 m (1H, NCH_2); 4.29-4.42 m, 4.61-4.73 m (1H, NCH_2); 5.88-5.98 m, 6.06-6.18 m (1H, CH); 6.84 s, 6.86 s (CH_{isox}); 7.29-7.34 m ($1H_{pyr}$); 7.40-7.51 m ($3N_{arom}$); 7.61-7.68 m ($1H_{pyr}$); 7.70-7.84 m ($2N_{arom}$); 8.52 d ($1H_{pyr}$, J 4.5 Hz); 8.55-8.66 m ($1H_{pyr}$). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 19.62 (CH_2); 25.55, 26.41 (CH_2); 27.07, 28.39 (CH_2); 39.06, 43.89 (NCH_2); 50.36, 55.37 (CH); 100.73, 100.77 (CH_{isox}); 123.77 ($1CH_{pyr}$); 126.05 ($2CH_{arom}$); 129.24 ($1CH_{arom}$); 130.81 ($2CH_{arom}$); 134.84, 134.93 ($1CH_{pyr}$); 148.39, 148.43

($1CH_{pyr}$); 148.53, 148.60 ($1CH_{pyr}$); (126.73, 126.82); (134.15, 134.28); (159.21, 159.29); (160.80, 160.98); 170.63 ($5\ C_{quaternary}$). It was found, %: C 72.35; H 5.81; N 12.44. Mass spectrum, *m/z* (Irel, %): 334.20 $[M+H]^+$ (100), 356.10. $[M+Na]^+$ (6.0), 667.30. $[2M+H]^+$ (34.3), 689.30. $[2M+Na]^+$ (34.2). $C_{20}H_{19}N_3O_2$. It was calculated, %: C 72.05; H 5.74; N 12.60. *M* is 333.39.

Adamantan-1-yl[(*S*)-(2-pyridin-3-ylpiperidin-1-yl)]methanone (KN-10). Yield is 79%, thick viscous oil. IR spectrum (thin layer), ν, cm^{-1}: 3083, 3034, 2938, 2906, 2852, 1621 (C=O), 1573, 1478, 1453, 1401, 1353, 1345, 1327, 1300, 1266, 1243, 1181, 1157, 1131, 1102, 1078, 1027, 1005, 973, 936, 855, 812, 766, 727, 715, 647, 633. λ_{max}, nm (ε): 255 (32000), 263 (2500), 269 (2000), 314 (200). ^1H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.46-1.62 m (2H, CH_2), 1.64-1.78 m (6H, CH_{2adam}); 1.85-1.93 m (3H, $3CH_{2adam}$), 1.97-2.14m (10H, $3CH_{2adam}+2CH_2$); 2.33-2.43 m (1H, CH_2); 4.27-4.38 m (1H, CH_2); 5.88-6.02 m (1H, CH); 7.22-7.29 m ($1H_{pyr}$); 7.43-7.51 m ($1H_{pyr}$); 8.41-8.52 m ($2H_{pyr}$). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 19.70 (CH_2); 26.39, 27.27 (CH_2); 27.80 (CH); 28.66 ($3CH_{adam}$); 36.43 (CH_2); 36.77 ($3CH_{2adam}$); 38.40 (CH2); 39.30 ($3CH_{2adam}$); 123.64 ($1CH_{pyr}$); 134.90 ($1CH_{pyr}$); 147.94 ($1CH_{pyr}$); 148.73 ($1CH_{pyr}$); 42.23, 135.24, 176.73 ($3\ C_{quaternary}$). It was found, %: C 77.98; H 8.76; N 8.52. Mass spectrum, *m/z* (Irel, %): 325.30 $[M+H]^+$ (100), 671.40 $[2M+Na]^+$ (10.0). $C_{21}H_{28}N_2O$. It was calculated, %: C 77.74; H8.70; N 8.63. *M* is 324.47.

Procedure for the cytisine amides synthesis (KN-4, 5, 6, 9). 1.9 g (0.01 mol) of cytisine was dissolved in 100 ml of dry dichloromethane, which was preliminarily kept for 1 day over $CaCl_2$ (to remove 0.5% of ethanol used to stabilize dichloromethane). 1.2 g (0.012 mol) of triethylamine and 0.011 mol of 4,5-dichloroisothiazole-3-carboxylic acid chloride (KN-4), 5-tolylisoxazole-3-carboxylic acid (KN-5), 5-phenylisoxazole-3-carboxylic (KN-6) or 1-adamantanecarboxylic acid (KN-9) were added successively with stirring to the resulting solution. The mixture was stirred for 1 hour and left for 15 hours at 20-23°C. The mixture was washed with water (2 x 200 ml, 1 hour each with stirring) and 5% sodium bicarbonate solution (2 x 200 ml, 1 hour each with stirring). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed; the residue was crystallized from a mixture of ether and hexane (KN-4, 6) or isolated as a viscous oil (KN-5).

(1*R*,5*S*)-3-(4,5-Dichloroisothiazole-3-carbonyl)-3,4,5,6-tetrahydro-1*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8(2*H*)-one (KN-4). Yield is 81%, m.p. is 232-233°C. IR spectrum (KBr), ν, cm^{-1}: 3066, 3022, 2995, 2960, 2945, 2924, 2873, 1656 (C=O), 1636 (C=O), 1577, 1565, 1547, 1501, 1465, 1453, 1445, 1423, 1380, 1365, 1356, 1335, 1305, 1258, 1240, 1230, 1218, 1186, 1160, 1143, 1130, 1109, 1100, 1090, 1061, 1036, 996, 968, 929, 919, 902, 889, 862, 833, 810, 798, 777, 760, 742, 735, 719, 694, 644, 610, 573, 560, , 540, 510, 453. UV spectrum (MeOH, c = $9\cdot10^{-5}$ mol/l), λ_{max}, nm (ε): 232 (12000), 267 (8000), 311 (7000). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.99-2.09 m (2H, CH_2), 2.42-2.49 m, 2.59-2.67 m (1H, CH); 2.93-3.01m, 3.16-3.22 m (1H, CH_2); 3.05-3.11 m, 3.37-3.44 m (1H, CH_2); 3.08-3.14 m, 3.45-3.51 m (1H, CH_2); 3.57-3.64 m, 3.77-3.83 m (1H, CH); 3.81-3.94 m (1H, CH_2); 4.11-4.23 m (1H, CH_2); 4.74-4.82 m, 4.84-4.92m (1H, CH_2); 5.81 dd, 6.11 dd ($1H_{pyr}$, J 6.8, 0.9 Hz); 6.39-6.49 m ($1H_{pyr}$); 7.19 dd, 7.28 dd ($1H_{pyr}$, J 9.1, 6.8 Hz). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 26.21, 26.29 (CH_2); 27.44, 27.68 (CH); 34.51, 34.89 (CH); 48.22, 48.68 (CH_2); 48.83, 49.19 (CH_2); 52.72, 53.92 (CH_2); 105.23, 106.00 ($1CH_{pyr}$); 117.87, 118.40 ($1CH_{pyr}$); 138.65, 139.13 ($1CH_{pyr}$); (123.24, 123.31); (147.80, 147.84); (149.07, 149.72); (159.35, 159.46); (161.61, 161.74); (163.44, 163.51) (6 $C_{quaternary}$). It was found, %: C 48.89; H 3.66; Cl 19.01; N 11.13; S 8.58. Mass spectrum, *m/z* (I_{rel}, %): 370.00 $[M]^+$ (100), 371.00 $[M+H]^+$ (16.5), 392.00 $[M+Na]^+$ (43.2), 763.00 $[2M+Na]^+$ (25.1). $C_{15}H_{13}Cl_2N_3O_2S$. It was calculated, %: C 48.66; H 3.54; Cl 19.15; N 11.35; S 8.66. *M* is 370.25.

(1*R*,5*S*)-3-[5-(*p*-tolyl)isoxazole-3-carbonyl]-3,4,5,6-tetrahydro-1*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8(2*H*)-one (KN-5). Yield is 84%, thick viscous oil. IR spectrum (in a thin layer), ν, cm^{-1}: 3125 (CH_{isox}), 3090, 3055, 3030, 2922, 2853, 1657 (C=O), 1610, 1577, 1545, 1488, 1441, 1414, 1391, 1355, 1342, 1305, 1258, 1230, 1180, 1165, 1155, 1138, 1120, 1091, 1070, 1045, 1036, 1020, 1003, 983, 945, 910, 840, 795, 760, 740, 720, 678, 507. UV spectrum (MeOH, c = $9\cdot10^{-5}$ mol/l), λ_{max}, nm (ε): 237 (11000), 275 (21000), 313 (5000). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 2.01-2.11 m (2H, CH_2), 2.37 s (3H, Me), 2.46-2.53 m, 2.56-2.64 m (1H, CH); 2.99-3.09 m (1H, CH_2); 3.09-3.13 m, 3.49-3.55 m (1H, CH_2); 3.13-3.21 m, 3.39-3.48 m (1H, CH_2); 3.76-3.90 m (1H, CH); 4.25 m (1H, CH_2); 4.45-4.53 m, 4.59-4.68 m (1H, CH_2); 4.73-4.83 m, 4.87-4.96 m (1H, CH_2); 5.89 d, 6.12 d ($1H_{pyr}$, J 6.3 Hz); 6.27 s, 6.57 s (CH_{isox}); 6.44 d ($1H_{pyr}$, J 9.1 Hz); 7.15 dd, 7.28 dd ($1H_{pyr}$, J 9.0, 6.9 Hz); 7.23 d ($2H_{arom}$, J 8.0 Hz); 7.55 d, 7.63 d ($2H_{arom}$, J 8.1 Hz). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 21.51, 21.52 (Me); 26.26, 26.35 (CH_2); 27.70, 27.84 (CH), 34.72, 35.10 (CH); 48.35, 48.38 (CH_2); 48.87, 49.46 (CH_2); 52.87, 53.99 (CH_2); 99.54, 99.93 (CH_{isox}); 105.64, 105.99 ($1CH_{pyr}$); 117.60 ($1CH_{pyr}$); 125.72, 125.94 ($2CH_{arom}$); 129.76, 129.80 ($2CH_{arom}$); 138.62, 139.09 ($1CH_{pyr}$); 123.88; 141.06; (148.04, 148.12); (158.32, 158.45); (160.37, 160.53); (163.32, 163.37); (170.28, 170.83) (7 $C_{quaternary}$). It was found, %: C 70.61; H 5.88; No. 11.01. Mass spectrum, m/z (Irel, %): 376.20 $[M+H]^+$ (100), 398.10 $[M+Na]^+$ (30.4), 751.30 $[2M+H]^+$ (40.8), 773.30 $[2M+Na]^+$ (65.1). $C_{22}H_{21}N_3O_3$. It was calculated, %: C 70.38; H 5.64; N 11.19. M *is* 375.43.

