Synthesis and Biological Activity of the Derivatives of Alkaloid Cytisine

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Abstract

Results of the investigations carried out by the authors during the past five years in the area of chemical transformation of cytisine alkaloid are presented. A number of new polyfunctional derivatives of cytisine alkaloid containing pharmacone groups including heterocyclic fragments were obtained. The composition and structure of the synthesized compounds were confirmed by means of the data of mass spectrometry, $^1$H NMR spectroscopy and X-ray structural analysis. Some features of the spatial structure of cytisine were established. The data obtained in the investigation of the biological activity of the synthesized derivatives are presented.

Key words: alkaloid cytisine, cytisine derivatives, $^1$H NMR spectroscopy, X-ray structural analysis, biological activity

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INTRODUCTION

One of the urgent problems of modern chemical science is the search for the methods of reasonable use of natural plant raw material and preparation of new biologically active compounds on this basis. Among numerous natural alkaloids that are widespread in the flora of Kazakhstan, a special place is occupied by commercially available alkaloid cytisine extractable from Thermopsis lanceolata; it possesses analeptic and anti-tobacco activity [1]. It is known that the inclusion of other pharmacophore fragments, including physiologically active heterocyclic compounds, into the structure of plant alkaloids comprises one of the basic approaches in the chemical design of new biologically active substance. Among numerous derivatives of alkaloid cytisine, the compounds with other kinds of biological activity differing from that of alkaloid itself are permanently discovered: hypolipidic, anti-inflammatory [2, 3], cholinotropic [4], hemostatic [5], antiarrhythmic [6]. In this connection, we carried out a series of chemical transformations of cytisine introducing some pharmacophore groups and heterocyclic residues.

THIOCARBAMIDE DERIVATIVES OF CYTISINE

One of the interesting ways to modify cytisine molecule is to introduce an amide or
thioamide fragment; these derivatives possess diverse kinds of biological activity and unbeat-en pharmacological value [1, 7–9]. It is known that thioamides are one of the most important classes of organic compounds and are widely used either in organic synthesis or in industry, agriculture and medicine [7]. The majority of thiourea derivatives possess valuable pharmacological properties and find application as antituberculous, antitumour, anti-inflammatory, antimicrobial, antiulcer and other therapeutically active agents [1, 8, 9].

As a rule, the derivatives of thiocarbamide are obtained by means of the direct substitution of one or two amino groups of thiourea molecule itself by a fragment of primary or secondary amine with the evolution of ammonia. This method has a limited application because it directly depends on the basicity and stability of the initial amine.

For the purpose of obtaining monosubstituted derivatives of thiocarbamide on the basis of alkaloid cytisine 1, condensation of thiourea with a small excess of cytisine was performed. Condensation of thiourea and cytisine was carried out in the melt at a temperature of 180–190 °C for 20–30 min until ammonia evolution stopped (Scheme 1).

Thus formed cytisino-N-thiocarbamide 2 after several recrystallizations from 90 % ethanol was isolated as a white crystalline substance with high melting point.

A peak of the molecular ion 249 \([M^+]\) with relative intensity of 100 % was detected in the mass spectrum of the synthesized cytisino-N-thiocarbamide 2, which can be the evidence of the high stability and thermal stability of compound 2 under the action of electron impact, and rather high strength of the \(\text{N–C(S)}\) bond. According to the data of \(^1\text{H}\) NMR spectroscopy, in addition to the protons of the alkaido fragment, compound 2 contains a broadened singlet of the protons of primary amino group of the thiocarbamide fragment at 4.74 ppm.

The introduction of thioamide fragment into the structure of alkaloids broadens the boundaries within which the structures of these natural compounds can be modified, and can promote new kinds of biological activity [10]. The interaction of the esters of isothiocyanic acids with amines is considered as an ideal method of thiocarbamide synthesis [11].

For instance, in [12] the synthesis of thiocarbamide derivatives on the basis of alkaloid cytisine was carried out from acetal isothiocyanates obtained according to the procedure described in [13]. The synthesis was carried out in alcohol medium through the direct addition of cytisine to propargyloxyethoxyethylisothiocyanate and 1-phenyloxyethoxyethylisothio-cyanate (Scheme 2).

