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# SYNTHESIS AND STUDY OF THE ANTIBACTERIAL PROPERTIES OF CHOLIC ACID WITH GAMMA-AMINO ACIDS

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***ABSTRACT***

*Binding of choline acid to γ-amino acids allows complicate molecular structure and raise her pharmacological efficiency. Amino acids contain atoms nitrogen, which are playing important role in antimicrobial activities, so sow they capable interact with the cell membranes and enzymatic systems of microorganisms, disrupting their vital processes. Due to this, the synthesized compounds have a dual effect: bacteriostatic, when the growth of microorganisms is suppressed, and bactericidal, when their complete destruction is observed. The results of our research have shown that choline compounds withγ-amino acids demonstrate activity in narrow range concentrations, what is important indicator for pharmacological efficiency and follows for precise drug dosing. This opens the possibility of using such compounds as the basis for the creation of new antibacterial agents suitable for the prevention and treatment of mixed infections and infections resistant to traditional antibiotics. Furthermore, these compounds have the potential for complex action: they simultaneously protect liver function and effectively combat pathogenic microorganisms, making them particularly promising for use in hepatology and infectious diseases. Overall, the study of the antibacterial properties of cholic acid in combination withγ-amino acids Maybe become important step by step V development new therapeutic strategies, providing high efficiency at minimal side effects.*



## 1. INTRODUCTION

Steroid compounds occupy a special place in modern medicinal chemistry due to their pronounced biological activity, structural diversity, and ability to selectively influence a wide range of physiological processes. They are considered one of the most promising platforms for the creation of new therapeutic agents, including those intended for the treatment of diseases of the digestive and hepatobiliary systems. [1] The high biocompatibility of the steroid scaffold, its stability in biological environments, and the possibility of targeted modification make steroids convenient structural matrices for the development of pharmacologically active substances. In recent years, researchers have increasingly focused on naturally occurring steroids, particularly

bile acid derivatives, among which cholic acid occupies a key position. [2] It has pronounced amphiphilic properties, is bioavailable, and contains functional groups suitable for chemical transformations, which makes it possible to create a wide range of new compounds based on it. Modification of cholic acid opens up the possibility of obtaining substances with anti-inflammatory, antioxidant, antibacterial, cytoprotective, and immunomodulatory properties, which is especially relevant for the treatment of liver and gastrointestinal diseases [3]. For organic chemists and pharmacists, cholic acid is a convenient and well-studied substrate. The presence of reactive hydroxyl and carboxyl groups makes it possible to carry out a variety of esterification, acylation, amidation, reduction, and formation of complex biologically active fragments [4]. This allows for fine-tuning the lipophilicity, acid-base properties, spatial configuration, and biodistribution of the created compounds [5]. In parallel, the direction of designing steroid drugs capable of acting on specific cellular targets in the liver is developing, including FXR receptors, TGR5, and other elements of signaling pathways involved in the regulation of bile formation, lipid metabolism, and inflammatory processes. Such compounds are already demonstrating promising results in the treatment of cholestatic diseases, steatohepatitis, cirrhosis of various etiologies and intestinal diseases [6]. The binding of choline acid to γ-amino acids allows for a more complex molecular structure and increased pharmacological efficacy [7]. Amino acids contain nitrogen atoms, which play a key role in antimicrobial activity because they are capable of interacting with the cell membranes and enzymatic systems of microorganisms, disrupting their vital processes. This allows the synthesized compounds to have a dual effect: bacteriostatic, inhibiting microbial growth, and bactericidal, resulting in their complete destruction [8]. Our research demonstrated that choline compounds with γ-amino acids exhibit activity over a narrow concentration range, which is an important indicator of pharmacological efficacy and allows for precise drug dosing. This opens the possibility of using such compounds as the basis for the creation of new antibacterial agents suitable for the prevention and treatment of mixed infections and infections resistant to traditional antibiotics. Furthermore, these compounds have the potential for complex action: they simultaneously protect liver function and effectively combat pathogenic microorganisms, making them particularly promising for use in hepatology and infectious disease practice [9-16]. Overall, studying the antibacterial properties of cholic acid in combination with γ-amino acids could be an important step in the development of new therapeutic strategies that provide high efficacy with minimal side effects and a broad spectrum of action against microorganisms.

## 2. EXPERIMENTAL PART

**Synthesis of γ-aminobutyric acid benzyl ester hydrochloride.** The following method was used to obtain benzyl ester of γ-aminobutyric acid hydrochloride. 6 ml of thionyl chloride ($SOCl_2$) was added to a cooled solution.) 3 g (2.3372 g pure substance) of γ-aminobutyric acid were gradually added to 24 ml of absolute benzyl alcohol with constant, vigorous stirring using a magnetic stirrer. This step ensured complete wetting and partial dissolution of the initial precipitate, which facilitated a uniform reaction between the amino acid and $SOCl_2$ and the formation of intermediate products.

After thorough stirring, the reaction mixture was left at room temperature for 50 minutes, allowing the reaction to reach a significant degree of completion. Next, 100 ml of dry diethyl ether was added to liquefy and stabilize the reaction mixture. The mixture was cooled in a water

bath with ice and table salt, which facilitated the precipitation of benzyl γ-aminobutyrate hydrochloride as an amorphous precipitate. The precipitate was carefully filtered on a glass Schott filter and dried in a vacuum desiccator over granulated NaOH to remove residual moisture and acidic gases, which maintained the chemical stability of the product.