(1*R*,5*S*)-3-(5-phenylisoxazole-3-carbonyl)-3,4,5,6-tetrahydro-1*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8(2*H*)-one (KN-6). Yield is 77%, m.p. is 74-76°C. IR spectrum (KBr), ν, cm^{-1}: 3113 (CH_{isox}), 3055, 3030, 2924, 2865, 1656 (C=O), 1574, 1545, 1503, 1479, 1446, 1393, 1356, 1342, 1306, 1258, 1229, 1152, 1139, 1092, 1070, 1055, 1002, 983, 950, 920, 845, 797, 767, 735, 689. UV spectrum (MeOH, c = $9\cdot10^{-5}$ mol/l) λ_{max}, nm (ε): 239 (14000), 267 (21000), 311 (6000). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 2.06-2.13 m (2H, CH_2), 2.49-2.55 m, 2.59-2.64 m (1H, CH); 3.03-3.08 m, 3.16-3.22 m (1H, CH_2); 3.08-3.11 m, 3.45-3.50 m (1H, CH_2); 3.11-3.15 m, 3.52-3.57 m (1H, CH_2); 3.80-3.91 m (1H, CH); 4.22-4.30 m (1H, CH_2); 4.53-4.60 m, 4.66-4.72 m (1H, CH_2); 4.78-4.85 m, 4.92-4.98 m (1H, CH_2); 5.90 dd, 6.13 dd ($1H_{pyr}$, J 6.8, 0.8 Hz); 6.37 s, 6.65 s (CH_{isox}); 6.46 d ($1H_{pyr}$, J 9.1, 1.5 Hz); 7.16 dd, 7.29 dd ($1H_{pyr}$, J 9.1, 6.8 Hz); 7.42-7.49 m ($3H_{arom}$); 7.66-7.71 m, 7.74-7.78 m ($2H_{arom}$). ^{13}C

NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 26.51, 26.60 (CH_2); 27.88, 28.03 (SN); 34.91, 35.30 (CH); 48.49, 48.59 (CH_2); 49.00, 49.67 (CH_2); 53.07, 54.14 (CH_2); 100.45, 100.78 (CH_{isox}); 105.69, 106.10 ($1CH_{pyr}$); 117.80 ($1CH_{pyr}$); 125.96, 126.18 ($2CH_{arom}$); 129.22, 129.27 ($2CH_{arom}$); 130.81 ($1CH_{arom}$); 138.69, 139.20 ($1CH_{pyr}$); 126.75; (148.09, 148.13); (158.56, 158.65); (160.43, 160.51); (163.46, 163.52); (170.25, 170.83) (6 $C_{quaternary}$). It was found, %: C 70.05; H 5.47; N 11.49. Mass spectrum, m/z (I_{rel}, %): 362.10 $[M+H]^+$ (100), 384.10 $[M+Na]^+$ (50.2), 723.30 $[2M+H]^+$ (5.3), 745.20 $[2M+Na]^+$ (32.6). $C_{21}H_{19}N_3O_3$. It was calculated, %: C 69.79; H 5.30; N 11.63. *M* is 361.40.

(1*R*,5*S*)-3-(3*R*,5*R*,7*R*)-Adamantane-1-carbonyl-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-*a*] [1,5]diazocin-8-one (KN-9). Yield is 81%, m.p. is 203-204°C. IR spectrum (KBr), ν, cm^{-1}: 3100, 3052, 3000, 2941, 2913, 2898, 2863, 2846, 1657 (C=O), 1619 (C=O), 1570, 1548, 1473, 1452, 1430, 1399, 1354, 1337, 1305, 1254, 1237, 1225, 1217, 1179, 1160, 1142, 1099, 1062, 1051, 1032, 943, 880, 817, 806, 747, 723, 642, 567, 507. UV spectrum (MeOH, c = 9·10-5 mol/l), λ_{max}, nm (ε): 233 (8000), 312 (7000). ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.53-1.63 m (6H, $3CH_{2adam}$), 1.63-1.71 m (6H, $3CH_{2adam}$), 1.82-1.88 m (3H, $3CH_{adam}$), 1.88-2.03 m (2H, CH_2), 2.41-2.48 m (1H, CH), 2.86-2.92 m (1H, CH), 3.09-3.18 m (2H, CH_2), 3.57-3.66 m (1H, CH_2), 3.85-3.94 m (1H, CH_2), 4.37-4.47 m (1H, CH_2), 4.51-4.59 m (1H, CH_2), 6.16-6.25 m ($2H_{pyr}$), 7.28-7.35 m ($1H_{pyr}$). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 26.13 (CH_2), 27.68 (CH), 28.38 ($1CH+3CH_{adam}$), 34.75 (CH), 36.49 ($3CH_{2adam}$), 38.74 ($3CH_{2adam}$) , 48.85 (CH_2), 50.39 (CH_2), 51.94 (CH_2), 105.48, 116.49 ($1CH_{pyr}$), 139.29 ($1CH_{pyr}$), 41.67, 150.10, 162.61, 175.95 (4 $C_{quaternary}$). It was found, %: C 75.33; H8.15; N 7.79. Mass spectrum, m/z (I_{rel}, %): 353.20 $[M+H]^+$ (100), 375.20 $[M+Na]^+$ (42.5), 705.40 $[2M+H]^+$ (21.3), 727.40 $[2M+\mathrm{Na}]^+$ (58.0). $C_{22}H_{28}N_2O_2$. It was calculated, %: C 74.97; H 8.01; N 7.95. *M* is 352.48.

Procedure for the synthesis of diiodomethylates of quinine esters (KN-1-MeI, KN-11-MeI) and iodomethylates of anabasine amides (KN-2-MeI, KN-3-MeI, KN-7-MeI). A mixture of 0.02 mol of a tertiary amine (KN-1, KN-2, KN-3, KN-7, or KN-11), 30 ml of dry dichloromethane previously kept for 1 day over $CaCl_2$ (in order to remove 0.5% of the ethanol used to stabilize dichloromethane) and 3 ml of dry iodomethane were kept in the dark for 5 days, after which they were boiled for another 1 day. After removal of solvents and evacuation, finished target products were obtained with almost quantitative yield.

(2*S*,4*S*,5*R*)-2-[(*R*)-(4,5-Dichloroisothiazole-3-carbonyloxy)(6-methoxy-1-methylquinolin-1-ium-4-yl)methyl]-1-methyl-5-vinyl-quinuclidine-1-ium diiodide (KN-1-MeI). Yield is 98%, m.p. is 174-175°C. IR spectrum (KBr), ν, cm^{-1}: 3072, 2997, 2925, 2855, 1742 (C=O), 1615, 1591, 1532, 1475, 1460, 1440, 1430, 1415, 1378, 1350, 1274, 1243, 1185, 1160, 1118, 1077, 1034, 1020, 1002, 970, 835, 910, 829, 795, 726, 715, 690, 514. UV spectrum (MeOH, $c = 2 \cdot 10^{-5}$ mol/l), λ_{max}, nm (ε): 254 (30000), 278 (5000), 318 (4000), 356 (5000). ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.70-1.81 m (1H, CH_2), 2.01-2.11 m (1H, CH_2), 2.13-2.20 m (1H, CH), 2.22-2.30 m (1H, CH_2), 2.50-2.59 m (1H, CH_2), 2.86-2.95 m (1H, $C\underline{H}–CH=CH_2$), 3.51 s (3H, MeN), 3.42-3.62 m (1H, CH_2) , 3.72-3.81 m (1H, CH_2), 3.83-3.90 m (1H, CH-N), 4.06-4.15 m (2H, CH_2), 4.18 s (3H, OMe), 4.64 s (3H, MeN), 5.04-5.09 d (1H, $CH=C\underline{H_2}$), 5.13-5.19 m (1H, $CH=C\underline{H_2}$), 5.72-5.83 m (1H, $C\underline{H}=CH_2$), 7.31 s (1H, CH–O), 7.60 d ($1H_{quin}$, J 9.2, 2.6 Hz), 8.03 dd ($1H_{quin}$, J 9.7, 2.5 Hz), 8.24 d ($1H_{quin}$, J 6.1 Hz), 8.56 d ($1H_{quin}$, J 9.7 Hz), 9.31 d ($1H_{quin}$, J 6.2 Hz). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 21.33 (CH_2), 24.76 (CH_2), 26.42 (CH), 37.98 ($\underline{C}H–CH=CH_2$), 46.40 (MeN), 49.65 (MeN), 54.91 (CH_2), 57.06 (OMe), 65.10 (CH–N), 65.25 (CH_2), 70.23 (CH–O), 104.30 ($1CH_{quin}$), 117.58 ($=CH_2$), 120.88 ($1CH_{quin}$), 122.71 ($1CH_{quin}$), 127.84 ($1CH_{quin}$), 138.19 ($\underline{C}H=CH_2$), 146.92 ($1CH_{quin}$), 125.86, 127.46, 134.66, 149.15, 151.15, 152.74, 156.94, 160.20 (8 $C_{quaternary}$). It was found %: C 39.89; H 3.79; Cl 8.72; I 31.80; N 5.05; S 5.68. Mass spectrum, *m/z* (I_{rel}, %): 534.10 $[M+H-I]^+$ (6.6). $C_{26}H_{29}Cl_2I_2N_3O_3S$. It was calculated, %: C 39.61; H 3.71; Cl 8.99; I 32.20; N 5.33; S 6.00. *M* is 788.30.