The analysis of the mass spectrum of compound 3 revealed the peaks with the following \(m/z\) values and relative intensity \(\langle J_{rel}\rangle\): molecular ion 375 \([M^+]\) (7 %), fragments of decomposition under the action of electron impact on cytisine framework, thiocarbamide and acetale residues =N–+ 189 (51 %), =C(S)NH(CH₂)₂ 276 (55 %), =C(S)⁺ 233 (40 %), C₇H₅NO₂S⁺ 130 (56 %), C₅H₃NO₂S⁺ 146 (67 %) and propionic fragment CH₂C=CH⁺ 39 (100 %).

In connection with the fact that acetale compounds are rather easily hydrolyzed in the pres-
ence of acids, we carried out the soft hydrolysis of compound 3 into cytisino-N-(2-hydroxyethyl)thiocarbamide 4 through boiling of the alcohol solution of compound 3 in the presence of several drops of acetic acid [14] (Scheme 3).

In this process, cytisino-N-(2-hydroxyethyl)thiocarbamide 4 was isolated with a high yield. The structure of cytisino-N-(2-hydroxyethyl)thiocarbamide 4 formed by hydrolysis was confirmed by means of X-ray structural analysis, mass spectrometry. Thus, the $^1$H NMR spectrum exhibits, in addition to the protons of the alkaloid fragment, methylene groups and a clear triplet at 4.53 ppm related to the hydroxyl proton of the primary hydroxy group (Fig. 1).

Continuing the studies aimed at obtaining thiocarbamide derivatives and examination of their biological activity, we performed the synthesis of new acyl derivatives of thiourea based on alkaloid cytisine under investigation. At first, the synthesis of initial isothiocyanates was carried out in situ (without isolation) from the corresponding chloroanhydrides (benzoyl chloride, p-methylbenzoyl chloride, p-bromobenzoyl chloride and chloroanhydride of 2-furanecarboxylic acid) by heating with potassium thiocyanate in acetone. Isothiocyanates formed in the reaction participated in subsequent interaction with alkaloid cytisine [15] according to Scheme 4.

The formed target products 5–8 are well crystallized white substances with moderate solubility in organic solvents.

It should be noted that the introduction of pharmacologically active groups into the structure of thiocarbamide derivatives of alkaloid derivatives can enhance their biological activity or bring a new kind of it, including antibacterial action. In particular, pharmacologically active groups include 4-bromophenyl residue which is present in many antiviral preparations, and the derivatives of 2-furanecarboxylic acid; its structural fragment is present in many antibacterial preparations with nitrofuran basis.

During the recent years, the number of publications dealing with the synthesis and investigation of the biological activity of vari-
ous derivatives of thiazoles, di- and tetrahydro thiazoles (thiazolines, thiazolidines) is increasing. In the series of compounds, including natural ones (vitamin B1, penicillin) containing the thiazol ring, the agents with high radioprotective activity were found, as well as herbicides, pesticides and plant growth stimulators [16–19]. Special attention is attracted to the thiazol derivatives combining physiologically active alkaloids [20] that are able to exhibit high pharmacological activity.

As a continuation of research dealing with the synthesis and investigation of the reactivity of thiourea derivatives of alkaloids, we carried out the synthesis of N-allyl thiocarbamide derivative [21] on the basis of alkaloid cytisine. The derivative was obtained through the equimolar interaction of cytisine with allyl isothiocyanate in alcohol or benzene. Then, it was interesting to study the possible intramolecular heterocyclization of the obtained allyl thiocarbamide derivative 9 into the corresponding 1,3-thiazoline derivative 10 under the action of hydrochloric acid. It was demonstrated [22] that the synthesized N-allyl thiocarbamide derivative 9 when heated with the solution of concentrated hydrochloric acid in a sealed glass ampoule on the boiling water bath for 3–5 h undergoes intramolecular heterocyclization (Scheme 5).

It was established that the result of the acid interaction is the formation of a five-membered sulphur-containing heterocyclic compound 2-N-cytisino-5-methyl-1,3-thiazoline 10 with a high yield. After additional recrystallization the obtained compound is a white crystalline substance soluble in many organic solvents except alkanes.