The described procedure yielded 1.5466 g of amorphous α-aminobutyric acid benzyl ester hydrochloride, representing a yield of 74.41% of the theoretical maximum. Thin-layer chromatography (TLC) was used to monitor purity and identify the product. These indicators confirm the stability and reproducibility of the synthesis, and the resulting hydrochloride is ready for further use in condensation reactions with epichlorohydrin and other stages of the synthesis of potentially biologically active compounds.

**Synthesis of methyl ester hydrochloride** γ–aminobutyric acid. To obtain crystalline benzyl ether hydrochlorideγ–aminobutyric acid, the following method was used. 3 g ofγ–aminobutyric acid, after which dry hydrogen chloride was passed through the resulting suspension without prior cooling. This step ensured complete dissolution of the amino acid and the formation of the hydrochloride salt in the alcohol solution, creating the basis for crystallization of the highly pure product.

After complete dissolution, the reaction mixture was carefully cooled in an ice-water bath, continuing to saturate with HCl at a temperature of 0–50°C. This facilitated a gentle crystallization process, preventing decomposition of the intermediate product and ensuring the formation of a proper crystal lattice.

To protect against moisture, the reaction mixture was passed through a calcium chloride tube and left overnight at room temperature to complete the reaction and stabilize the intermediate hydrochloride. The next day, the mixture was cooled again in an ice-salt water bath, which promoted the precipitation of crystalline hydrochloride. The precipitate was carefully filtered on a glass Schott filter and washed with cold benzyl alcohol and absolute diethyl ether to remove impurities. The resulting product was dried in a vacuum desiccator over granulated NaOH with the addition of absolute ether to remove all moisture and ensure crystal stability.

As a result of the described procedure, 2.7 g of crystalline benzyl ether hydrochloride were obtained. γ–aminobutyric acid, which corresponds to a yield of 77.14%. The resulting product was characterized by: Melting point: 211–2130C**.** These indicators confirm the high purity and crystalline nature of the compound, which makes it ready for further use in condensation reactions with epichlorohydrin and other stages of the synthesis of potentially biologically active derivatives.

**Synthesis of 1-chloro-3-benzyl ether of α–bityryl propan-2-ol.** A similar method used for the synthesis of α-aminobutyric esters was used to obtain this compound. The starting amounts were 0.54 g of epichlorohydrin and 1.5 g of benzyl γ-aminobutyrate hydrochloride. The reaction was carried out at a controlled temperature, allowing for the opening of the epichlorohydrin epoxide ring and the addition of γ-aminobutyric acid to the amine group, forming the glycidyl ester of propan-2-ol. The process proceeded without side reactions and yielded a stable, amorphous product. As a result of the reaction, 2.54 g of 1-chloro-3-benzyl ester of γ-aminobutyric acid propan-2-ol were obtained, which corresponds to a yield of 72.15% of the theoretically possible.

Thin-layer chromatography (TLC) was used to control purity and identification: The resulting amorphous product is ready for further use in the synthesis of glycerol and amino acid derivatives, and can also be used to study its biological activity and physicochemical properties.

**Synthesis of 1-chloro-3-benzyl ether of γ–butyryl-propan-2-ol.** Prepared using a similar method to 3., starting from 0.54 g of epichlorohydrin and 1.5 g of benzyl chlorohydride of γ-aminobutyric acid. Obtained are 2.54 g (72.15%) of 1-chloro-3-benzyl ester of γ-aminobutyric acid of propan-2-ol in amorphous form, Rf=0.56 (A), Rf=0.58 (B).

**1. Synthesis of 3α,7α,12α-trihydroxycholanate-3-γ-aminobutyryl-propan-2-ol**

A three-necked round-bottomed flask equipped with a reflux condenser, mechanical stirrer, and dropping funnel was charged with 1.09 g (0.01 mol) of 1-chloro-3-carbobenzoxy-γ-butyrylpropan-2-ol and 10 ml of absolute 1,4-dioxane. The reaction mixture was stirred for 6 hours at room temperature until the starting material was completely dissolved.

After complete dissolution, the reaction temperature was gradually raised to 75–80°C. Then, 0.75 ml (0.01 mol) of a solution of cholanic acid salt in an appropriate solvent was added dropwise using a dropping funnel. After complete addition, the reaction mixture was stirred and heated for another 4–4.5 hours. The total reaction time was approximately 7 hours.

After the reaction was complete, the mixture was kept at room temperature for 16 hours. The solvent was removed by vacuum evaporation, yielding a white crystalline solid. The resulting product was purified by recrystallization from absolute benzene or isopropanol.

The yield of the target product was 1.8 g (86.7%). Melting point: 189–190°C.

**Found, %:**

C - 70.90; H - 11.70; N - 1.40; O - 16.01.

**Calculated for $C_{59}H_{116}NO_{10}$, %:**
C - 65.98; H - 11.05; N - 1.32; O - 15.12.

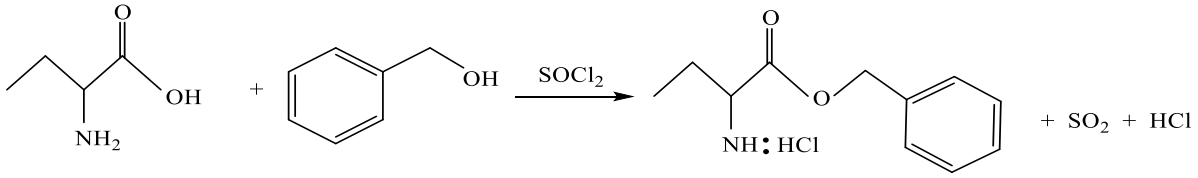
Thin layer chromatography data:
Rf = 0.61 (system A);
Rf = 0.59 (system B);
Rf = 0.56 (system B).