(*S*)-3-[1-(4,5-Dichloroisothiazole-3-carbonyl)piperidin-2-yl]-1-methylpyridin-1-ium iodide (KN-2-MeI). Yield is 99%, m.p. is 161-162^{o}C. IR spectrum (KBr), ν, cm^{-1}: 3151, 3080, 3036, 2990, 2933, 2870, 2845, 1631 (C=O), 1590, 1504, 1464, 1446, 1378, 1355, 1345, 1328, 1315, 1289, 1250, 1218, 1171, 1137, 1126, 1110, 1083, 1031, 1010, 964, 937, 920, 899, 860, 840, 828, 816, 770, 745, 738, 720, 690, 670, 638, 586, 555, 510, 486. UV spectrum (MeOH, c = 7·10^{-5} mol/l), λ_{max}, nm (ε): 218 (25000), 266 (12000). ^{1}H NMR spectrum (500 MHz, DMSO-d$_6$), δ, ppm: 1.34-1.75 m (4H, 2CH$_2$), 1.82-1.94 m, 1.95-2.06 m (1H, CH$_2$); 2.43-2.56 m (1H, CH$_2$), 2.71-2.81 m, 3.04-3.14 m (1H, NCH$_2$); 3.46-3.55 m, 4.44-4.52 m (1H, NCH$_2$); 4.38-4.43 m (3H, MeN), 5.20-5.25 m, 5.93-5.99 m (1H, CH); 8.13-8.18 m, 8.19-8.25 m (1H$_{pyr}$); 8.36-8.42 m, 8.43-8.49 m (1H$_{pyr}$); 8.92-9.01 m (2H$_{pyr}$). ^{13}C NMR spectrum (125 MHz, DMSO-d$_6$), δ, ppm: 19.25, 19.45 (CH$_2$); 25.34, 25.62 (CH$_2$); 27.42, 29.03 (CH$_2$); 38.86, 43.85 (NCH$_2$); 48.83, 48.91 (NMe); 50.38, 55.27 (CH); 128.11, 128.40 (1CH$_{pyr}$); 143.42, 143.83 (1CH$_{pyr}$); 144.54, 144.84 (2CH$_{pyr}$); (122.05, 122.15); (134.24, 134.35); 149.82; (159.93, 160.03); (161.98, 162.49) (5 C$_{quaternary}$). It was found, %: C 37.55; H 3.61; Cl 14.15; N 8.39; S 8.67. Mass spectrum, *m/z* (I_{rel}, %): 357.10 $[M\text{-}I]^+$ (17.1). $C_{15}H_{16}Cl_2IN_3OS$. It was calculated, %: C 37.21; H 3.33; Cl 14.64; I 26.21; N 8.68; S 8.97. *M* is 484.18.

(*S*)-1-Methyl-3-{1-[5-(*p*-tolyl)isoxazole-3-carbonyl]piperidin-2-yl}pyridin-1-ium iodide (KN-3-MeI). Yield is 99%, m.p. is 101-102^{o}C. IR spectrum (KBr), ν, cm^{-1}: 3031, 2931, 2856, 1636 (C=O), 1595, 1567, 1506, 1486, 1453, 1439, 1413, 1392, 1350, 1323, 1253, 1218, 1187, 1177, 1156, 1130, 1100, 1023, 983, 947, 900, 823, 807, 757, 730, 673, 667, 502. UV spectrum (MeOH, c = 6·10^{-5} mol/l), λ_{max}, nm (ε): 219 (29000), 273 (25000). ^{1}H NMR spectrum (500 MHz, CDCl$_3$), δ, ppm: 1.52-1.82 m (4H, 2CH$_2$); 1.98-2.11 m, 2.43-2.51 m (2H, CH$_2$); 2.31-2.43 m (3H, Me); 2.64-2.77 m, 2.97-3.13 m (1H, NCH$_2$); 4.29-4.42 m, 4.61-4.73 m (1H, NCH$_2$); 5.88-5.98 m, 6.08-6.18 m (1H, CH); 6.78 s, 6.80 s (CH$_{isox}$); 7.19-7.28 m (2N$_{arom}$); 7.28-7.35 m (2H$_{pyr}$); 7.57-7.66 m (2N$_{arom}$); 7.67-7.72 m (1H$_{pyr}$); 8.52 d (1H$_{pyr}$, J 4.5 Hz); 8.58-8.62 m (1H$_{pyr}$). ^{13}C NMR spectrum (125 MHz, CDCl$_3$), δ, ppm: 19.62 (CH$_2$); 21.63 (Me); 25.55, 26.41 (CH$_2$); 27.07, 28.37

(CH_2); 39.02, 43.87 (NCH_2); 50.32, 55.35 (CH); 100.09 (CH_{isox}); 123.75 ($1CH_{pyr}$); 125.81, 125.99 ($2CH_{arom}$); 129.91, 130.18 ($2CH_{arom}$); 134.90 ($1CH_{pyr}$); 148.44, 148.52 ($1CH_{pyr}$); 148.65 ($1CH_{pyr}$); 124.13, (134.15, 134.30); 141.20, 159.15; 161.08; 170.82 (6 $C_{quaternary}$). It was found, %: C 54.41; H 5.05; I 25.74; N 8.25. Mass spectrum, *m/z* (I_{rel}, %): 362.20 $[M\text{-}I]^+$ (100), 353.20 [*M*+*H*-*I*]+ (22.0). $C_{22}H_{24}IN_3O_2$. It was calculated, %: C 54.00; H 4.94; I 25.93; N 8.59. *M* is 489.35.

(*S*)-1-Methyl-3-(1-(5-phenylisoxazole-3-carbonyl)piperidin-2-yl)pyridin-1-ium iodide (KN-7-MeI). Yield is 95%, m.p. is 64-65^{0}C. IR spectrum (KBr), ν, cm^{-1}: 3030, 2926, 2855, 1633 (C=O), 1589, 1571, 1500, 1472, 1445, 1391, 1254, 1220, 1160, 1129, 1070, 1020, 982, 945, 900, 830, 810, 767, 687, 672. UV spectrum (MeOH, c = $7{\cdot}10^{-5}$ mol/l), λ_{max}, nm (ε): 222 (28000), 267 (25000). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.49-1.75 m (4H, $2CH_2$), 1.77-1.83 m, 1.93-2.11 m (2H, CH_2); 2.52-2.72 m (1H, NCH_2); 3.15-3.25 m, 4.19-4.24 m (1H, NCH_2); 4.65 s (3H, NMe); 5.84-6.01 m (1H, CH); 6.84 s, 6.94 s (CH_{isox}); 7.33-7.41 m ($3N_{arom}$); 7.62-7.76 m ($2N_{arom}$); 8.07 t ($1H_{pyr}$, J 6.9 Hz); 8.27-8.46 m ($1H_{pyr}$); 9.03-9.20 m ($2H_{pyr}$). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 19.63 (CH_2); 25.07, 25.40 (CH_2); 27.39, 28.32 (CH_2); 39.43, 44.64 (NCH_2); 50.25 (NMe); 51.10 (CH); 100.99 (CH_{isox}); 126.16 ($2CH_{arom}$); 128.54 ($1CH_{arom}$); 129.31 ($2CH_{arom}$); 130.96 ($1CH_{pyr}$); 144.01 ($1CH_{pyr}$); 144.27 ($1CH_{pyr}$); 144.37 ($1CH_{pyr}$); 126.64; 141.57; 158.81; 161.92; 170.85 (5 $C_{quaternary}$). It was found, %: C 53.44; H 4.81; I 26.55; N 8.74. Mass spectrum, *m/z* (I_{rel}, %): 348.20 $[M\text{-}I]^+$ (100). $C_{21}H_{22}IN_3O_2$. It was calculated, %: C 53.06; H 4.67; I 26.70; N 8.84. *M* is 475.33.

(1*S*,2*R*,4*S*,5*R*)-2-(*R*)-(6-Methoxy-1-methylquinolin-1-ium-4-yl)(5-(*p*-tolyl)isoxazole-3-carbonyl)oxymethyl-1-methyl-5-vinyl-quinuclidin-1-ium iodide (KN-11-MeI). Yield is 95%, m.p. is 193-194^{0}C. IR spectrum (KBr), ν, cm^{-1}: 3072, 3000, , 2946, 1749 (C=O), 1614, 1592, 1533, 1510, 1477, 1443, 1378, 1275, 1223, 1209, 1163, 1129, 1111, 1035, 1020, 993, 947, 917, 825, 794, 759, 715, 677, 500. UV spectrum (MeOH, c = $4{\cdot}10^{-5}$ mol/l), λ_{max}, nm (ε): 212 (54000), 254 (38000), 277 (17000), 302 (5000), 357 (5000). ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.7-1.84 m (1H, CH_2), 2.07-2.16 m

(1H, CH_2), 2.17-2.23 m (1H, CH), 2.32-2.39 m (2H, CH_2), 2.40 s (3H, Me), 2.52-2.60 m (1H, CH_2), 2.87-2.97 m (1H, $C\underline{H}$–$CH{=}CH_2$), 3.54 s (3H, NMe), 3.56-3.64 m (1H, CH_2), 3.72-3.80 m (1H, CH_2), 3.84-3.93 m (1H, CH_2), 4.03-4.15 m (2H, CH_2 +CH–N), 4.18 s (3H, OMe) , 4.65 s (3H, NMe), 5.06-5.10 m (1H, $CH{=}C\underline{H_2}$), 5.14-5.21 m (1H, $CH{=}C\underline{H_2}$), 5.75 s (1H, CH–O), 5.78-5.84 m (1H, $C\underline{H}{=}CH_2$), 7.41 d ($2H_{arom}$, J 8.1 Hz), 7.57-7.64 m ($1H_{quin}$), 7.61 s (CH_{isox}), 7.92 d ($2H_{arom}$, J 8.1 Hz), 8.05 dd ($1H_{quin}$, J 9.8, 2.5 Hz) , 8.30 d ($1H_{quin}$, J 6.1 Hz), 8.58 d ($1H_{quin}$, J 9.7 Hz), 9.32 d ($1H_{quin}$, J 6.2 Hz). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 21.18 (CH_2), 21.65 (Me), 24.81 (CH_2), 26.49 (CH), 37.97 ($\underline{C}H$–$CH{=}CH_2$), 46.43 (NMe), 49.71 (NMe), 54.81 (CH_2), 57.09 (OMe), 65.13 (CH–N), 65.31 (CH_2), 70.24 (CH–O), 101.12 (CH_{isox}), 104.29 ($1CH_{quin}$), 117.62 (=CH2), 120.90 ($1CH_{quin}$), 122.75 ($1CH_{quin}$), 126.45 ($2CH_{arom}$), 127.84 ($1CH_{quin}$), 130.57 ($2CH_{arom}$), 138.17 ($\underline{C}H{=}CH_2$), 147.04 ($1CH_{quin}$), 123.76, 127.46, 134.67, 141.91, 141.91. 156.90, 158.31, 160.24, 172.34 (9 $C_{quaternary}$). It was found, %: C 50.21; H 4.81; I 31.64; N 5.11. $C_{33}H_{37}I_2N_3O_4$. It was calculated, %: C 49.95; H 4.70; I 31.99; N 5.30. *M* is 793.48.