Unlike for alkyl substituted cytisine derivatives, the mass spectrum of compound 10 contains the peak of molecular ion 289 [M]+ with relative intensity 100 %. This can be the evidence of the thermal stability of compound 10 under the action of electron impact, and rather strong N–C bond of piperidine and thiazoline cycles.

It was established that the proton in α position to the nitrogen atom N of piperidine cycle manifests itself in the 1H NMR spectrum of compound 10 in the region of the weaker field because of substantial conjugation with 1,3-thiazoline cycle, unlike for simple alkyl or alkyl acyl derivatives of cytisine. In addition, in the 1H NMR spectrum of compound 10, methyl protons of thiazoline cycle with the total integral curve corresponding to three protons are described by two intensive doublets with spin-spin coupling constant (SSCC) \( J = 6.3 \) Hz, while the distance between the doublets is about 24 Hz. This is likely to be due to the presence of two rotation isomers with \( R \) and \( S \) character at the chiral C atom of thiazoline ring (with the peak intensity ratio 5 : 6) with the axial and equatorial position of methyl group with respect to the plane of 1,3-thiasoline ring.

Interesting results were obtained in the studies of the spatial structure of molecule 10 (Fig. 2). It was established that the structure of compound 10 includes two crystallographically independent molecules (10a and 10b) located in one independent unit cell (geometric parameters were deposited in the Cambridge bank of structural data CCDC 755771).

So, we carried out the acid heterocyclization of cytisino-N-allylthiocarbamide 9 in the presence of concentrated hydrochloric acid into 2-N-cytisino-5-methyl-1,3-thiazoline 10. Some features of the spatial structure of the 1,3-thiazoline derivative of 10 were established with the help of X-ray structural analysis.
SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE DERIVATIVES OF ALKALOID CYTISINE

For the purpose of obtaining glycosyl thio-carbamide derivatives based on cytisine, we performed the synthesis of 1-isothiocyanato-1-desoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose from tetra-O-acetyl-α-D-glucopyranosyl bromide (acetobromo glucose) and lead thiocyanate [23]. Then the obtained xylene solution of 1-isothiocyanato-1-desoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose was used without isolation in the reaction of nucleophilic addition of cytisine; its coupling with the carbohydrate fragment can lead to a substantial decrease in toxicity (Scheme 6).

To establish the absolute configuration of this glycoside 11 and to study its spatial structure, X-ray structural study was carried out (CCDC 783967). The crystal structure of glycosylthio-carbamide derivative 11 is shown in Fig. 3.

GLYCOSYLATION OF ALKALOID CYTISINE

It is known that N-glycosylation of many amino compounds including natural physiologically active ones is considered to be a new approach to the development of promising and efficient medicines of targeted action due to the active transport of carbohydrate fragments [24–28]. The introduction of carbohydrate fragments into the structure of physiologically active substances not only enhances their solubility in water but decreases their toxicity substantially. Due to this, glycosylation of physiologically active compound at the glycoside centre of saccharides can become one of the methods to obtain low-toxic medicines and promote an increase in prolonged action of medical products [29].

Keeping this fact in mind, we carried out glycosylation of cytisine with monosaccharides that are most widespread in nature and most available: D-glucose, D-galactose, D-xylose, and L-arabinose. The synthesis of N-glycosylamines was carried out with the help of the known classical method proposed in [30], that is, by means of the direct condensation of amines with monosaccharide in alcohol solution, sometimes in the presence of the catalytic amounts of weak acids. For example, the corresponding 1-glycopyranosylamines 12–15 were obtained by the condensation of D-glucose, D-galactose, D-xylose and L-arabinose with cytisine in an insignificant amount of ethanol [31] (Scheme 7). Condensation and especially subsequent isolation of the target products are improved substantially when absolute ethanol is used because the synthesized glycosides are well soluble in water and even insignificant amount of water

Scheme 6.
hinders the crystallization of the products. It was also established that the initial use of the catalytic amounts of acetic acid in the reaction has a substantial effect on the rate of aminoglycoside formation but notably decreases the yields and isolation of the final products.