Iodomethylate vapor was used as a developer, which made it possible to visualize the spots of the compound being studied on chromatographic plates.

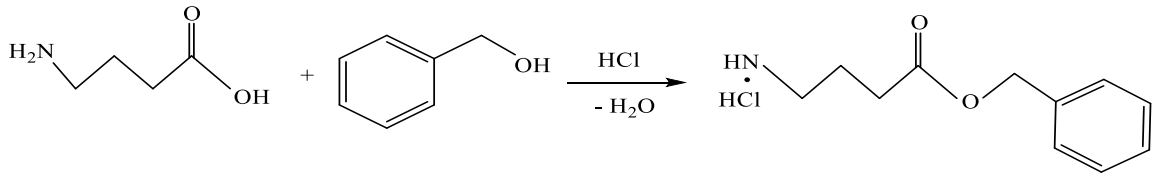
## 3. RESULTS AND DISCUSSION

For this study, we used α-aminobutyric acid (I) and γ-aminobutyric acid (II) benzyl ester hydrochlorides as starting compounds. We obtained these compounds using classical peptide chemistry methods, ensuring high purity and reproducibility of the products. The hydrochloride synthesis was carried out in several steps: first, α- and γ-aminobutyric acids were converted to the corresponding benzyl esters by esterification in the presence of an acid catalyst, after which the resulting esters were treated with hydrogen chloride to obtain the target hydrochloride compounds. All intermediate and final products were monitored using thin-layer chromatography (TLC) and IR spectroscopy to confirm their structure and purity.

The obtained hydrochlorides (I) and (II) served as key reagents in subsequent condensation reactions with epichlorohydrin and glycerol chlorohydrins, which made it possible to study the influence of the spatial arrangement of the amino group ($\alpha$- or $\gamma$-position) on the reaction rate, the yield of the target product and the formation of side compounds.



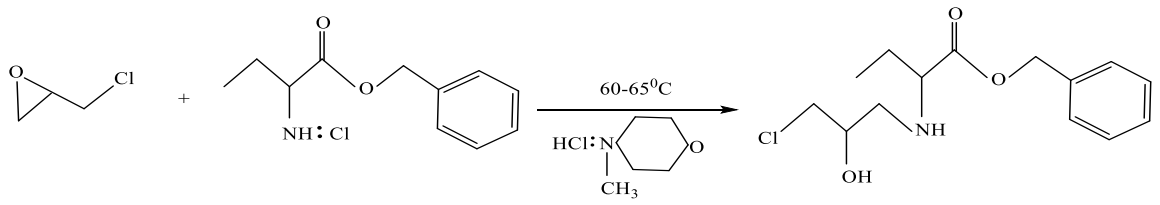

Benzyl $\gamma$-aminobutyrate hydrochloride was synthesized as follows: a solution of $\gamma$-aminobutyrate benzyl ester in anhydrous methanol was saturated with dry gaseous hydrogen chloride until the reaction mixture was completely absorbed. The process was carried out at a controlled temperature to ensure uniform formation of the hydrochloride and prevent decomposition of the starting ester. After completion of the reaction, the formed precipitate was filtered, washed with cold methanol to remove impurities and dried in a vacuum desiccator over granulated NaOH until a pure product was obtained. The process was carried out at a temperature of 0-50C for 1-1.5 hours with constant stirring to ensure uniform interaction of the reagents. After completion of the reaction, the solution was left at a low temperature for crystallization of the product. The precipitated crystals of the hydrochloride were filtered, washed with cold dry ether to remove residual solvent and dried in a vacuum over $P_2O_5$to constant mass.




(II)

The condensation reaction of synthesized $\alpha$- and $\gamma$-aminobutyric acid benzyl ester hydrochlorides with epichlorohydrin was studied in absolute dioxane at 60–650°C. Experiments revealed that the reaction proceeds with high selectivity, forming stable condensation products without significant side reactions. The use of absolute dioxane ensured a homogeneous reaction medium and prevented epichlorohydrin hydrolysis, thereby maximizing the yield of the target compounds. The reaction was monitored using thin-layer chromatography, where the formation of new chromatographic bands corresponding to the condensation products was observed, while the starting hydrochlorides were completely separated.

The experiments conducted showed that the interactionThe reaction of benzyl $\alpha$-aminobutyrate hydrochloride with epichlorohydrin produces a glycidyl ester. The reaction begins with the opening of the epoxide ring under the action of the amino group, leading to the formation of an active intermediate. Subsequently, a glycerol moiety attaches to the resulting epoxide center, resulting in the formation of a stable glycidyl ester. This process occurs at a controlled temperature of 60–650°C in an absolute dioxane environment, minimizing side reactions and yielding a highly purified product. The structure of the resulting compound was confirmed by thin-layer chromatography, IR spectroscopy, and elemental analysis, allowing for the reliably determination of the formation of the epoxide and glycerol moieties in the molecule.

