ORIGINAL ARTICLE

A study of hypoglycemic activity of acids and salts containing 1,2,4-triazole

Štúdium hypoglykemickej aktivity kyselín a solí obsahujúcich 1,2,4-triazol

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Summary

This article presents the study results of the hypoglycemic properties among 1,2,4-triazole derivatives synthesized at the Department of Physical and Colloidal Chemistry of the Zaporizhzhia State Medical University. Today, many people have a sedentary way of life, also most of their diet contains products that they frequently use, which increase the level of glucose in the blood, which can provoke the development of serious diseases. Therefore, to date, the creation of drugs that exhibit hypoglycemic activity and have a low level of toxicity remains an urgent task for medicine and pharmacy. In the first stage of our research, acute toxicity prediction was performed. The hypoglycemic activity of the synthesized compounds was assessed by performing an intraperitoneal glucose tolerance test (IPGTT) with a change in the blood glucose concentration of the animal after its single intraperitoneal administration in the form of a 40% solution at a dose of 2 g/kg of rat body weight. Thirty-eight compounds of the different classes were studied for hypoglycemic activity. Zinc (II) 2-{5-[(3,4-methoxyphenyl)-3*H*-1,2,4-triazole-3-yl]thio} acetate (3.18) showed the highest efficiency in terms of the ability to lower blood glucose levels, namely, by 27.3% (approximately 1.3 times).

Key words: 1,2,4-triazole • hypoglycemic activity • intraperitoneal glucose tolerance test

Súhrn

Článok prezentuje výsledky štúdie hypoglykemických vlastností medzi 1,2,4-triazolovými derivátmi syntetizovanými na Katedre fyzikálnej a koloidnej chémie Štátnej lekárskej univerzity v Zaporizhzhia. V súčasnosti má veľké množstvo ľudí sedavý spôsob života, aj väčšina ich stravy obsahuje často používané produkty, ktoré zvyšujú hladinu glukózy v krvi, čo môže vyvolať rozvoj závažných ochorení. Preto do dnešného dňa zostáva vytvorenie liekov, ktoré vykazujú hypoglykemickú aktivitu a majú nízku úroveň toxicity, naliehavou úlohou pre lekárne. V prvej fáze nášho výskumu bola vykonaná predpoveď akútnej toxicity. Hypoglykemická aktivita syntetizovaných zlúčenín bola hodnotená vykonaním intraperitoneálneho glukózového tolerančného testu (IPGTT) so zmenou koncentrácie glukózy v krvi zvieraťa po jeho jednorazovom intraperitoneálnom podaní vo forme 40 % roztoku v dávke 2 g/kg telesnej hmotnosti potkana. Zlúčeniny (38) rôznych tried boli študované na hypoglykemickú aktivitu. 2-{5-[(3,4-dimetoxyfenyl)-3*H*-1,2,4-triazol-3-yl]sulfanyl}acetát zinočnatý (3.18) vykazoval najvyššiu účinnosť z hľadiska schopnosti znižovať krv hladiny glukózy, a to o 27,3 % (približne 1,3-krát).

Klíčová slova: 1,2,4-triazol • hypoglykemická aktivita • intraperitoneálny glukózový tolerančný test

Introduction

The 21st century is one of the most successful in developing scientific and technological progress. All the works of researchers in different fields of science, countries, and nations have a common goal of work, which is to improve people's quality and life expectancy. But not only science is improving, the existing diseases are also improving and spreading, and new ones also appear for which the created drugs are ineffective. Today, a large number of people have a sedentary way of life, and also most of their diet contains products which due to their frequent use increase the level of glucose in the blood, which can provoke the development of serious diseases. Therefore, to date, the creation of drugs that exhibit hypoglycemic activity

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and have a low level of toxicity remains an urgent task for medicine and pharmacy.

An analysis of modern literature1–12) indicates that the search for biologically active substances among 1,2,4-triazole-3-ylthio acids and their salts is promising. Analysis of the works of the ZSMU scientific school, namely the thesis1) and articles2–15), show that substances that have methoxyphenyl substituents are highly active antimicrobial, antifungal agents and can be the basis for the creation of promising drugs. At the same time, the pharmacological activity in a number of {5-[(2,4-, 3,4-dimethoxyphenyl)-3*H*-1,2,4-triazole-3-yl] thio}(acetic, propanoic, benzoic) acids and their salts have not been sufficiently studied. So synthesis, study of the physical-chemical and biological properties of 1,2,4-triazole-3-ylthio(acetic, propanoic, benzoic) acids and salts that have 2,4- and 3,4-dimethoxyphenyl have scientific novelty, theoretical and practical significance.