Chapter 4

STUDY OF THE ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED DERIVATIVES OF CYTISINE, QUININE AND ANABASINE

Evaluation of antimicrobial activity of samples by diffusion into agar

In this work, the antimicrobial activity of 14 samples against strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis,* gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa*, and the yeast fungus *Candida albicans* was evaluated by diffusion into agar (wells).

The antimicrobial activity of 14 samples was studied on reference test microorganisms, namely facultative anaerobic Gram-positive cocci *Staphylococcus aureus* ATCC 6538, aerobic gram-positive spore-forming bacilli *Bacillus subtilis* ATCC 6633, gram-negative rods facultative anaerobes *Escherichia coli* ATCC 25922 aerobic *Pseudomonas aeruginosa* ATCC 27853 and yeast *Candida albicans* ATCC 10231 by diffusion into agar (wells). The test strains of microorganisms used in the study were obtained from the American Type Culture Collection.

Benzylpenicillin sodium salt, gentamicin for bacteria and nystatin for the yeast fungus *Candida albicans* are reference drugs.

The agar diffusion method is based on the assessment of the growth inhibition of test microorganisms by certain concentrations of the test agent.

For the study, pure cultures of test strains were taken, which had been preliminarily grown in a liquid medium pH 7.3 ± 0.2 at a temperature of 30 to 37^0C for 24-48 hours on slant beef agar. A standard bacterial suspension was prepared by diluting the culture 1:1000 in a sterile 0.9% isotonic sodium chloride solution. 1.0 ml of the appropriate bacterial suspension was added to Petri dishes with appropriate elective, nutrient media for the studied test strains and inoculated according to the “solid lawn” method. After drying, 6.0 mm wells were formed on the agar surface, into which 20 μl of the test sample was added. In the control, water for injection was used, which was used to dilute samples in equivolume amounts. The cultures were incubated at 37^{0}C for 24 hours for bacteria and at 30^{0}C for 48 hours for *Candida albicans*.

The sample antimicrobial activity was assessed by the diameter of the growth inhibition zones of test strains (mm) around the well, including the diameter of the well itself. If there was no zone of growth inhibition, the test culture was not sensitive to a given sample concentration; the diameter of the zones of growth inhibition was less than 10 mm and continuous growth in the Petri dish was assessed as the absence of antibacterial activity, 10-15 mm was weak activity, 15-20 mm was moderately pronounced activity, more than 20 mm was pronounced one. Each sample was tested in three parallel experiments. For comparative characterization of antimicrobial activity, solutions of antibiotics were used, namely benzylpenicillin sodium salt, gentamicin, nystatin [79, 80].

Statistical processing was carried out by parametric statistics methods with the calculation of the arithmetic mean and standard error.

The antimicrobial activity investigation results of 14 samples by diffusion into agar are shown in Table 2.

Table 2

Antimicrobial activity

Sample code	*Staphylococcus aureus ATCC 6538*	*Bacillus subtilis ATCC 6633*	*Escherichia coli ATCC 25922*	*Pseudomonas aeruginosa ATCC 27853*	*Candida albicans ATCC 10231*
KN-1	-	12 ± 0.2	-	-	-
KN-1-MeI	-	-	-	-	-
KN-2	21± 0.1	14± 0.1		-	12 ± 0.1
KN-2- MeI	-	-	-	-	-

KN-3	20± 0.2	15 ± 0.1	-	-	14± 0.1
KN-3- MeI	23± 0.1	13 ± 0.2			-
KN-4	11± 0.2	-	-	-	-
KN-5	15± 0.1	-	16± 0.1	-	12 ± 0.1
KN-6	17 ± 0.1		12 ± 0.2		
KN-7	24 ± 0.2		15± 0.1		
KN-7- MeI	20± 0.1	14± 0.2	-		
KN-9	12 ± 0.1				
KN-10	23 ± 0.2		16± 0.1		
KH-11	-				
Benzylpenicillin sodium salt	16 ± 0.1	14 ± 0.1	15 ± 0.1	-	-
Gentamicin	24 ± 0.1	21 ± 0.2	26 ± 0.1	27±0.1	-
Nystatin	-	-	-		21 ± 0.2

Note - * - significance of differences $p<0.05$ compared with the comparison group

As a result of the antimicrobial study, it was found that samples KN-2, KN-3, KN-3-MeI, KN-7, KN-7-MeI, and KN-10 exhibited pronounced antimicrobial activity against the gram-positive test microorganism *Staphylococcus aureus* ATCC 6538. Compounds KN-5, KN-7, KN-10 showed moderate antibacterial activity against the gram-negative test strain *Escherichia coli* ATCC 25922. Compounds KN-2, KN-3, and KN-5 showed weak antifungal activity against the yeast fungus *Candida albicans* ATCC 10231.

Study of samples cytotoxic activity

According to the results of studying the cytotoxic activity of KN1-11; KN-1-MeI-KN-3-MeI; KN-7-MeI samples against larvae of marine crustaceans *Artemia salina (Leach)*. The research objective was to assess the cytotoxic activity of KN1-11; KN-1-MeI-KN-3-MeI; KN-7-MeI samples against larvae of marine crustaceans *Artemiasalina (Leach)* under *in vitro* cultivation conditions [81, 82].

The samples cytotoxicity was assessed in the larval survival test of marine crustaceans *Artemiasalina (Leach)*. The experiments were carried out on 2-day-old larvae under *in vitro* cultivation conditions. The larvae were grown by immersing the eggs of the marine crustaceans *Artemiasalina (Leach)* into artificial sea water and incubated for 48 hours at a temperature of 37^0C. A portion of the test sample was dissolved in 2 ml of ethanol, then 500 μl (3 parallels), 50 μl (3 parallels), 5 μl (3 parallels) were taken from this solution. After evaporation of the ethanol, 5 ml of artificial sea water was added to each vial. Thus, if the initial sample weight was 2 mg, then the final sample concentrations were 100 μg/mL, 10 μg/mL, and 1 μg/mL, respectively, of each concentration in 3 repetitions. Using a Pasteur pipette, 10 2-day-old *Artemiasalina* marine crustacean larvae were planted in each bottle. After that, all vials were left at room temperature in the light for 24 hours. After 24 hours, the surviving and dead larvae were counted. Then, using the obtained data on the upper and lower toxic limit, the half toxic dose of the sample was calculated. DMSO in equivolume quantities was the control.

The test was carried out using ready-made samples, as well as the reference drug, dactinomycin (actinomycin D), which has antitumor (cytotoxic) activity.

The results statistical processing was carried out using the FNI computer program.

The results of testing the cytotoxic activity of KN1-11; KN-1-MeI-KN-3-MeI; KN-7-MeI samples against larvae of marine crustaceans *Artemia salina (Leach)* under *in vitro* cultivation conditions are shown in Table 3.

Table 3

Cytotoxic activity of KN1-11; KN-1-MeI-KN-3-MeI; KN-7-MeI samples

Test sample code	LD50, µg/ml	Activity
KN-7	50.2	possess
KN-3	57.4	possess
KN-10	59.7	possess
KN-2	68.5	possess
KN-7-MeI	74.0	possess
KN-3- MeI	79.6	possess
KN-6	80.3	possess
KN-5	87.1	possess
KN-1-MeI	-	does not possess
KN-2- MeI	-	does not possess
KN-2- MeI	-	does not possess
KN-9	105.4	possess
KN-4	-	does not possess
KN-11	-	does not possess
Reference drug is dactinomycin (actinomycin D)	46.8	

As a result of the study, it was found that KN-7, KN-3, KN-10, KN-2, KN-7-MeI, KN-3-MeI, KN-6, KN-5 samples exhibit cytotoxic activity against larvae of marine crustaceans *Artemia salina (Leach)*. At the same time, the cytotoxicity of KN-7, KN-3, KN-10 samples is higher than that of other compounds.

Chapter 5

QUANTUM-CHEMICAL STUDY OF THE ALKALOID MOLECULES REACTIVITY

5.1 Quantum-chemical study of the quinine molecule reactivity

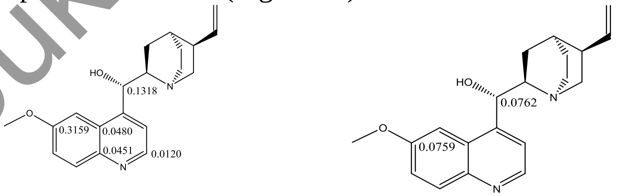
The charge, orbital, and energy parameters were studied by semi-empirical methods of quantum chemistry AM1 and PM6 in order to study the quinine molecule reactivity. The results of quantum-chemical calculations of energy characteristics indicate the thermodynamic stability of this molecule (Table 4).