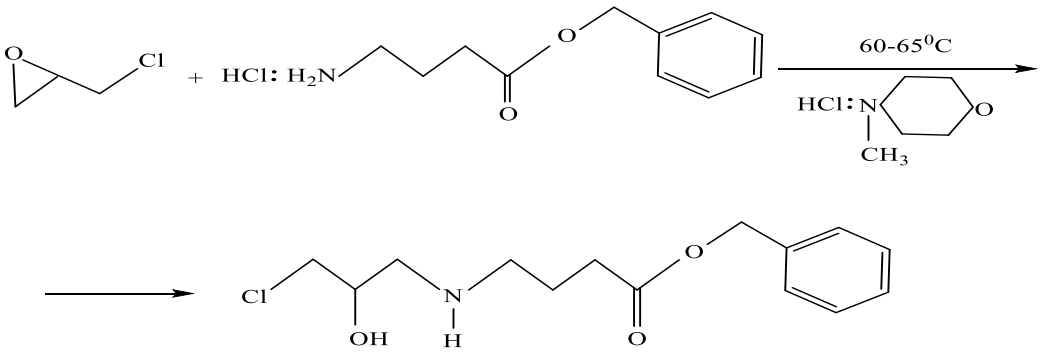



The structure of the synthesized products 4 and 5 was confirmed using a comprehensive spectral analysis, including H NMR and C NMR spectroscopy. Characteristic proton signals were identified in the H NMR spectramethine and methylene groups, as well as for protons associated with the amino group and the epoxide fragment of the glycidyl ether. The singlet and multiplet signals allowed us to determine the positions of the substituents and confirm the preservation of the stereochemistry of the original amino acid residues.

NMR$^{13}$C analysis revealed the presence of all key carbonyl and aliphatic carbon atoms characteristic of glycidyl esters and benzyl esters of $\alpha$- and $\gamma$-aminobutyric acid. The spectra revealed signals from the ester carbons, as well as those in the epoxide ring, confirming successful condensation with epichlorohydrin.

Comparison of the obtained data with published chemical shift values and proton integration ratios allowed for the reliably identification of products 4 and 5 and the exclusion of possible condensation byproducts. This approach ensures high accuracy of structure determination and confirms the validity of the synthetic scheme. A similar condensation reaction of epichlorohydrin with $\gamma$-aminobutyric acid benzyl ester hydrochloride was carried out. The reaction was carried out in absolute dioxane at a controlled temperature of 60–650°C to form the corresponding glycidyl ester. During the reaction, the nitrogen atom of the $\gamma$-aminobutyric acid amino group attacks epichlorohydrin, opening the epoxide ring and forming a new C–N bond while retaining the benzyl ester on the carboxyl group.

The resulting products were isolated using standard purification methods, including extraction and crystallization, after which their structure was confirmed by spectral analysis. H NMR spectra revealed characteristic signals for the protons of the methylene and methine groups, as well as for protons associated with the amino group and glycidyl structure. C NMR spectroscopy identified the carbonyl and aliphatic carbon atoms, including signals from the epoxide ring, confirming the successful completion of the reaction and the formation of the target product.



It has been experimentally established that under the influence ofThe amine group of γ-aminobutyric acid opens the epoxide ring of epichlorohydrin at a temperature of 60–650°C and a molar ratio of the reactants of 1:1 over a period of 3.5–4.0 hours. According to Krasusky's rule, described in the literature, the process proceeds with the preferential formation of propan-2-ol derivatives. The reaction mechanism involves the nucleophilic addition of the amine group to the less substituted carbon atom of the epoxide ring, which ensures the selective formation of the target product. During the reaction, the amine attacks the less sterically hindered carbon atom of the epoxide ring, leading to its opening and the formation of a new C–N compound while maintaining the carboxyl ester functional group.

The reaction progress and purity of the resulting compounds were monitored using thin-layer chromatography (TLC) on Sulufo plates.

Three eluent systems were used to separate the components:

1. **Chloroform-methanol (8:2)**– effective for isolating polar reaction products.
2. **n-Butanol–water–acetic acid (10:4:2)**– was used to separate hydrophilic compounds and amino acid fragments.
3. **Benzene-acetone-acetic acid (8:2:1)**– was used to evaluate non-polar and semi-polar components.

To visualize the chromatograms, iodine vapor was used as a developer, ensuring the appearance of distinct spots of all components. This approach allowed for rapid monitoring of reaction completion, identification of byproducts, and assessment of the purity of the synthesized compounds.

TLC analysis allowed us to identify the individuality of each compound obtained and to ensure the absence of by-products, ensuring control over the reaction progress. After confirming the structure and composition of the synthesized compounds (1–4) using elemental analysis and IR spectroscopy, the next stage of the research was carried out – studying the reaction of α-glycerol monochlorohydrin with these aminobutyric acids.

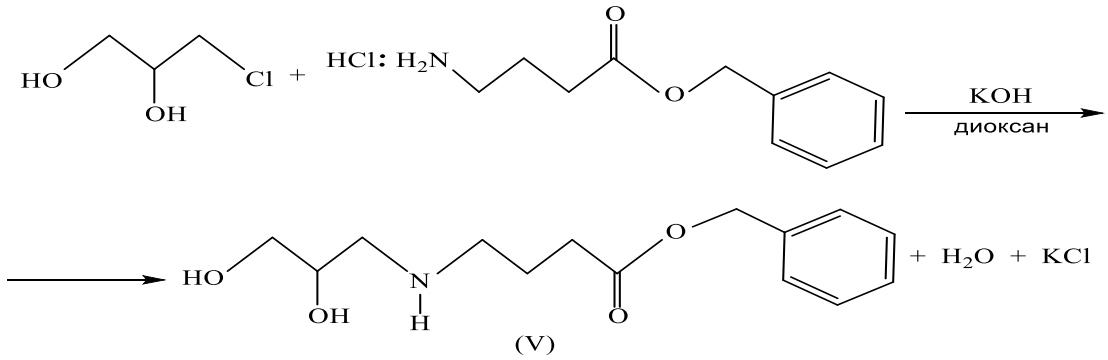
IR spectra of previously synthesized compounds showed characteristic bands:

- **760–820 $cm^{-1}$**— C–Cl stretching vibrations indicating the presence of organochlorine fragments,
- **1850–1965 $cm^{-1}$** — stretching vibrations of $CH_2$and CH, confirming the preservation of the carbon chain,
- **1645 $cm^{-1}$** — stretching vibrations of C=O of the amide group (CO–NH), which confirms the formation of an amide bond,
- **3355–3375 $cm^{-1}$** — stretching vibrations of the hydroxyl group OH, indicating the presence of free hydroxyls that can participate in further reactions with glycerol.