The aim of our work was to investigate the possible hypoglycemic activity of {5-[(2,4-, 3,4-dimethoxyphenyl)-3*H*-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids and their salts.

Materials and methods

Chemistry

The synthetic part of the work is a step-by-step formation of target products of chemical transformation based on well-T-known methods of organic synthesis using available reagents and solvents with their additional purification. All investigated compounds were synthesized at the Department of Physical and Colloidal Chemistry at Zaporizhzhia State Medical University. The structure of all synthesized substances was proved using physical-chemical analysis methods. Modern analysis methods were used to establish the structure and confirm the purity of the obtained compounds. Melting points were set in open capillaries using a "Stanford Research Systems Melting point Apparatus 100" (SRS, USA). Elemental analysis (C, H, N, S) was performed using an "Elementary vario EL cube" analyzer (Elementary Analysensysteme, Germany). IR spectra (in the frequency range 4000–400 cm^{-1}) were obtained on an ALPHA-T module of the Bruker ALPHA FT-IR spectrometer (Bruker optics, Germany). ^1H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 spectrometer using tetramethylsilane as the internal standard in a DMSO-d6 solution. Chromato-mass spectra were obtained using an Agilent 1260 Infinity HPLC li-quid chromatograph equipped with an Agilent 6120 spectrometer (electrospray ionization method (ESI)).

Toxicity

At the first stage of the study of hypoglycemic activity of the 1,2,4-triazole derivatives, the predicting acute toxicity was performed with the program GUSAR-online. Computer prediction of acute toxicity of the 1,2,4-triazole derivatives was carried out according to structural formulas compounds in the online version of GUSAR-online[16].

Animals

The studies were carried out on white sexually mature rats of the Wistar line weighing 180–220 g. The rats were obtained from the breeding nursery of the State Institution "Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine". Experimental rats were kept in a vivarium in standard conditions with access to water *ad libitum*.

All manipulations were carried out in accordance with the accepted bioethical standards, observing the relevant rules of the ICH (International Conference on Harmonization), the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (No. 2447-IV of 04.08.2017), the rules of the European Convention for the Protection of Vertebrate Animals used in experimental research and for different purposes dated March 18, 1986, and according to the statement of a committee approving animal experiments conclusion of ZSMU No. 1 dated January 12, 2022.

Hypoglycemic activity

The hypoglycemic activity of the synthesized compounds was assessed by performing an intraperitoneal glucose tolerance test (IPGTT) with a change in the blood glucose concentration of the animal after its single intraperitoneal administration in the form of a 40% solution at a dose of 2 g/kg of rat body weight[17].

The experiment used rats males of the Wistar line weighing 180–220 g, kept under standard vivarium conditions. The animals were divided into 3 groups of 10 experimental in each cage (n = 10) as follows: a group of animals that received a 40% aqueous solution of glucose, a group of animals that received a 40% aqueous solution of glucose, and a reference drug generally accepted in the clinic, antidiabetic agent – metformin at a dose of 200 mg/kg, which was administered orally, and the experimental group, which received a 40% aqueous solution of glucose with propane substance at a dose of 1/10 of the LD_{50}.

The studies were carried out on animals that received a standard food with a sufficient amount of carbohydrates for a week since the expressiveness of the glucose-lowering effect of drugs in intact animals also depends on the nature of the diet. In order to exclude the influence of food on the absorption of the test substance, they were left hungry throughout the night.

The investigated substances were administered orally using a probe in the form of an aqueous solution. The dose of the investigated substances was chosen in the amount of 1/10 of the LD_{50}, which was predicted using the PASS (Prediction of Activity Spectra for Substances) computer program in silico by QSAR modeling based on GUSAR 2011 (General Unrestricted Structure-Activity Relationships).

The glucose in the blood was determined using an express analyzer of the "Gamma mini" glucometer in units of mmol/L. Blood samples for analysis were taken from the tail vein 60 and 180 minutes after the injection of the substances.