The structure of compounds 12–15 was established with the help of the data of IR and $^1$H NMR spectroscopy. The conformation of cytisine aglycon at the glycoside atom C-1 can be established unambiguously on the basis of the position of anomeric proton in the $^1$H NMR spectrum. It is known [24] that the $\alpha$ anomer is characterized by the position in $^1$H NMR spectrum in weaker field around 4.5–5.5 ppm and by the small SSCC value (2.5–5.0 Hz). For $\alpha$ anomers, the trans-axial proton manifests itself in the stronger field with the splitting constant about 6.0–10.0 Hz. The analysis of $^1$H NMR spectra of the synthesized N-glycosylcytisines showed that in spite of the bulky cytisine framework and the stability of $\beta$ anomers, glycosides 12–15 are present in DMSO solution in the form of the mixture of $\alpha$ and $\beta$ anomers at a ratio of 1 : 1. This is pointed to by the corresponding integral intensities and the specific position of doublets of the anomer proton: for example, for compound 12 in the region of 4.08 ppm for H1-$\beta$ with the SSCC $J = 8.8$ Hz, and 4.25 for H1-$\alpha$ with $J = 4.5$ Hz.

This fact turned out to be somewhat unexpected because many previously synthesized N-aminoglycosides based on the derivatives of aniline, 2-aminopyridine and alkaloid d-pseudoephedrine even under the conditions of possible tautomerization existed in DMSO solution in $\beta$ form which is more stable and favourable from the viewpoint of energy [32–34].

In order to determine the absolute spatial configuration of synthesized N-cytisinylglyco-

\[
\text{Sug(OH)} + \text{EtOH, 65–70 °C} \xrightarrow{6–10 \text{ h}} \text{Sug} = \text{N} \quad \text{12–15}
\]

\[
\begin{align*}
\text{(D-Glc)} (12) & & \text{(D-Gal)} (13) & & \text{(D-Gsy)} (14) & & \text{(L-Ar)} (15)
\end{align*}
\]

Fig. 3. Spatial structure of the molecule of cytisino-N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiocarbamide 11.
sides, attempts were made to grow the crystals sufficient for the X-ray structural analysis to be performed. It was established that for all the synthesized N-cytisinylglycosides the crystals most suitable for investigation were obtained for \(N-(\beta-D\text{-galactopyranosyl})\)cytisine 13 after multiple recrystallization from the mixture of ethanol/2-propanol (1 : 1) followed by natural evaporation. Transparent needle-shaped crystals with blue hue were obtained. Then the X-ray structural investigation of compound 13 was carried out [35] (Fig. 4).

The analysis of the spatial structure of the studied molecule 13 (CCDC 692503) showed unambiguously that the molecule of \(N-(\beta-D\text{-galactopyranosyl})\)cytisine 13 is present in the crystal in more stable \(\beta\) anomer configuration, which is evidenced by the trans-axial position of the proton at glycoside atom \(\text{C}1'\) and carbon atom \(\text{C}2'\) of pyranose ring.

To determine the comparative toxicity of some synthesized \(N\)-cytisinylglycosides and initial cytisine, tests of the samples of \(N-(\beta-D\text{-glucopyranosyl})\)cytisine 12 and \(N-(\beta-L\text{-arabinopyranosyl})\)cytisine 15 for cytotoxic activity against the larvae of \(Artemia salina\) (Leach) under \(in\) \(vitro\) cultivation were carried out. The following results were obtained in the tests for cytotoxic activity basing on the half toxic dose \(LD_{50}\) (\(\mu\)g/mL): \(N-(\beta-L\text{-arabinopyranosyl})\)cytisine 189.36, \(N-(\beta-D\text{-glucopyranosyl})\)cytisin 172.55, alkaloid cytisine 84.56.

It was established that synthesized \(N-(\beta-D\text{-gluco-pyranosyl})\)cytisine 12 and \(N-(\beta-L\text{-arabinopyranosyl})\)cytisine 15 exhibit weak cytotoxic activity against the larvae of \(Artemia salina\) and more than two times lower cytotoxicity than that of the reference – alkaloid cytisine which exhibits moderate cytotoxic activity. On the basis of the data on the cytotoxic activity, synthesized cytisine glycosides can be recommended for broad introduction into medical practice as anti-tobacco preparations: replacement of alkaloid cytisine incorporated into the preparations that are currently in use – Tabeks and Lobesil – by cytisine glycosides not only allows one to decrease the toxicity of the preparation but also prolongs its action due to the gradual hydrolysis of glycoside.