These data indicated the correct formation of target products with an open epoxy ring and amine functionality.

In a subsequent study, glycerol α-monochlorohydrin was used in a reaction with benzyl esters of α- and γ-aminobutyric acids, enabling the study of the mechanisms of epoxide ring opening and the formation of new propan-2-ol compounds with potential biological activity. The reaction was

conducted under controlled conditions of temperature and molar ratio of the reactants, followed by analysis of the resulting products using thin-layer chromatography and IR spectroscopy to confirm the structure and purity of the compounds. The reaction was carried out under similar conditions—in absolute dioxane at 60–650°C with a controlled molar ratio of the reactants of 1:1. The results demonstrated high selectivity and the formation of glycidyl esters with preservation of the stereochemical structure, as confirmed by subsequent spectral and chromatographic analyses.




(V)

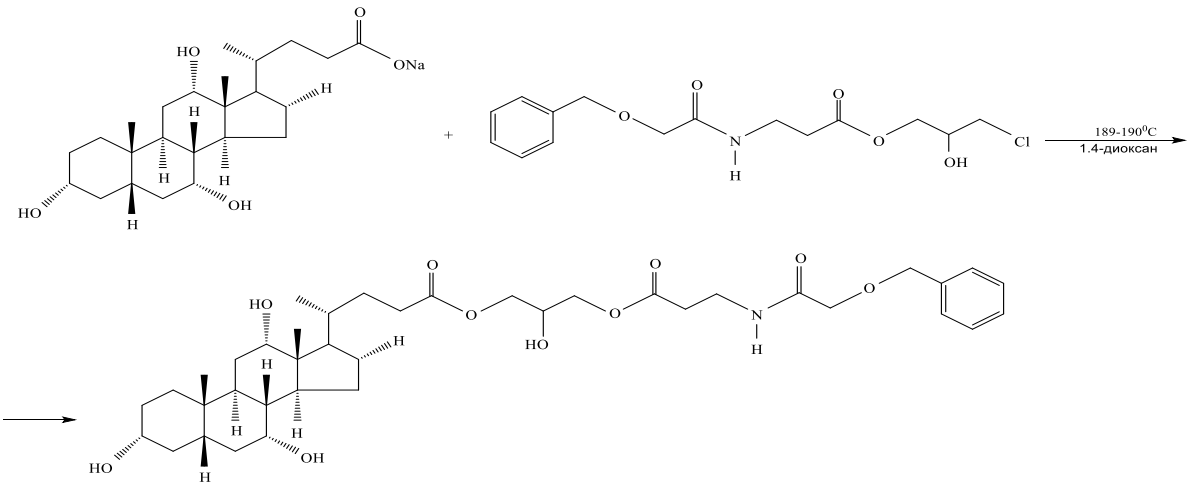
The reaction of $\alpha$-monochlorohydrin glycerol with benzyl ester of $\gamma$-aminobutyric acid is carried out at a temperature of 60–650°C and a molar ratio of 1:1. The process proceeds through the opening of the epoxide ring of $\alpha$-monochlorohydrin by the amine group of the ester, followed by the addition of a glycerol fragment, which leads to the formation of a glycidyl ester.

The reaction mechanism includes the following stages:

1. Nucleophilic attack of the amine group of $\gamma$-aminobutyric acid on the epoxide ring of glycerol monochlorohydrin.
2. Opening of the epoxy ring to form a secondary hydroxyl group.
3. Formation of a stable glycidyl ester with retention of amide and benzyl functionalities.

The synthesis of cholanic acid derivatives with aromatic and aliphatic amino acids represents a promising direction in the development of new biologically active compounds with pronounced antifungal activity due to the presence of reactive functional groups. To obtain these compounds, an approach was used involving the interaction of amino acid esters and dipeptides with 2-chloromethyloxirane and cholanic acid, which enabled the formation of new synthetic molecules with complex properties. Among the synthesized derivatives, compounds with multifaceted biological activity were observed, including antitumor, anti-inflammatory, antiviral, and antibacterial effects. Particular attention was paid to the bactericidal properties of the obtained compounds. Experimental evaluation was conducted on cultures of microorganisms of clinical and epizootic significance, such as Staphylococcus aureus (including saprophytic and epidermal strains), Escherichia coli, and Salmonella. The results showed that the compounds exhibited both bacteriostatic and bactericidal effects, depending on the microorganism species and drug concentration. These data demonstrate the potential of the developed compounds for the prevention and treatment of bacterial infections, as well as the possibility of reducing the development of pathogenic microbial resistance when using combination chemotherapeutic agents. An additional advantage is the ability to reduce the required drug dosage, which increases the cost-effectiveness of their use in human and veterinary practice.

For the first time, the study proposed drug screening among compounds structurally related to natural pyrimidines, such as thymine, uracil, and cytosine. This allowed for the identification of new avenues for the development of synthetic biologically active substances with predictable pharmacological activity.




The compound is a new compound not described in the literature,3α,7α,12α–trihydroxycholanate-3-γ–aminobutyryl-propan-2-ol, which belongs to the group of ester compounds.