Experimental part

Chemistry

{5-[(2,4-, 3,4-dimethoxyphenyl)-3H-1,2,4-triazole--3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 2.1-2.6)

The preparation of 1,2,4-triazole-3-ylthio acetate acids was carried out by two methods.

Method A. In the first case, obtaining acids produced a reaction between 5-(2,4-, 3,4-dimethoxy phenyl)-3*H*--1,2,4-triazole-3-thiones (compounds 1.1, 1.2) (Fig. 1) and chloroacetic acid in puddle environment.

A round-bottom flask with a volume of 250 ml, equipped with a reflux condenser, was loaded with 0.1 mol of the ((5-(2,4-, 3,4-dimethoxy phenyl)-3*H*-1,2,4--triazole-3-thione (compounds 1.1, 1.2), which was dissolved in 20 ml of dimethylformamide. Then it was added 0.1 mol of NaOH (which previously had been dissolved in 20 ml of water) and 0.1 mol of chloroacetic acid. It was heated to transition pH of the solution to a weakly acidic value, after which the resulting solution was evaporated (Fig. 1).

Method B. This method was based on the interaction of {5-[(2,4-, 3,4- dimethoxy phenyl)-3*H*-1,2,4-triazole-3--yl]thio}aceto-, propano-, benzo-) nitriles (compounds 1.3–1.8) (Fig. 1) with hydrochloric acid.

In a 250 ml flask, 0.1 mol of the aceto-, propano-, or benzonitrile (compounds 1.3–1.8), 65 ml of hydrochloric acid were loaded, dissolved, and left at room temperature for 5 days. Then, 200 ml of water were added, and a precipitate formed, which was filtered off and dried.

Most 1,2,4-triazole-3-thio acids are sparingly soluble in water; therefore, to improve solubility in water, salts {5-[(2,4-, 3,4-dimethoxyphenyl)-3*H*-1,2,4-triazole--3-yl]thio}(acetic, propanoic, benzoic) acid were obtained. It was known from scientific sources[1–7] that salts can change biological activity depending on the cation or anion. Therefore, many of these