Table 4

Thermodynamic parameters of the quinine molecule

Method	Total energy, eV	Heat of formation, kJ	HOMO, eV	LUMO, eV
PM 6	-3716.57	-102.11	-8.549	-0.490
AM1	-3944.59	-33.63	-8.619	-0.302

Based on the obtained values of the boundary molecular orbitals (Table 4), it can be assumed that, in general, the molecule exhibits electrophilic properties. To determine the reaction centers in the quinine molecule, the Mulliken charge distribution on non-hydrogen atoms was calculated by the RHF semi-empirical method in the PM6 and AM1 parameterization (Figure 21).



PM6 AM1

Figure 21. Distribution of partial charges on non-hydrogen atoms in a quinine molecule

It can be predicted based on the data presented that the C1 and C11 atoms will be the reaction centers for the attack of nucleophilic reagents, which is probably due to the neighboring arrangement of the oxygen atoms of the methoxy and hydroxy groups. Quantum-chemical semi-empirical calculations were performed using the MOPAC 2009 program with the help of the Modified Neglect of Diatomic Overlap (MNDO) in the MNDO-AM1 (Austine Model 1) and MNDO-PM6 (Parametric Method 6) parameterization [83].

In [84], a critical analysis of the MNDO method for calculating various physico-chemical characteristics of molecules was carried out. Comparison with the data of photoelectron spectroscopy indicates that the MNDO calculations, in general, correctly reproduce the order of the molecular levels. Relatively small errors are also observed in the calculation of geometric parameters. The MNDO method makes it possible to fairly accurately calculate the main properties of molecules, including those for which no parameterization has been performed.

The introduction of Gaussians into the expression for calculating the EAW energy has two goals: firstly, to modify the interatomic repulsion at distances within which chemical interactions are significant and secondly, to correct excessive long-range interactions. The values of the parameters akA, bkA and ckA are found from the optimization procedure.

AM1 method: The introduction of a new expression for the core-core repulsion and the redefinition of the values of the variable and dependent semi-empirical parameters were carried out in a modified scheme, called the AM1 method (Austin Model 1). If in the MNDO method the selection of parameters was carried out on the basis of 34 molecules, and then in the AM1 method about a hundred compounds were used.

The AM1 method describes hydrogen and hypervalent bonds better, better estimates the activation energies of chemical reactions, but reproduces the main physico-chemical characteristics of molecules with approximately the same accuracy as the MNDO method. The MNDO and AM1 methods are based on the MO LCAO approximations [85].

Method PM6: The modified version of PM3 developed by Stewart and based on the formalism of the AM1 method [86] has been called the PM3 (Parameterized Model revision 3) or PM3 (parametric method 3) method. The PM3 method combines the theoretical structure of the MNDO method and an updated set of parameters that differ from those of the MNDO and AM1 methods. This method uses derived functions from calculated properties to obtain optimized variable parameters. At the same time, the speed of calculations is increased due to the use of a series of simple expressions for the calculated values of these properties.

Method of quantum-chemical calculations

The semi-empirical quantum-chemical method can use different types of test wave functions. The specific form of the wave function is determined by the molecules class characteristics that must be reproduced within its framework. So, it is important to be able to describe the breaking and formation of chemical bonds for descriptions of chemical transformations. However, the trial wave function of the one-electron approximation is known to have an incorrect limit when the bond is broken. Errors in the calculated quantities can be introduced both due to the approximation used and due to semi-empirical parameters. The search for variable parameters is carried out by minimizing the objective function, namely the sum of the squared deviations of the calculated and experimental characteristics for a number of test molecules. Heats of formation, geometric parameters, ionization potentials, and dipole moments are used as such characteristics. The electronic structure and geometry of molecular systems are calculated by the quantum-chemical method of molecular orbitals of a self-consistent Hartree-Fock field in semi-empirical approximations with full geometry optimization.

5.2 Quantum-chemical calculations of combined derivatives of natural alkaloids with fragments of 1,2-azoles and adamantane

At present, synthetic transformations of natural compounds are firmly among the leading areas of pharmaceutical science [87, 88]. This is due to the unique structure and biological properties of substances synthesized as a result of complex biochemical processes of vital activity. Alkaloids are one of the first compounds of plant origin that attracted the attention of pharmacologists for the creation of medicinal preparations based on them. Many alkaloids representatives have been widely used in clinical practice for several decades, for example, the antitumor drugs Vinblastine, Kolhamin, the antihypertensive Vincamine, Reserpine, the analgesic Morphine, the antitussive Codeine, and many others [89]. In addition, it has been proven that some alkaloids have neuroprotective properties, so they are used in the treatment of Alzheimer’s disease [90].

For a long time, the quinolizidine alkaloid $(-)$-cytisine did not find wide therapeutic application, in addition to being used as an analeptic and for the treatment of tobacco dependence. However, over the past two decades, it has become a popular initial matrix for the synthesis of substances with potential neurotropic properties [91-94] due to the presence of high affinity for nicotinic acetylcholine receptors (nAChRs), which are associated with an ever-growing list of diseases [95].

It should be noted that in the presence of a unique physiological effect on the human body, alkaloids at the same time have a side, toxic effect [96, 97]. In this regard, the researchers took the path of chemical modification of these substances in order to obtain derivatives that would retain their main physiological effect and would be deprived of unwanted side effects. So anabasine, although used as a hydrochloride to reduce the craving for smoking, in large doses has an analeptic effect and is a “ganglionic poison”, acting similarly to nicotine. It has been shown in a number of works that the substitution of hydrogen at the nitrogen of the piperidine ring of anabasine leads to a decrease in toxicity and the appearance of interesting biological properties [98-102].

Much attention has recently been paid to the chemical modification of quinine due to the wide spectrum of biological activity of derivatives derived from it [103-107]. Quinine is the main alkaloid of cinchona bark (Cinchona L.) and has antipyretic, analgesic properties, as well as a pronounced effect against malarial plasmodia. The latter made it possible to use quinine as an effective treatment for malaria for a long time [108, 109]. It was found that quinine also had an anti-lipid peroxidase, antioxidant effect on cancer cells [110]. Quinine derivatives have also been considered as potential treatments for COVID-19, in particular, promising results have been obtained using hydroxychloroquine [111].

In this regard, the development of rational synthesis methods using alkaloids, aimed at obtaining new drugs, is a very urgent task of the synthetic drugs chemistry. The aim of this work was to develop methods for the synthesis of new combined derivatives of the natural alkaloids quinine, anabasine, and cytisine with fragments of 1,2-azoles and adamantane. Isoxazole and isothiazole heterocycles are fragments of molecules of a large number of pharmaceutical substances and agrochemical preparations [112]. Previous studies have shown that isoxazole and isothiazole derivatives have a synergistic effect in their composition with known insecticides as well as chemotherapeutic agents, which allows reducing the dose of these agents and thus reducing the risk of toxic side effects in cancer chemotherapy and improve the quality of patients' life [113]. The combination of fragments of alkaloids and 1,2-azoles in one molecule can impart new useful properties to their conjugates, and the high lipophilicity, along with the bulk structure of the adamantane radical, when introduced into the molecules of various biologically active compounds. It can significantly promote and modify their pharmacological action in connection with the creation of favorable conditions for their transport through biological membranes [114].

Quinine esters and amides of cytisine and anabasine with heterocyclic fragments of substituted derivatives of isoxazole and isothiazole, as well as an adamantane fragment (KN-9, KN-10) were produced. Based on the synthesized alkaloid derivatives, quaternary salts (iodine methylates) were obtained. Synthesis procedures are given in the experimental part. The prepared samples were submitted for biological activity testing.

In the ^{1}H and ^{13}C NMR spectra of the amides of cytisine and anabasine with isoxazole and isothiazole fragments, a bifurcation of proton signals is observed. It is probably due to the presence of rotational isomers caused by inhibition of internal rotation around the C(O)–N amide bond when heterocyclic fragments are included in the molecule. For alkaloids derivatives with an adamantane fragment, this phenomenon is not observed in the NMR spectra. However, it was not possible to explain this fact using quantum-chemical calculations of the difference between the total energies of two isomers (the enthalpy of transition between two energy states of the molecule ΔH), since the results turned out to be rather contradictory (see Tables 5 and 6), so this fact requires further study.

Ab initio quantum-chemical calculations of the compounds were carried out by the DFT method with the help of the B3LYP1/MIDI theory level using the GAMESS software package [115] and the MIDI basis set [116]. In the course of calculations, all geometrical parameters were completely optimized until the minima of total electron energies were reached. The total energy of a molecule is the sum of the electron energy and the repulsion energy of the nuclei, i.e. (in atomic units):

$$E_{tot} = E + E_{NN} = E + \sum_{A=1}^{N_A-1} \sum_{B=A+1}^{N_A} \frac{Z_A Z_B}{\left|\mathbf{R}_A - \mathbf{R}_B\right|}.$$

The set of approximations described above is often referred to as the Self-Consistent Field Molecular Orbitals Method.