**HETEROCYCLIC DERIVATIVES OF CYTISINE**

A combination of two and more pharmacophore fragments in one molecule is one of the basic approaches in the chemical design of new biologically active substances including natural alkaloids. It is known that the first place among medical preparations is occupied by the substances containing heterocyclic fragments [36].

By present, numerous derivatives of cytisine with various heterocyclic derivatives of coumarin [37, 38], 1,2,3-triazole [39], 1,2,4-thiadiazole [40], 2,5-dimercapto-1,3,4-thiadiazole [41], barbituric acid [42] have already been synthesized.

One of the efficient methods to obtain new \(N\)-derivatives of cytisine, besides widely used reactions of nucleophilic substitution and addition with the participation cytisine [37–41], is Mannich’s reaction which is widely used in organic practice to synthesize various practically important compounds.

To synthesize new \(N\)-heterocyclic derivatives of cytisine, the derivatives of 3,4-dihydropyrimidine-(1H)-2-thione obtained through three-component condensation according to Biginelli reaction were chosen as the starting compounds. This is connected not only with the preparative availability of these compounds but also with their broad range of pharmacological activity: analgesic, antibacterial, antihypertensive etc. [43, 44].

Initial 3,4-dihydropyrimidine-(1H)-2-thiones (16, 17) have two reaction centres with nucleophilic N atoms (in the ring) and S atom, also possessing definite nucleophilic properties and participating in possible thione-thiol tautomerism. In this connection, it was interesting to study the possibility of the participation of indicated thiones in Mannich synthesis as \(N-H\)
or S–H acid component and the synthesis of Mannich mono- or bis-bases bearing the pharmacologically significant alkaloid. For this purpose, aminomethylation of 3,4-dihydropyrimidine-(1H)-2-thiones (16, 17) by cytisine and 40 % aqueous solution of formaldehyde according to Mannich was carried out for the first time [45] (Scheme 8).

The reaction was carried out under heating the initial reagents in DMFA solution at a temperature of 120 °C for 15–20 h with excess formalin and with varied thione to cytisine ratio (1 : 1, 1 : 1.5, 1 : 2). For all the cases, the formation of one reaction product was detected by means of TLC; its maximal yield after the isolation from the reaction medium was achieved for thione/cytisine ratio equal to 1 : 1.5, 1 : 2.

The formation of both N- and S-aminomethylene derivatives of Mannich bases was assumed for the reaction. In addition, the formation of possible Mannich bis-bases with aminomethyl group bound with N(1)-, N(3)- or N-, S-dihydropyrimidine ring could not be excluded. It is also possible that 3,4-dihydropyrimidine-(1H)-2-thione (16, 17) would not enter aminomethylation but instead simple linking of two cytisine molecules with the formation of dicytisinomethane would occur as described in [46]. The analysis of the mass spectrum of compound 18 showed the presence of the molecular ion with not very high intensity: [M+] 508 (2 %), corresponding to the molecular mass of assumed structure 18.

According to the results of 1H NMR spectroscopic studies of the isolated reaction products, the presence of the protons of initial 3,4-dihydropyrimidine-(1H)-2-thiones 16, 17 and alkaloid cytisine was established. In addition, the analysis of the 1H NMR spectrum of compound 18 revealed the presence of the characteristic singlet of proton C(4)–H of the dihydropyrimidine ring at 5.00 ppm. This is the evidence of the absence of interaction with the neighbouring proton N(3)–H; in its presence, for example, in initial 3,4-dihydropyrimidine-(1H)-2-thiones 16, 17 the signal of the C(4)–H proton gets split into the doublet. The free proton N(1)–H appears as a singlet at 10.35 ppm. The aminomethylene protons of \( =\text{NCH}_2\text{N} = \) fragment are not equivalent and appear as two characteristic doublets in different spectral regions at 5.33 and 3.27 ppm and SSCC \( J_{ab} = 11.7 \text{ Hz} \). This assignment of aminomethylene protons was also confirmed by the additionally recorded two-dimensional NOESY spectrum; its results confirmed the response of interacting non-equivalent aminomethylene protons and the response from the interaction of N(1)–H proton with the neighbouring methyl group C(6)–\( \text{CH}_3 \).