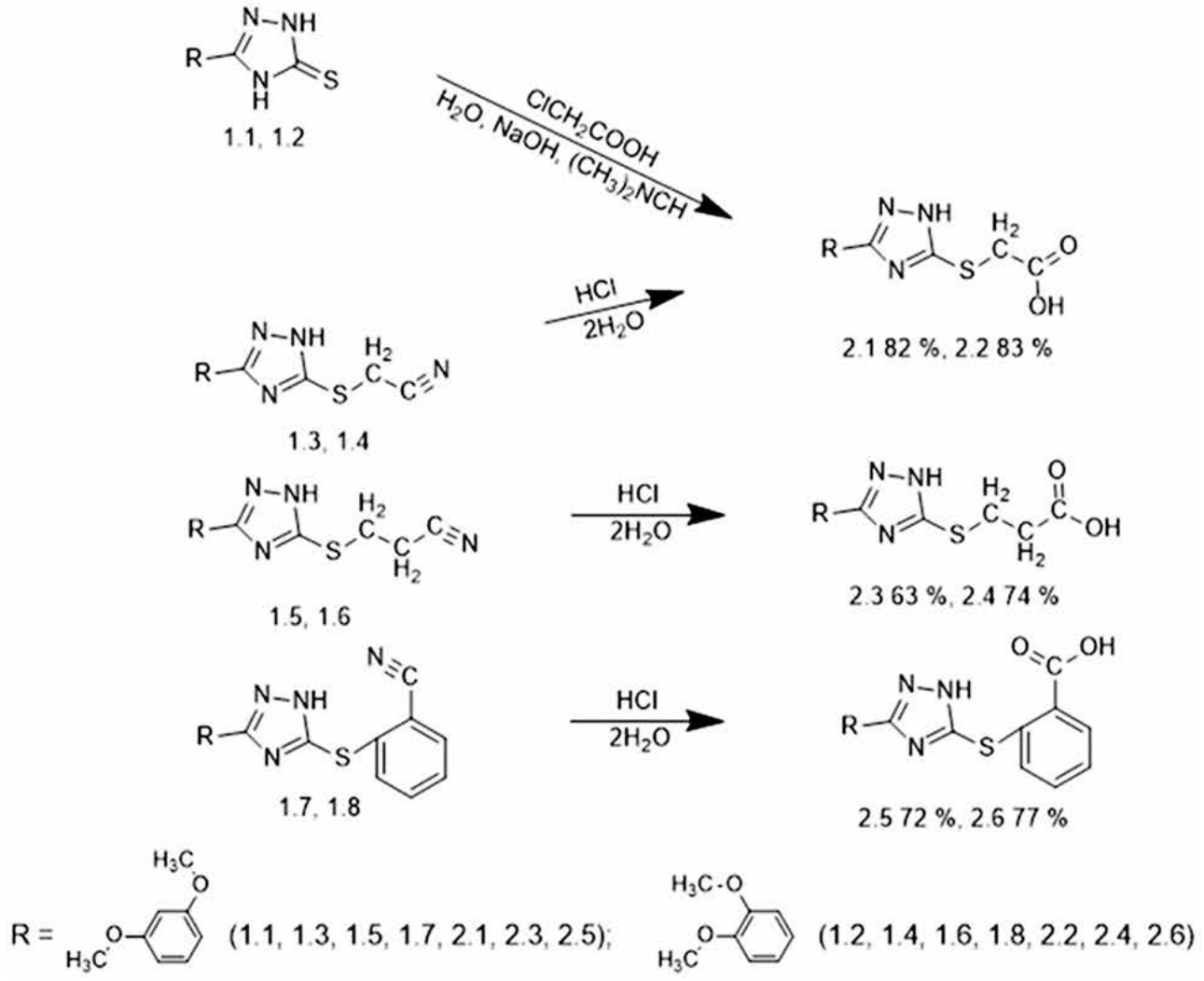

Fig. 1. Scheme of synthesis of {5-[(2,4-, 3,4-dimethoxy phenyl)-3H-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 2.1–2.6)