**Physicochemical constants are presented in Table 1.**

| No. | Name of the substance | Gross formula | Exit % | Tpl. 0C | Rf* | | | %N | %Cl |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | A | b | With | | |
| 1 | Benzyl ether butanate hydrochloride | $C_6H_{12}O_2NCl$ | 74.42 | Amorphous | 0.31 | 0.37 | 0.51 | 27.45<br>27.65 | 26.69<br>26.89 |
| 2 | Benzyl ether hydrochloride-butanate | $C_{11}H_{14}O_2N_2Cl$ | 77.14 | 211-213 | 0.37 | 0.41 | 0.52 | 23.58<br>23.88 | 22.61<br>22.91 |
| 3 | 1-chloro-3-benzyl ether butyropropan-2-ol | $C_9H_{16}O_3NCl$ | 76.12 | Amorphous | 0.62 | 0.71 | 0.67 | 23.31<br>23.61 | 23.31<br>23.61 |
| 4 | 1-chloro-3-cabobenzoxyγ-butyrylpropan-2-ol | $C_{25}H_{48}Cl\ NO_5$ | 81 | 120-121 | 0.56 | 0.58 | 0.65 | 24.62<br>24.82 | 23.53<br>23.83 |
| 5 | 3α,7α,12α–trihydroxycholanate-3-γ– aminobutyryl-propan-2-ol | $C_{59}H_{116}NO_{10}$ | 86.7 | 189-190 | 0.63 | 0.68 | 0.70 | 24.72<br>24.82 | 23.63<br>23.83 |

**Study of biological activity.** For the first time, the study proposed drug screening among compounds structurally related to natural pyrimidines, such as thymine, uracil, and cytosine. This allowed for the identification of new avenues for the development of synthetic biologically active substances with predictable pharmacological activity.

The antimicrobial activity of a specific compound, 3α,7β-dihydroxycholic acid with gamma-amino acids, was assessed using serial dilutions in meat-peptone broth (MPB) against various epizootic and field strains of microorganisms: Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Proteus vulgaris, Salmonella enteritidis, Salmonella pullorum, Pasteurella multocida, Pseudomonas aeruginosa, and Klebsiella pneumoniae. For the experiment, a stock solution of 1-chloro-3-methyltryptophan-2-ol was prepared at a concentration of 5 mg per 100 ml of sterile purified water. Then, two-fold serial dilutions of the solution were performed in 2 ml MPB. Each ml of prepared media was supplemented with 200 μl of a suspension of test microorganism cultures in isotonic sodium chloride solution, which enabled the precise

determination of minimum inhibitory concentrations of the drug for different strains. Thus, this study demonstrates the successful synthesis of new cholanic acid derivatives with amino acid fragments and confirms their broad biological activity. The obtained data open up prospects for further study of these compounds as potential antifungal, antibacterial, and antiviral agents, as well as for the development of complex chemotherapeutic agents with improved pharmacological properties.

Microorganism samples were incubated under strictly controlled conditions at 37°C for 24–48 hours, allowing for accurate assessment of culture growth and the effect of the synthesized compound 3$\alpha$,7$\beta$-dihydroxycholic acid with gamma-amino acids on them. Incubation was performed using optimal conditions for each bacterial species to eliminate the influence of external factors on microbial activity and reliably determine the pharmacological potential of the test substance. After the incubation period, bacterial growth was quantified and the minimum bacteriostatic concentration (MBC) was determined. This is the concentration of the substance at which inhibition of microbial growth and reproduction is observed, which serves as a criterion for its effectiveness as a bacteriostatic agent.

To more accurately determine the minimum bactericidal concentration (MBC), repeat experiments were conducted on meat-peptone agar (MPA), which serves as an indicator of microbial death. The absence of growth on MPA indicates complete cell destruction, allowing one to distinguish bactericidal action from a purely bacteriostatic one. These tests also used positive and negative controls on MPA and MPA, ensuring the reliability of the results and distinguishing the effect of the test compound from the possible influence of the incubation medium or natural fluctuations in microbial growth. Experimental data demonstrated that the synthesized 3$\alpha$,7$\beta$-dihydroxycholic acid with gamma-amino acids exhibits high activity against a wide range of microorganisms, including pathogenic and opportunistic strains: Staphylococcus aureus, Streptococcus pyogenes, Pasteurella multocida, Proteus vulgaris, Salmonella enteritidis, Pseudomonas aeruginosa, Escherichia coli, Salmonella pullorum, and Klebsiella pneumoniae. For these strains, MBC concentrations ranged from 0.33 to 1.26 μg/ml, indicating the substance's high efficacy in suppressing bacterial growth. Analysis of the results also revealed that different microorganisms exhibit varying sensitivity, which is important for determining the compound's spectrum of activity and planning its practical application. The stability of the synthesized substance was additionally assessed. Experiments have shown that 3$\alpha$,7$\beta$-dihydroxycholic acid with gamma-amino acids retains bactericidal activity over a wide concentration range—from 0.54 to 3.96 μg/ml—and exhibits high thermal stability, confirming its suitability for use in various pharmaceutical formulations. These characteristics are important for the development of drugs with a long shelf life and predictable therapeutic effect, as well as for use under variable external factors, such as changes in temperature or environmental composition. The practical significance of the obtained data lies in confirming that the synthesized compound is capable of acting as an effective antimicrobial agent against a wide range of pathogenic microorganisms. The low MBC range and high stability of the substance allow its use in pharmacological preparations for the prevention and treatment of bacterial and mixed infections, reducing the risk of pathogenic microorganism resistance. Furthermore, effective action at relatively low doses may reduce the need for increased therapeutic dosages, improving the safety and cost-effectiveness of the drug. Thus, the studies conducted confirm the high efficacy and stability of 3$\alpha$,7$\beta$-dihydroxycholic acid with gamma-amino acids, demonstrating its potential for further clinical study as a broad-spectrum antimicrobial and antifungal agent. These tables reflect