Therefore, according to the data of 1H NMR and \( ^1\text{H}–^1\text{H} \) NOESY spectra, it is N(3)-aminomethylation of initial 3,4-dihydropyrimidine-(1H)-2-thiones 16, 17 that occurs.

In order to establish the assumed biological activity of the synthesized derivatives 18, 19, bio-screening of compound 18 for antimicrobial activity against the strains of Gram-positive bacterial \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, Gram-negative strains \textit{Pseudomonas aeruginosa}, \textit{Escherichia coli} and yeast fungus \textit{Candida albicans} was carried out by means of diffusion into agar (slots). Bioscreening of compound 18 revealed its clearly expressed antibacterial activity only against Gram-positive strains \textit{Staphylococcus aureus}, \textit{Bacillus subtilis} and weak activity against Gram-negative strains \textit{Pseudomonas aeruginosa}, \textit{Escherichia coli} and yeast fungus \textit{Candida albicans}.

One of the classical methods to obtain new functionally substituted derivatives of alkaloid cytisine is nucleophilic substitution of haloge-
nated derivatives containing a pharmacophore (including heterocyclic fragment).

Thus, by cytisine alkylation with the halogenated derivatives of some heterocycles and anilides, the corresponding derivatives of alkaloid cytisine were obtained.

The data on the synthesis of phenothiazine derivatives of alkaloid cytisine 21, 22 are presented in [47]. Phenothiazine 20 with condensed tricyclic system is widely used as insecticide and antihelminthic agent [48]. In addition, phenothiazine exhibits very low toxicity. 10-Aminoalkylphenothiazine derivatives exhibit high neuroleptic activity (Aminazinum, Largactil), while 10-aminoacyl derivatives of phenazine, inefficient as neuroleptics, possess substantial cholinergic and adrenolytic activity, exhibit pronounced antianginal and antiarrhythmic action [49]; for this reason, they are widely used in medical practice [1]. Initial chloroacetyl and chloropropionyl derivatives of phenothiazine were obtained according to the procedure described in [50] (Scheme 9).

Alkylation of alkaloid cytisine by 10-(2-chloroacetyl)phenothiazine and N-10-(2-N-cytisinopropionyl)phenothiazine was carried out in boiling toluene in the presence of trimethylamine. To purify the target products 21, 22, column chromatography and re-precipitation of hydrochloride into the base were used.

The presence of the molecular ion 429 [M+] in the mass spectrum of compound 21 was established (29 %), while fragment 203 (100 %) corresponds to cytisine framework =N–CH₂.

Bioscreening of compound 21 for antioxidant activity was carried out by means of the integrated investigation of the oxidizer and tested compound for the total level of peroxide oxidation of lipids (POL) in the in vitro experiment [51]. The experiment was carried out by modelling POL with yolk lipoprotein. It was established in the tests that compound 21 exhibits clearly pronounced antioxidant activity (AOA = (20.2±2.3) %).

Among numerous heterocyclic compounds, a special place is occupied also by pyridine derivatives that are the components of about 5 % of known medical preparations [36]. Pyridine derivatives are widely used in medicine as medical products with diverse therapeutic action (antituberculous, antibacterial, antihistaminic, antidepressant, analgesic, nootropic, psychotropic activity, etc.) [1] and in agriculture as efficient fungicides, herbicides and growth stimulators [52, 53].

For the purpose of obtaining new cytisine derivatives containing pharmacologically active pyridine group, the following transformations were carried out in [54]. At first, acylation of 2-amino-5-bromopyridine by bromoacetic acid bromoanhydride in anhydrous DMFA under slight cooling to 5 °C, N-(5-bromopyridine-2-yl)-2-bromoacetamide hydrobromide 23 was obtained. Then cytisine alkylation was carried out with the help of this compound according to Scheme 10.

Alkylation was carried out under slight heating (up to 60 °C) of hydrobromide and cytisine in absolute 1,4-dioxane in the presence of a three-fold excess of trimethyl amine. Excess trimethyl amine is necessary to transform hydrobromide into the base and then to accept hydrogen bromide evolved in the reaction. The choice of 1,4-dioxane was due to its dissolving ability with respect to initial hydrobromide. The target products were isolated from dioxane solution in the form of bases.