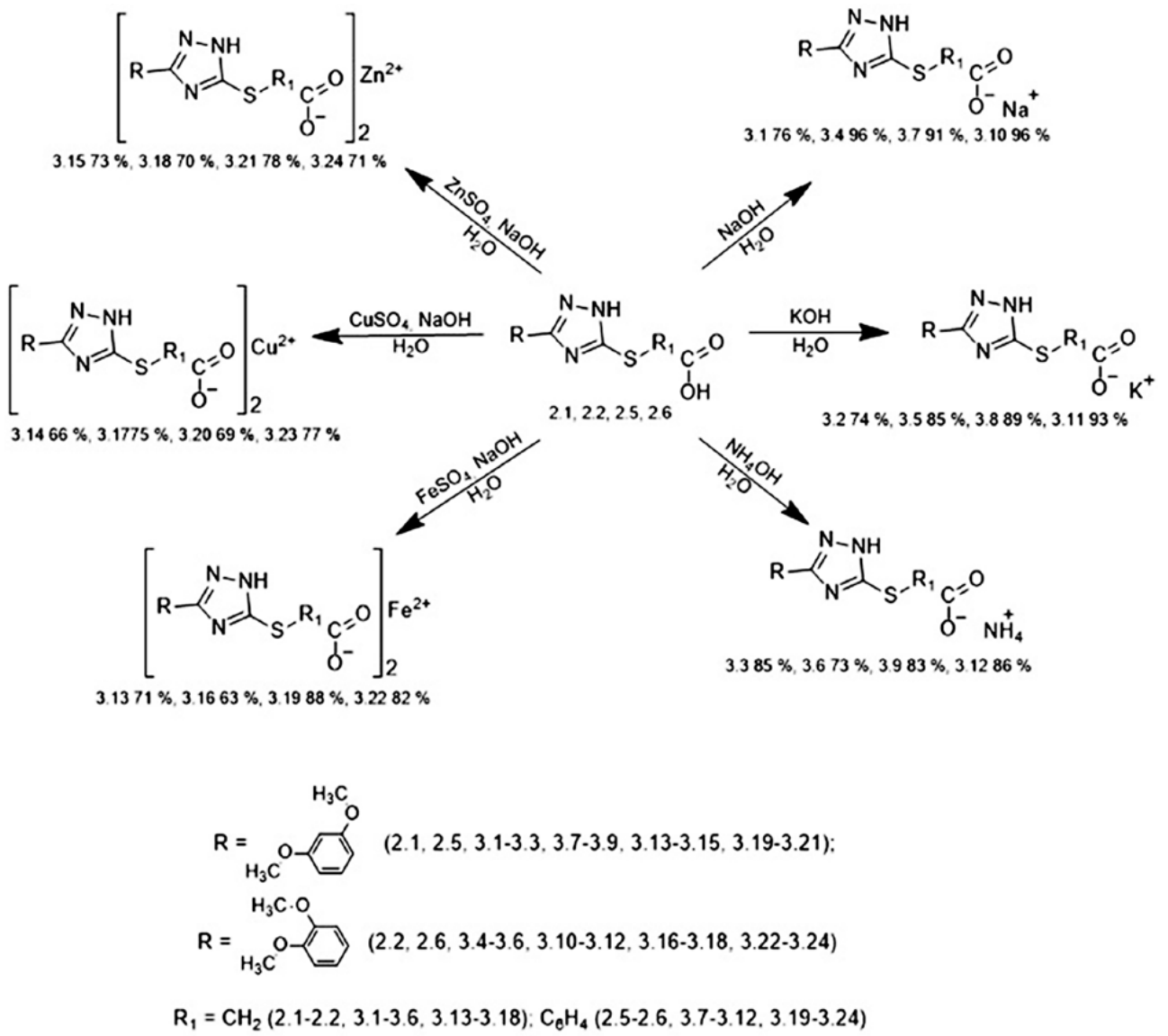

Fig. 2. Scheme of synthesis of salts {5-[(2,4-, 3,4-dimethoxy phenyl)-3H-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids with inorganic bases (compounds 3.1–3.24)

substances were synthesized to expand the spectrum of pharmacological action. Also, scientific literature[1, 6–7, 9–12] indicates that 1,2,4-triazole-3-ylthio(acetic, propanoic, benzoic) acid salts have a wide spectrum of pharmacological activity and exhibit a low level of toxicity.

Salts {5-[(2,4-, 3,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}(acetate, propane, benzoic) acids (compounds 3.1-3.40)

• *Ammonium, sodium, and potassium salts {5-[(2,4-, 3,4-dimethoxy phenyl)-3H-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 3.1–3.12)* (Fig. 2). 5 ml of an aqueous solution of 0.01 mol of sodium hydroxide was loaded into an evaporation bowl. Then 0.01 mol of 5 ml of an aqueous solution of the corresponding 3*H*-1,2,4-triazole-3-ylthio acid was added. After dissolution, it was evaporated in a water bath to form a crystalline substance.

• *Zinc (II), copper (II), and iron (II) salts {5-[(2,4-, 3,4-dimethoxy phenyl)-3H-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 3.13–3.24)* (Fig. 2). A measuring beaker was charged with 0.01 mol of the corresponding 3*H*-1,2,4-triazole-3-ylthio acid, 10 ml of an aqueous solution of 0.01 mol sodium hydroxide, 10 ml of an aqueous solution of 0.01 mol of the corresponding sulfate. After dissolving the resulting solution was left for 24 hours at room temperature and then filtered off.

• *Salts {5-[(2,4-, 3,4-dimethoxy phenyl)-3H-1,2,4-triazole--3-yl]thio}(acetic, propanoic, benzoic) acids with organic bases (ethylamine, dimethylamine, diethylamine (in the reaction with hexamethylenediamine, methanol was mixed with an aqueous solution of NaOH for a successful reaction)(compounds 3.25–3.40)* (Fig. 3). 0.01 mol of the corresponding acid was loaded into the evaporation bowl, 25 ml of methyl alcohol, and 0.01 mol of an organic base (ethylamine, dimethylamine, diethylamine,