the ranges of the minimum bacteriostatic concentration (MBc) and minimum bactericidal concentration (MBC) of the synthesized compound 3α,7β-dihydroxycholic acid with gamma-amino acids against various microorganisms. These data demonstrate that the synthesized compound has a broad spectrum of antimicrobial activity. Gram-positive bacteria (St. aureus, Str. pyogenes) are most sensitive to it, while gram-negative microorganisms (E. coli, S. pullorum, Kl. pneumoniae) require higher concentrations to achieve a bacteriostatic and bactericidal effect. The overall range of MBcK and MBC confirms the efficacy of the compound in pharmacological applications and its potential for the development of broad-spectrum anti-infective drugs.

***Table 2.*** *Antibacterial activity 3α,7β-dihydroxy cholevaya acids With gamma-amino acids,mcg/ml*

| Microorganism | MBsK | MBsK |
|---|---|---|
| St. aureus | 0.29 – 1.19 | 0.55 – 2.66 |
| Str. Pyogenes | 0.44 – 1.14 | 0.77 – 2.55 |
| E. coli | 1.22 – 4.55 | 2.42 – 9.03 |
| Pr. Vulgaris | 0.58 – 2.65 | 1.32 – 4.54 |
| S. enteritidis | 0.55 – 2.66 | 1.33 – 4.88 |
| S. pullorum | 1.32 – 4.77 | 2.33 – 9.78 |
| P. multocida | 0.77 – 1.44 | 0.55 – 2.77 |
| Ps. Aeruginosa | 0.65 – 2.65 | 1.33 – 4.55 |
| Kl. Pneumonia | 1.33 – 4.77 | 2.55 – 9.88 |

3α,7β-dihydroxycholic acid with aminobutyric acid derivatives exhibits pronounced antimicrobial activity against a wide range of microorganisms, including epizootic strains and laboratory cultures used in control studies. This compound has the ability to inhibit bacterial growth in the concentration range of 0.72–9.22 μg/ml, indicating its high biological efficacy. Experimental studies have shown that this substance has both bacteriostatic and bactericidal effects. In particular, at the lower end of the concentration range (approximately 0.72 μg/ml), a slowdown in microorganism growth was observed, reflecting a bacteriostatic effect, and at higher concentrations (up to 9.22 μg/ml), complete cessation of growth and cell death of microorganisms were observed, indicating bactericidal activity. In addition, the compound exhibits high efficacy against strains obtained from field conditions, which is especially important for epizootic and clinical studies. A comparison of the results with laboratory strains showed that 3α,7β-dihydroxycholic acid with gamma-amino acids exhibits a more pronounced bactericidal effect on field strains, indicating its potential for use in veterinary and clinical practice to combat bacterial infections. The compound's mechanism of action is likely related to the interaction of the molecule's functional groups with bacterial cell membranes, disrupting their integrity and inhibiting key enzymatic processes. The compound's high activity is also explained by the presence of hydroxyl groups 3α and 7β, which enhance the molecule's polarity and increase its ability to interact with microbial membrane structures. The obtained data highlight the practical significance of the compound as a potential broad-spectrum antimicrobial agent capable of effectively inhibiting the growth of both laboratory and field strains of microorganisms. This makes it a promising candidate for further study as a pharmacological agent for the prevention and treatment of bacterial infections in veterinary and human medicine.

## 4. CONCLUSIONS

As a result of the research, six new compounds belonging to the class of propan-2-ols and propane-1,2-diols, previously undescribed in the scientific literature, were synthesized, isolated, and characterized in detail. These compounds are derivatives of $\alpha$- and $\gamma$-aminobutyric acid condensed with epichlorohydrin and glycerol mono- or dichlorohydrin, forming a unique combination of hydroxyl and amino groups in the molecule. The structure of the obtained compounds was confirmed using IR spectroscopy and elemental analysis, which allowed for the identification of key functional groups, including hydroxyl (–OH), amide (–CO–NH), and chlorine-containing (–C–Cl) moieties. Purity and reaction progress were monitored using thin-layer chromatography, using various eluent and developer systems, ensuring high reliability of the obtained data. Each of the isolated compounds was subjected to comprehensive spectroscopic analysis, including IR spectroscopy and H and C NMR, as well as elemental analysis to confirm composition and structure. The IR spectra revealed characteristic absorption bands confirming the presence of hydroxyl groups, amino groups, and carbonyl moieties, while NMR spectra allowed the arrangement of substituents within the carbon skeleton to be determined and the structure of the propan-2-ols and propane-1,2-diols to be confirmed.

These new compounds exhibit high chemical stability and purity, making them promising for further study as potentially biologically active molecules. The isolation of these previously undescribed derivatives opens up opportunities for the development of new drugs and for in-depth studies of the reactivity of propane-2-ols and propane-1,2-diols in organic and pharmaceutical chemistry.