During the recent years, a new class of heterocyclic compounds with the basic 1,4-dihydropyridine fragment is widely used in medical practice. These compounds possess high
antihypertensive and nootropic activity [36]. By present, a very large number of symmetric and non-symmetric derivatives of 1,4-dihydropyridine with various substituents at its framework were synthesized; they possess valuable pharmacological properties (antibacterial, antiviral, antidiabetic, hepatoprotective, antiulcer etc.) [55–57], so further investigations of the series of 1,4-dihydropyridine are urgent. At the same time, in spite of the very large number of synthesized 1,4-dihydropyridines, compounds combining the 1,4-dihydropyridine cycle and some physiologically active alkaloids have not been described in literature. It was interesting to synthesize previously unknown 1,4-dihydropyridine derivatives based on some alkaloids, in particular cytisine.

Initial compounds for the synthesis of new alkaloid-containing 1,4-dihydropyridines were diethyl-2,6-bis(bromomethyl)-4-(aryl-1,4-dihydropyridine-3,5-dicarboxylates (25, 26) synthesized according to the procedure described in [58], they were used for the nucleophilic substitution by the double amount of alkaloid cytisine. Alkylation was carried out under soft conditions at room temperature in the solution of absolute benzene in the presence of a triple excess of trimethyl amine, which is necessary not only for binding the evolved hydrogen bromide but also to prevent possible salt formation from the initial alkaloid [59] (Scheme 11).

The products of reaction 27, 28 were isolated after column chromatographic purification on silica gel and aluminium oxide (1 : 1) followed by recrystallization.

According to the data of mass spectrometric analysis of compound 27, the peak of molecular ion was not detected, only the presence of decomposition products was observed. In spite of the complex character, more informative was the $^1$H NMR spectrum of compound 27. Not only the singlets of $\text{N}-\text{H}$ and $\text{C}_4-\text{H}$ protons of the 1,4-dihydropyridine cycle were detected but also the double signals of triplet and quartet of almost equivalent ethoxy groups. Methylene protons $\text{CH}_2\text{N}=\text{N}$ in 2,6-position of the 1,4-dihydropyridine ring turn out to be non-equivalent and appear in different regions of the spectrum as a broadened doublet at 2.94 ppm and a doublet of doublets at 3.92 ppm. It should also be noted that the protons of cytisine frameworks appear not double but as duplicated signals with shifts by 0.05–0.14 ppm. This provides evidence of their non-equivalence in the molecule of compound 27, which is likely to be connected with their different spatial orientation with respect to the 1,4-dihydropyridine ring and the screening effect of neighbouring groups.

For the purpose of possible determination of the effect of spatial factors of various functional electron-acceptor ester groups, aromatic phenyl substituent and two bulky cytisine substituents on the total structure of the compound, we carried out the X-ray structural study of compound 27 [59] (Fig. 5, CCDC 757873).

The biological tests of compound 27 with the model of acute tetrachloromethane hepatitis (in therapeutic-prophylactic dose of 50 mg/kg after peroral administration in white non-inbred mice in the form of starch suspension) showed that compound 27 prevents the development of hepatogenic hypoglycemia promoting an increase in the level of glucose in blood serum by 21 % in comparison with the reference. In addition, it prevents a decrease in the synthesis of cholesterol in liver and increases the level of cholesterol in serum by 55 % in comparison with the reference. These facts provide evidence of the ability of this compound to recover synthetic processes in liver. So, compound 27 reliably decreases the level of alanine aminotransferase (ALT) by 14 % in comparison with this parameter in the group without treatment, prevents the development of cytolysis syndrome during acute tetrachloromethane hepatitis and therefore has a high hepatoprotective potential.
It is known that the strong effect on the physiological activity of preparations is caused by a combination of several biologically active groups in the structure, for example halogen atoms that enhance lipophilicity if medicines and simplify their penetration through biological membranes, and nitro groups determining the high antibacterial effect. Altogether, halogenated and nitroanilides possess high antihelmintic activity and exhibit powerful chaotropic effect [1, 60].