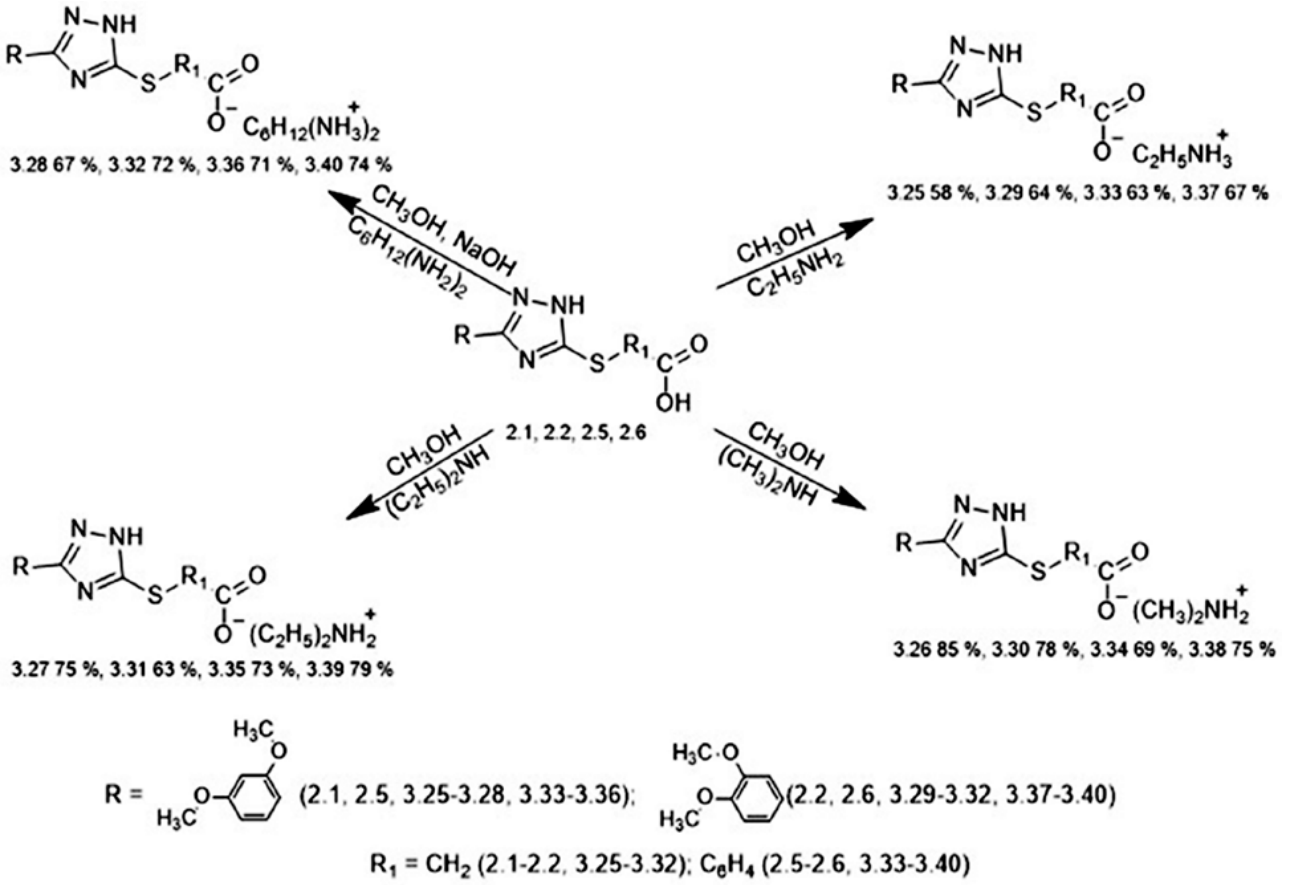

Fig. 3. Scheme of synthesis of salts {5-[(2,4-, 3,4-dimethoxy phenyl)-3H-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids with organic bases (compounds 3.25–3.40)

hexamethylenediamine dihydrochloride). After the dissolution, it was evaporated in a water bath until a crystalline substance was formed.

Results and discussion

After recrystallization, the identity of {5-[(2,4-, 3,4-dimethoxy phenyl)-3*H*-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 2.1–2.6) and their salts (compounds 3.1–2.40) was confirmed by elemental analysis, IR-spectrophotometry, and chromatographically. The results of the elemental analysis confirm the data on the tangential percentage of elements (C, H, N, S) in the samples of the obtained compounds.

In the IR spectra of all synthesized compounds with absorption bands of -C=N- groups (in the cycle) at 1603–1563 cm^{-1}, C–S- group at 707–679 cm^{-1}, absorption bands of the aromatic ring at 1614–1598 cm^{-1}. The IR spectra of acids (compounds 2.1–2.6) had absorption bands of CH_2-COOH-group at 1757–1760 cm^{-1}. In addition, the IR spectra of salts (compounds 3.1–3.40) had symmetric and asymmetric absorption bands characteristic for carboxylic acid salts containing COO^--groups in the range of 1377–1344 cm^{-1} and 1595–1523 cm^{-1}. Also, salts contained absorption bands at 1510–1471 cm^{-1}, indicating aromatic radicals' presence in their structure[17, 18].