The thermal effect of any reaction is found as the difference between the sum of the formation heats of all products and the sum of the formation heats of all reactants in this reaction (a consequence of Hess’s law):

$$\Delta H^{o}_{\text{reaction}} = \Sigma\Delta H_{(\text{products})} - \Sigma\Delta H_{(\text{reagents}).}$$

For comparison, the thermal effect of the reaction for the synthesis of diethyl ether, used in medicine for anesthesia, at 298K:

$$2C_2H_5OH(l) \rightarrow C_2H_5OC_2H_5(l) + H_2O(\text{liquid})$$

standard enthalpies of combustion of substances involved in the reaction:

$\Delta H_{\text{combustion}}$ $C_2H_5OC_2H_5$ (liquid) = −2727 kJ/mol;
$\Delta H_{\text{combustion}}$ C_2H_5OH (liquid) = −1371 kJ/mol;
$\Delta H_{\text{combustion}}$ H_2O (liquid) = 0 kJ/mol;
$\Delta H_{\text{reaction}} = 2[\Delta H_{\text{combustion}} C_2H_5OH] - [\Delta H_{\text{combustion}} C_2H_5OC_2H_5 + \Delta H_{\text{combustion}} H_2O] =$
$= 2[(-1371)] - [(-2727)+0] = -15$ kJ/mol

Table 5

Rotamers of anabasine derivatives

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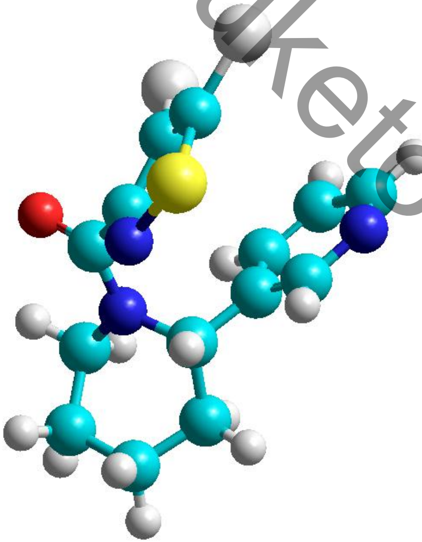
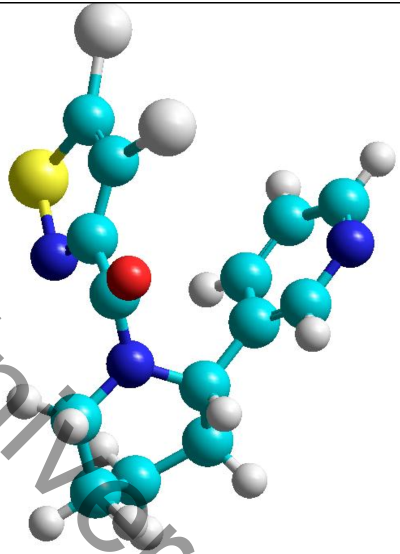
<tr><td></td><td></td></tr>
<tr><td>KN-2-1</td><td>KN-2-2</td></tr>
<tr><td>−2088.4064475735 a.u. 4.64 D</td><td>−2088.3980505218 a.u. 5.40 D</td></tr>
<tr><td colspan="2">ΔH = 0.0083970517 a.u. = 22.05 kJ/mol</td></tr>
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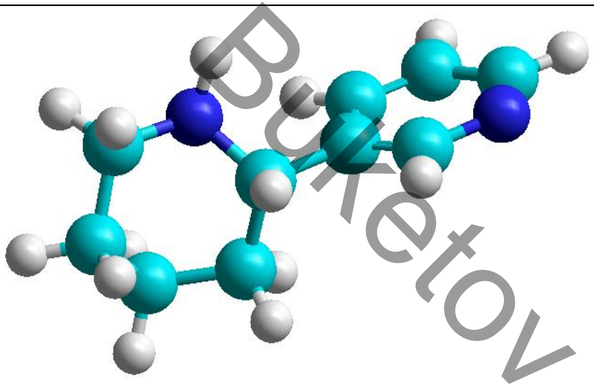	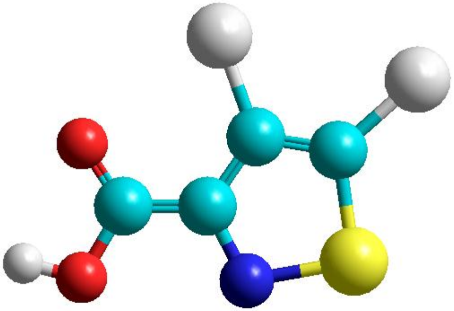
Anabasine	4,5-Dichloroisothiazolecarboxylic acid
−496.0462986461 a.u. 2.38 D	−1668.3037759076 a.u. 1.49 D

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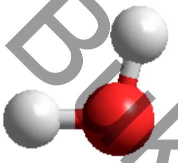
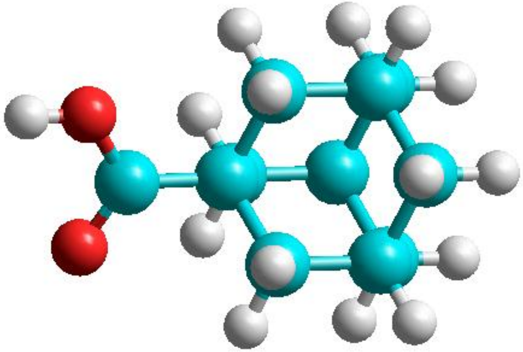
<tr><td></td><td></td></tr>
<tr><td>Water</td><td>1-Adamantanecarboxylic acid</td></tr>
<tr><td>−75.9472917827 a.u. 2.21 D</td><td>−575.8997748976 a.u. 1.34 D</td></tr>
<tr><td colspan="2">$\Delta E_{Int.(\mathbf{Amide})} = [E_{(\mathbf{Amide})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Anabasine})} + E_{(\mathbf{4,5\text{-}Dichloroisothiazolecarboxylic\ acid})}]$
KN-2-1: −0.0036648025 a.u. = −9.62 kJ/mol
KN-2-2: 0.0047322492 a.u. = 12.42 kJ/mol</td></tr>
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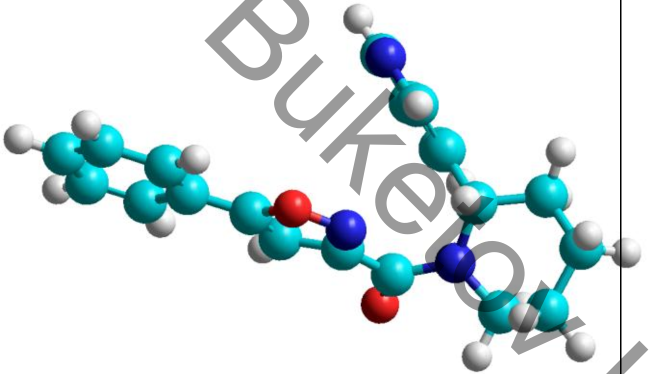
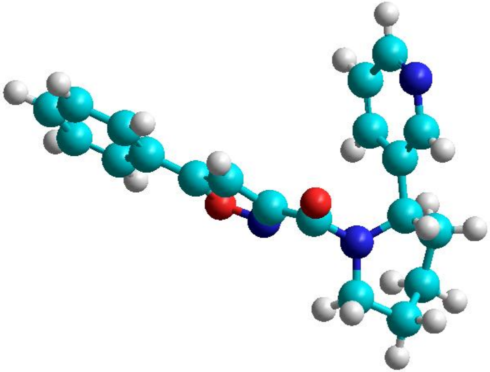
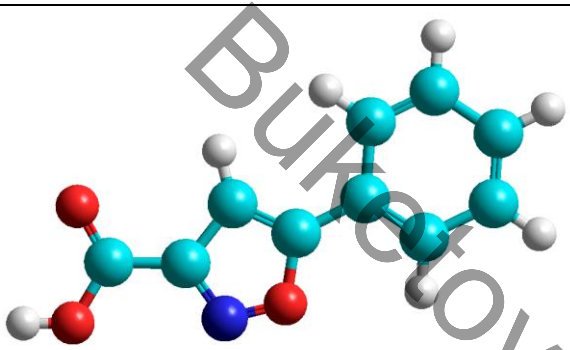
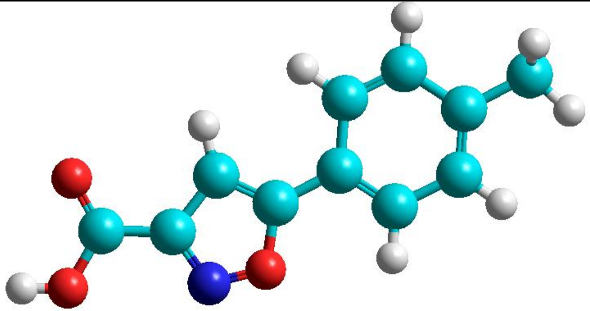
	
KN-7-1	KN-7-2
−1081.8459454116 a.u. 1.80 D	−1081.8465730531 a.u. 3.41 D
ΔH = 0.0006276415 a.u. = 1.65 kJ/mol ≈ 0 kJ/mol	

	
Phenylisoxazolecarboxylic acid	*p*-Tolylisoxazolecarboxylic acid
−661.7452529817 a.u. 2.67 D	−700.8334465619 a.u. 3.23 D
$\Delta E_{Int.(\mathbf{Amide})} = [E_{(\mathbf{Amide})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Anabasine})} + E_{(\mathbf{Phenylisoxazolecarboxylic\ acid})}]$ KN-7-1: −0.0016855665 a.u. = −4.43 kJ/mol KN-7-2: −0.0023132080 a.u. = −6.07 kJ/mol	