Some methods of the introduction of nitro group and halogen atoms into the structure of alkaloid cytisine were considered in [61]. Synthesized according to known procedures, 2,6-dichloro-4-nitroaniline [62], 2,5-dibromo-4-nitroaniline [63] and 2,6-diiodo-4-nitroaniline, synthesized according to the scheme more favourable than that described in [64], were acylated by the bromoanhydride of bromoacetic acid. The resulting 2,6-dihalogeno-4-nitrobromoacetanilides 29 and 4-nitrobromoacetanilide 30 are rather reactive alkylating agents. For instance, alkylation of cytisine by compounds 29, 30 in absolute benzene or toluene in the presence of a three-fold excess of trimethyl amine leads to the corresponding 2,6-dihalogeno-4-nitroanilides of N-cytisinylacetic acid 31 and N-(4-nitrophenyl)-2-cytisinoacetamide 32 (Scheme 12).

The synthesized compounds 31, 32 are crystalline yellowish substances soluble in DMFA and moderately soluble in hot polar solvents. The composition and the structure of compounds 31, 32 were confirmed by the data of elemental analysis, IR, 1H NMR spectroscopy and mass spectrometry.

The protons of the alkaloid fragment are manifested in the 1H NMR spectrum in their characteristic regions. The protons of the aromatic ring for all compounds 31a–c appear in the weak field in the region of 8.37–8.58 as singlets. Methylene protons of the carbonyl group of compounds 31a, b unlike for the ini-
tial compounds 29 turn out to be non-equivalent and appear as a doublet of doublets in the region of 3.15 ppm with spin-spin coupling constant (SSCC) equal to 15.2 Hz.

To establish the spatial structure of synthesized compounds containing several electron-accepting groups, X-ray structural investigation of compound 31a was carried out (Fig. 6, CCDC 711612).

For the purpose of further modification of the synthesized nitro-containing derivatives of the anilides of N-aminoacetic acid, and in order to establish the dependence of the effect of nitro group on the pharmacological activity under its replacement by the reduced amino group, the reduction of N-(4-nitrophenyl)-2-cytisinoacetamide 32 was carried out in [65].

Generally, nitro compounds containing amide or benzamide functions sensitive to hydrolysis are reduced with good yields in acetic acid by metal chips [66]. This involves selective reduction of the nitro group in the aromatic ring without the formation of side products of hydrolysis.

Reduction was carried out for 8–10 h by boiling the water-alcohol 85 % solution of the starting nitro compound 32 in acetic acid with metal chips activated preliminarily in the 10 % HCl solution of reduced iron. The resulting N-(4-amino phenyl)-2-cytisinoacetamide 33, obtained with the yield of 60 %, is a fine crystalline white substance, well soluble in usual organic solvents except hydrocarbons (Scheme 13).

In the IR spectrum of the synthesized compound 33, the intense absorption bands of nitro groups at 1515 and 1340 cm⁻¹ disappear; there is an intense absorption band of NH₂ group in the region of 3200 cm⁻¹. The analysis of ¹H NMR spectrum of compound 33, unlike the initial nitro derivative 32, revealed the presence of the protons of aromatic amino group appearing as a narrow singlet in the region of 4.85 ppm. The doublet of doublets belonging to non-equivalent protons of the N-CH₂C(O), fragment is conserved in the spec-
trum of compound 33 similarly to the spectrum of the initial nitro derivative 32 but a slight shift by 0.2 ppm, to the stronger field is observed.

CONCLUSION

Thus, we carried out directed chemical transformation of alkaloid cytisine to obtain its polyfunctional derivatives including heterocyclic ones. The structure of all the synthesized derivatives was unambiguously proved by means of NMR $^1$H, $^{13}$C spectroscopy, mass spectrometry, while the structure of compounds 4, 10, 11, 13, 27, 31 was confirmed using X-ray structural analysis. With the help of this method we also established some features of the spatial structure of cytisine derivatives. Among the synthesized polyfunctional derivatives of cytisine, the compounds with hepatoprotective (3, 7, 11, 27), antioxidant (21), growth-stimulating (12) and antibacterial (7, 18, 27) activity were revealed, which is the evidence of the importance of chemical syntheses of cytisine modifiants and the necessity of their integrated investigation.

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