**REFERENCES**

1. *Samandarzoda, N.Yu. Synthesis and learning structure acid and cholan bo ester and amino acid aroma / Sh.E. Olimova, N.Yu., Samandarzoda // Vestnik-DDD. 2024 No. 3 (29). P. 84-92.*
2. *Samandarzoda, N.Yu. Synthesis of steroid modification and amino acid aromaï/Sh.E. Olimova, N.Yu. Samandarzoda, Zh.A. Oliftaeva, S.I. Raҷabzoda //Vestnik-DDH. 2024 No. 3. pp. 224-229.*
3. *Patent No. TJ 1371. 1-chloro-3-methoxytryptophalopropan-2-ol-3α, 7β-dihydroxy acid and holonati dori tasiri ziddimicrobū. /N.Yu.Samandarov, S.I. Rajabzoda, Sh.E. Olimova, I.Z. Alimov,Ч.M. Obidov// Application No. 2201666, small patent No. TJ 1371 was issued for the invention. IPC C07J9/00, registered in the State Register of Inventions of the Republic of Tajikistan on April 18, 2023 - Dushanbe, 2023.*
4. *Nahustpatent No. TJ 1594.1-beta-d-ribofuranosyl-1n-1,2,4-triazole-3-carboxamide acid and holon dori tasiri ziddivirusū /N.Yu. Samandarov, S.S. Ismoilzoda, S.M. Abdullozoda, N.M. Mirzoeva, Sh.E. Olimova, I.Z. Alimov,Ч.M. Obidov // Application No. 2301881, small patent No. TJ 1594 was issued for the invention. Registered in the State Register of Inventions of the Republic of Tajikistan on September 29, 2023 - Dushanbe, 2025.*
5. *Nahustpatent No. TJ 1305. Malҳami Malonol" dori ta'siri ziddisuhtagū,ziddiltiҳaboutū va ҷaroҳatsiҳatkunanda/N.Yu. Samandarov, S.M. Abdullozoda, Sh.E. Olimova, B.Kh. Makhkamova, R.I. Begmurodov // Application No. 2201659, small patent No. TJ 1305 was issued for the invention. Registered in the State Register of Inventions of the Republic of Tajikistan on April 11, 2022, Dushanbe, 2022.*
6. *Ismoilzoda, S.S.Antimicrobial activity of new epichlorohydrin derivatives with amino acid residues /S.S. Ismoilzoda, A.J. Yusufzoda, S.I. Rajabov, Z. Ashurova// Scientific journal, science and innovation. Tajik National University; - Dushanbe, 2019. No. 4-P. 130-133.*
7. *Ismoilzoda, S.S. Interactions of epichlorohydrin with some amino acid esters. /S.I. Radjabov, S.S. Ismoilzoda, L.G. Mukhtorov, Ivanova E.V., Nikishina M.B., Atroshchenko Yu.M.// Proceedings of the XVI international scientific and practical conference. Prospects of world science-2020. Pp.11-13.*

8. *Ismoilzoda, S.S. These reactions mutated 2-chloromethyloxirane in the ester base and L-tyrosine. /S.I.Raabov, S.S. Ismoilzoda, A.A. Gulov, R.A. Mustafakulova//Payomi Donishgoi millii Tojikiston. Bakhshi ilmoi tabi. ISSN 2413-452X/ No. 1. Dushanbe-2020.Сањ. 182-192.*

9. *Ismoilzoda, S.S. Synthesis 1-0-( γ-aminobutyryl) glycerol and 1.2.-di-0-(γ-aminobutyryl) glycerol. /S.S. Ismoilzoda., F.N. Sharipov., S.I. Radjabov// Bulletin of the Pedagogical University. ISSN 2707-9996/ No. 1 (10-11). Dushanbe-2021. P-309-315.*

10. *Popadyuk, I.I. Modern approaches to modifications of bile acids for the purpose of synthesizing compounds with valuable physicochemical and biological properties / I.I.Popadyuk, O.V.Salomatina, N.F. Salakhutdinov // Advances in Chemistry. – 2017. – Issue. 86(5). – pp. 388–443.*

11. *Popadyuk, I.I. Novel derivatives of deoxycholic acid bearing aliphatic or cyclic diamine moieties at the C-3 position: synthesis and evaluation of anti-proliferative activity /I. Popadyuk, A.V. Markov, O.V. Salomatina, E.B. Logashenko, S A.V.hernyukov, M.A.Zenkova, N.F. Salakhutdinov // Bioorg. Med. Chem. Lett. –2017. DOI: 10.1016/j.bmcl.2017.06.072.*

12. *Hofmann, A.F. Key discoveries in bile acid chemistry and biology and the infrared clinical applications / Hofmann A.F., Hagey L.R. // J. Lipid Res. - 2014. - V. 55. - P. 1553-1595.*

13. *Nazarova, Z.D. Synthesis and properties of aminothiophene-containing bile acids. / Z.D. Nazarova, M.M. Murodova, A.Kh. Kadyrov, K.Kh. Khaidarov // Proc. rep. conf. "Achievements in chemistry and chemical technology". Dushanbe, 2002, - p. 77-79.*

14. *Sultonmamadova, M.P. Synthesis of 3α, 7α 12α-triketocholanic acid esters. / M.P. Sultonmamadova, A.Kh. Kadyrov, K.Kh. Khaidarov // Dokl. AN RT, 2011. T. 54 No. 8. -c. 649-652.*

15. *Murodova, M.M. Synthesis of new bile acid derivatives. / MM. Murodova, A.Kh. Kadyrov, Z.D. Nazarova, K.Kh. Khaidarov // Dokl. AS RT, 2005. T. XLVIII No. 2. -c. 21-25.*

16. *Rakhabov, S.I. Synthesis of vatagio and 2-chloromethyloxirane bo ester and methyl amino acid/S.I. Rakhabov // Payomi Donishgohi millii Tojikiston. Dushanbe: Sino, -2018. – No. 1. – P. 158-163.*