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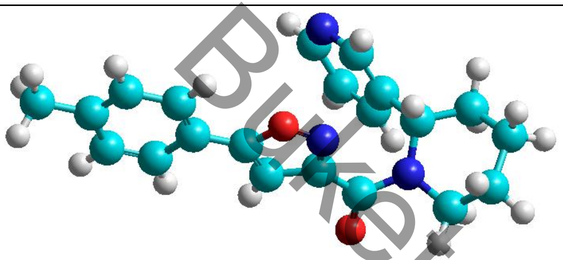
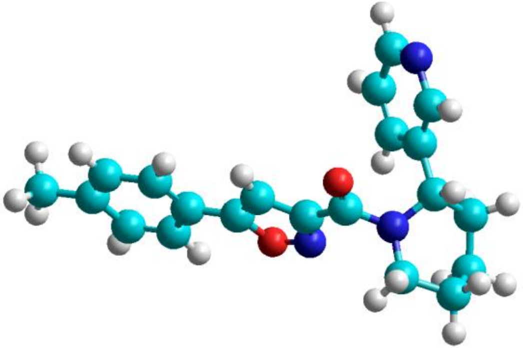
<tr><td></td><td></td></tr>
<tr><td>KN-3-1</td><td>KN-3-2</td></tr>
<tr><td>−1120.9340754442 a.u. 2.06 D</td><td>−1120.9406860121 a.u. 4.07 D</td></tr>
<tr><td colspan="2">ΔH = 0.0066105679 a.u. = 17.35 kJ/mol</td></tr>
<tr><td colspan="2">$\mathit{\Delta E}_{Int.(\mathbf{Amide})} = [E_{(\mathbf{Amide})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Anabasine})} + E_{(p\text{-Tolylisoxazolecarboxylic acid})}]$
KN-3-1: −0.0016220189 a.u. = −4.26 kJ/mol
KN-3-2: −0.0082325868 a.u. = −21.61 kJ/mol</td></tr>
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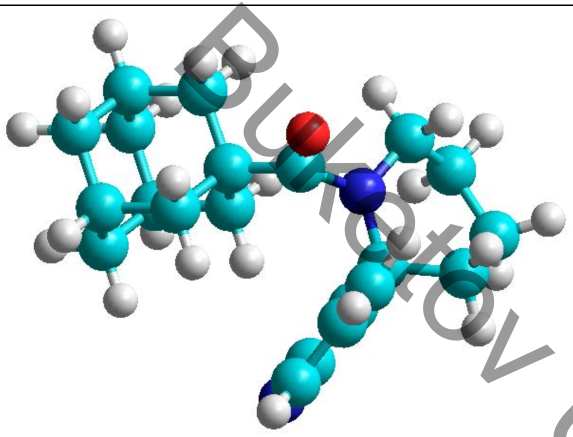
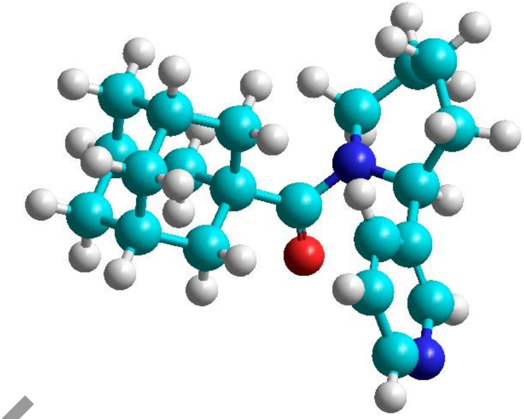
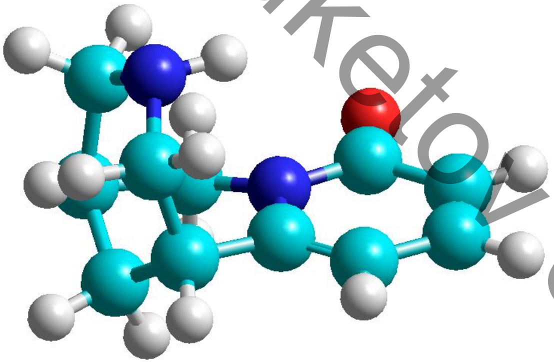
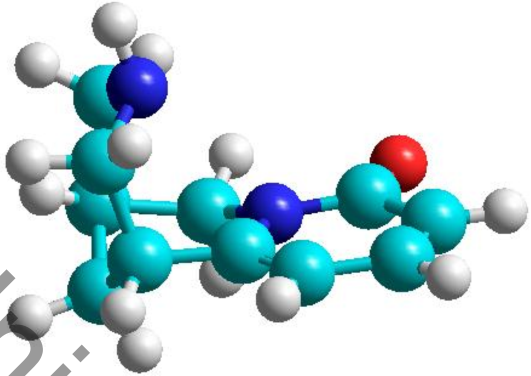
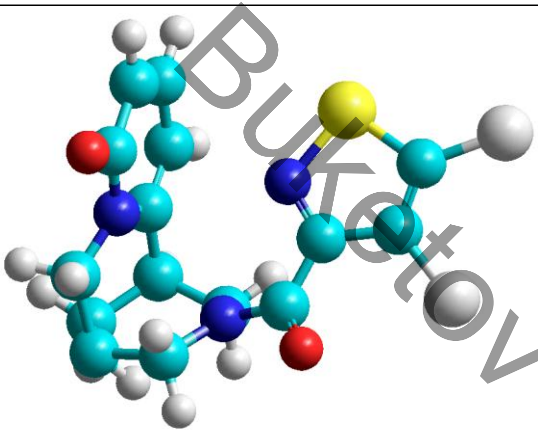
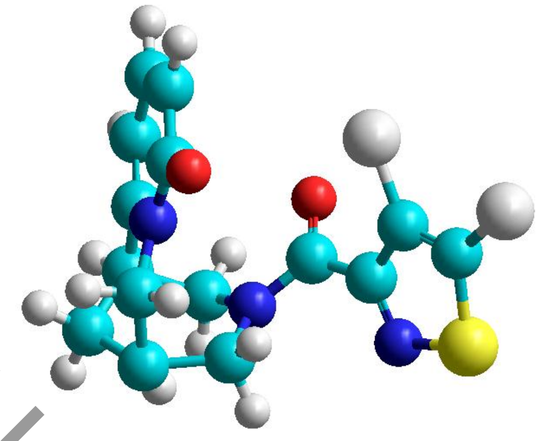
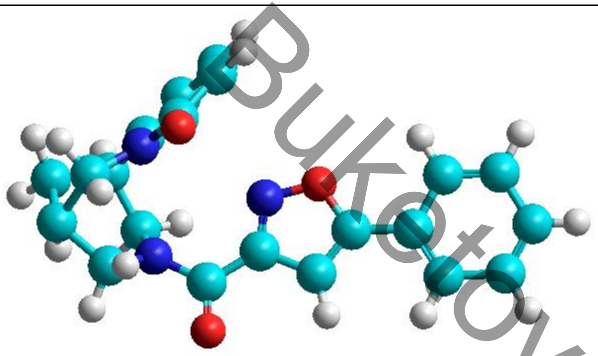
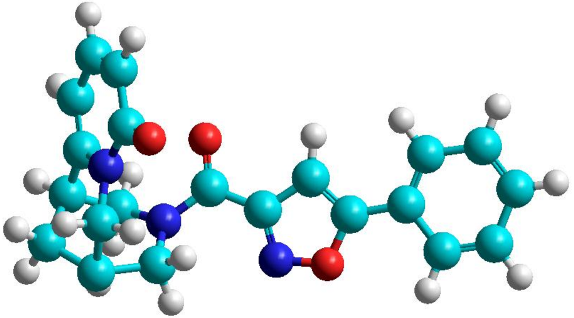
<tr><td></td><td></td></tr>
<tr><td>KN-10-1</td><td>KN-10-2</td></tr>
<tr><td>−995.9839497508 a.u. 0.94 D</td><td>−995.9839012747 a.u. 5.40 D</td></tr>
<tr><td colspan="2">ΔH = 0.0000484761 a.u. = 0.13 kJ/mol ≈ 0 kJ/mol</td></tr>
<tr><td colspan="2">$\mathit{\Delta E}_{\mathit{Int.}\mathbf{(Amide)}} = [E_{\mathbf{(Amide)}} + E_{\mathbf{(Water)}}] - [E_{\mathbf{(Anabasine)}} + E_{\mathbf{(1\text{-}Adamantanecarboxylic\ acid)}}]$
KN-10-1: 0.0148320102 a.u. = 38.94 kJ/mol
KN-10-2: 0.0148804863 a.u. = 39.07 kJ/mol</td></tr>
</table>

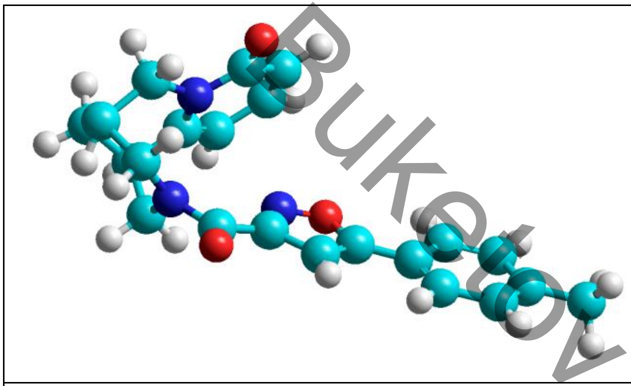
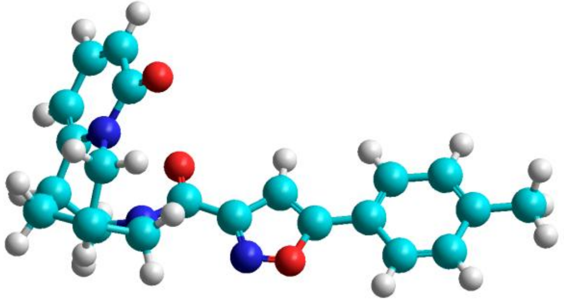
Table 6

Rotamers of cytisine derivatives

	
Cytisine-1 (endo)	Cytisine-2 (exo)
−608.7395792021 a.u. 3.80 D	−608.7401754518 a.u. 5.20 D
ΔH = 0.0005962497 a.u. = 1.57 kJ/mol ≈ 0 kJ/mol	

	
KN-4-1	KN-4-2
−2201.0972381247 a.u. 6.91 D	−2201.0973439503 a.u. 6.13 D
$\Delta H = 0.0001058256$ a.e. = 0.27 kJ/mol ≈ 0 kJ/mol	
$\Delta E_{Int.(\mathbf{Amide})} = [E_{(\mathbf{Amide})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Cytisine\text{-}2})} + E_{(\mathbf{4,5\text{-}Dichloroisothiazolecarboxylic\ acid})}]$ KN-4-1: −0.000578548 a.u. = −1.52 kJ/mol KN-4-2: -0.0006843736 a.u. = −1.80 kJ/mol	

	
KN-6-1	KN-6-2
−1194.5402013015 a.u. 4.12 D	−1194.5402363646 a.u. 3.30 D
ΔH = 0.0000350631 a.u. = 0.09 kJ/mol ≈ 0 kJ/mol	
$\mathit{\Delta E}_{Int.(\mathbf{Amide})} = [E_{(\mathbf{Amide})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Cytisine\text{-}2})} + E_{(\mathbf{Phenylisoxazolecarboxylic\ acid})}]$ KN-6-1: −0.0020646507 a.u. = −5.42 kJ/mol KN-6-2: -0.0020997138 a.u. = −5.51 kJ/mol	

	
KN-5-1	KN-5-2
−1233.6283876889 a.u. 4.07 D	−1233.6283950807 a.u. 3.20 D
ΔH = 0.0000073918 a.u. = 0.02 kJ/mol ≈ 0 kJ/mol	
$\Delta E_{Int.(\mathbf{Amide})} = [E_{(\mathbf{Amide})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Cytisine\text{-}2})} + E_{(p\text{-Tolylisoxazolecarboxylic acid})}]$ KN-5-1: −0.0020574579 a.u. = −5.40 kJ/mol KN-5-2: −0.0020648497 a.u. = −5.42 kJ/mol	

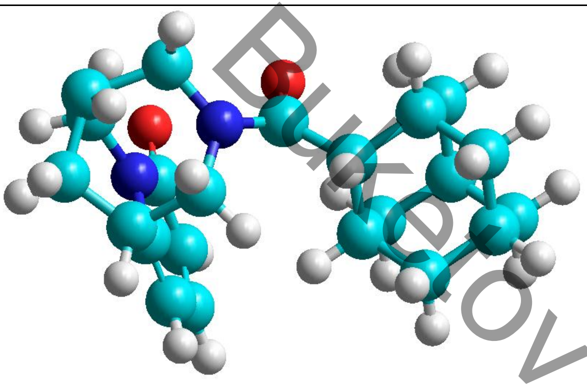
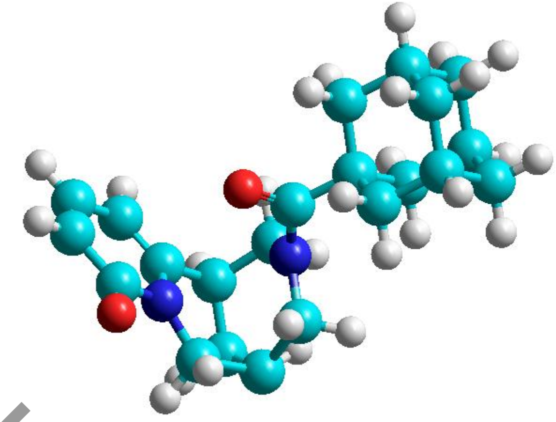
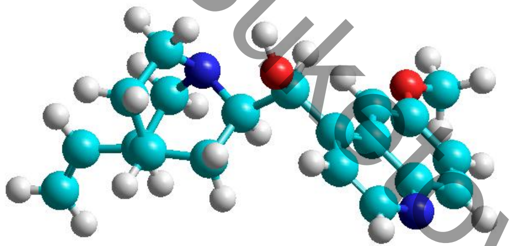
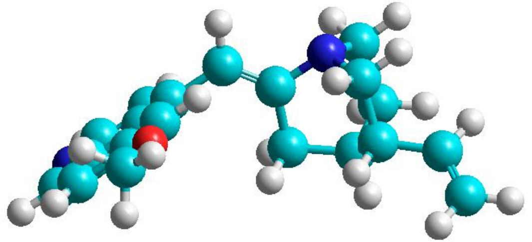
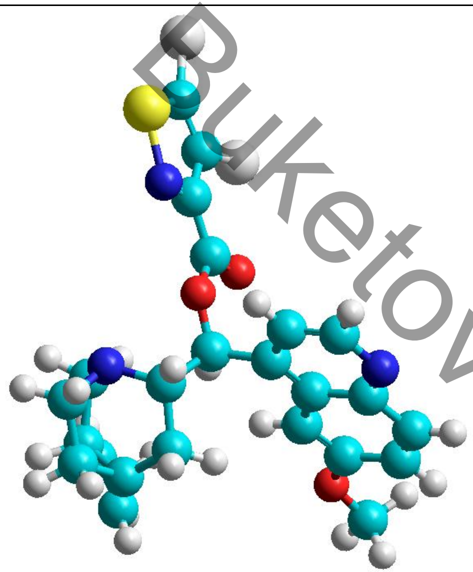
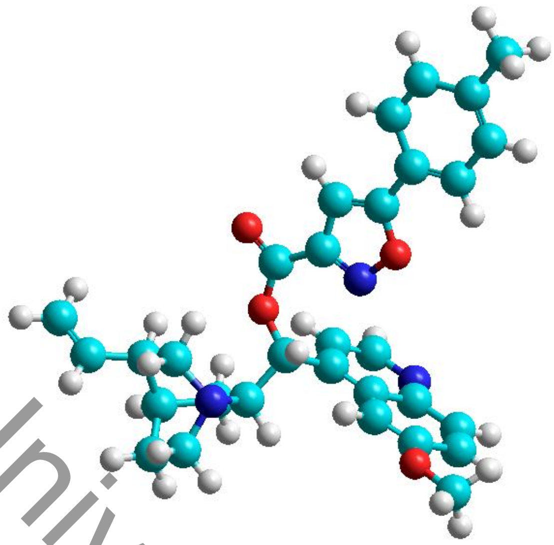
<table>
<tr><td></td><td></td></tr>
<tr><td>KN-9-1</td><td>KN-9-2</td></tr>
<tr><td>−1108.6840108051 a.u. 6.44 D</td><td>−1108.6773643453 a.u. 4.71 D</td></tr>
<tr><td colspan="2">ΔH = 0.0066464598 a.u. = 17.45 kJ/mol ≈ 0 kJ/mol</td></tr>
<tr><td colspan="2">$\mathit{\Delta E}_{Int.\textbf{(Amide)}} = [E_{\textbf{(Amide)}} + E_{\textbf{(Water)}}] - [E_{\textbf{(Cytisine-2)}} + E_{\textbf{(1-Adamantanecarboxylic acid)}}]$
KN-9-1: 0.0086477616 a.u. = 22.70 kJ/mol
KN-9-2: 0.0152942214 a.u. = 40.15 kJ/mol</td></tr>
</table>

Table 7

Quinine derivatives

	
Quinine	(4S,5R,E)-2-(6-Methoxyquinolin-4-yl)methylene-5-vinylquinuclidine
−1030.3971022808 a.u. 2.08 D	−954.4122535093 a.u. 1.85 D
$\Delta E_{Int.(\ \mathbf{Deoxyquinine})} = [E_{(\mathbf{Deoxyquinine})} + E_{(\mathbf{Water})}] - [E_{(\mathbf{Quinine})}] = [-954.4122535093 + -75.9472917827] - [-1030.3971022808] = 0.0375569888 = 98.61$ kJ/mol	

	
KN-1	KN-11
-2622.7471805293 a.u. 4.63 Db	
$\mathit{\Delta E}_{Int.(\textbf{Ester})} = [E_{(\textbf{Ester})} + E_{(\textbf{Water})}] - [E_{(\textbf{Quinine})} + E_{(\textbf{4,5-Dichloroisothiazolecarboxylic acid})}] =$ $= 0.0064058764$ a.u. $= 16.82$ kJ/mol	

1 a.u. (Hartree, *Eh*) = 2625.5 kJ/mol.

CONCLUSION

The organization of domestic original drugs production is the priority in the development of pharmaceutical science and industry of the Republic of Kazakhstan. In turn, this provides for the integrated use of its own raw materials based on innovative science-intensive technologies.

The chemistry of natural compounds, including alkaloids, is one of the rapidly developing areas of organic chemistry. The systematic study of new compounds requires not only the creation of efficient methods for the synthesis of these compounds, but also the preparation of their various derivatives. Given the valuable biological properties of alkaloids and their derivatives, the search for new ways of chemical modification of alkaloids is certainly relevant, and the attention of researchers is attracted by the production of increasingly complex heterocyclic systems.

The modern scientific and technical level of experimental chemistry also motivates many scientists to synthesize new natural compounds. The impetus for the study of alkaloids is their valuable physiologically active properties. They are widely used in the treatment of cardiovascular diseases, as antitumor, antimicrobial and anticholinesterase agents. The presence in the alkaloids molecules of one or more nitrogen atoms with lone pairs of electrons determines their chemical properties. The chemical synthesis of alkaloids derivatives makes it possible to expand the possible ways of searching for new medicines.

The relevance of carrying out chemical transformations of plant alkaloids is determined by the need to obtain new compounds characterized by improved physico-chemical properties, more pronounced biological activity, reduced toxicity, and prolonged action compared to the original natural analogues. Currently, interest is growing in the synthesis of combined compounds based on various alkaloids by introducing fragments of other biologically active compounds into their molecules.

The monograph presents the following research results:

1. For the first time, methods for isolation and production of alkaloids anabasine, cytisine and quinine were developed. The

structure of their molecules was established by modern spectroscopic methods.

2. There was carried out the chemical modification of alkaloids anabasine, cytisine and quinine as synthons in terms of hybrid molecules new transformations.

3. There was assessed the antimicrobial activity of the synthesized alkaloids derivatives against strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and yeast fungus *Candida albicans*.

4. There was assessed the cytotoxic activity of the synthesized alkaloids derivatives against larvae of marine crustaceans *Artemiasalina (Leach)* under *in vitro* cultivation conditions.

5. Quantum-chemical calculations of the electronic and spatial structure of molecules of new alkaloid derivatives were carried out.

This monograph summarizes the data on the synthesis of new combined derivatives based on molecules of alkaloids anabasine, cytisine and quinine. At the same time, methods for obtaining hybrid structures containing fragments of natural compounds molecules in combination with other biologically active plant metabolites were analyzed as leading compounds for the development of pharmacologically valuable agents in order to create new original domestic medicines.

The combination of pharmacophore residues in one molecule, namely, various heterocyclic substituents in the nucleoside position of natural alkaloids opens up new possibilities for both subsequent chemical modification of the obtained polyfunctional derivatives and their new diverse biological activity. Given that the preparation of combined derivatives based on alkaloids molecules has not been sufficiently studied, the directed synthesis of new compounds is of interest both in terms of obtaining new medicinal substances and developing new methods of organic synthesis.

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CONTENTS

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