^{1}H NMR spectrum of {5-[(2,4-, 3,4-dimethoxy phenyl)-3*H*-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 2.1–2.6) and their salts (compounds 3.1–3.40) was characterized by the presence of multiplet signals of aromatic protons at 6.57–7.85 ppm, two singlet signals of methoxy group protons at 3.65–3.93 ppm, and signals indicating a thiomethylene group at 3.32–3.60 ppm[18].

The homogenicity of the synthesized compounds had been proven using thin-layer chromatography. The acetone : hexane : propanol 2 : 1 : 1 system was used as a mobile medium in this case.

Physical-chemical properties of synthesized compounds

• *2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl] thio}acetic acid (2.1).* Yield 82%, m.p. = 143–145 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.38 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.90 (3H, s, O-CH_3); 4.20 (1H, s, CH); 6.67–7.81 (3H, m, C_6H_3), 12.34 (^{1}H, s, COOH) . Calcd for $C_{12}H_{13}N_3O_4S$ %: C, 48.81; H, 4.44; N, 14.23; S, 10.86. Found %: C, 48.76; H, 4.51; N, 14.23; S, 10.89.

• *2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl] thio}acetic acid (2.2). Orange* 83 %, m.p. = 132–134 °C.

^{1}H NMR (400 MHz, DMSO-d6) d 3.36 (2H, s, S-CH_2); 3.83 (3H, s, O-CH_3); 3.85 (3H, s, O-CH_3); 4.21 (1H, s, CH); 6.98–7.49 (3H, m, C_6H_3), 12.37 (1H, s, COOH) . Calcd for $C_{12}H_{13}N_3O_4S$ %: C, 48.81; H, 4.44; N, 14.23; S, 10.86. Found %: C, 48.78; H, 4.49; N, 14.27; S, 10.81.

• *3-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio} propanoic acid (2.3).* White 63 %, m.p. = 151–153 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 2.60–2.71 (4H, m, CH_2-CH_2); 2.71 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.90 (3H, s, O-CH_3); 4.21 (1H, s, CH); 6.66–7.80 (3H, m, C_6H_3), 12.18 (1H, s, COOH). Calcd for $C_{12}H_{13}N_3O_4S$ %: C, 50.48; H, 4.89; N, 13.58; S, 10.36. Found %: C, 50.55; H, 4.97; N, 13.49; S, 10.30.

• *3-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}propanoic acid (2.4). Orange* 74 %, m.p. = 153–155 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 2.62–2.70 (4H, m, CH_2-CH_2); 2.70 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.86 (3H, s, O-CH_3); 4.23 (1H, s, CH); 6.99–7.50 (3H, m, C_6H_3), 12.20 (1H, s, COOH) . Calcd for $C_{12}H_{13}N_3O_4S$ %: C, 50.48; H, 4.89; N, 13.58; S, 10.36. Found %: C, 50.40; H, 4.94; N, 13.66; S, 10.29.

• *2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl] thio}benzoic acid (2.5).* White 72 %, m.p. = 141–143 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.84 (3H, s, O-CH_3); 3.90 (3H, s, O-CH_3); 4.50 (1H, s, CH); 6.67–7.81 (3H, m, C_6H_3); 7.59–8.30 (4H, m, C_6H_4); 12.75 (1H, s, COOH). Calcd for $C_{17}H_{15}N_3O_4S$ %: C, 57.13; H, 4.23; N, 11.76; S, 8.97. Found %: C, 57.17; H, 4.27; N, 11.70; S, 8.91.

• *2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl] thio}benzoic acid (2.6).* White 77 %, m.p. = 157–159 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.83 (3H, s, O-CH_3); 3.85 (3H, s, O-CH_3); 4.50 (1H, s, CH); 6.98–7.49 (3H, m, C_6H_3); 7.59–8.30 (4H, m, C_6H_4); 12.75 (1H, s, COOH). Calcd for $C_{17}H_{15}N_3O_4S$ %: C, 57.13; H, 4.23; N, 11.76; S, 8.97. Found %: C, 57.17; H, 4.28; N, 11.71; S, 8.92.

• *Sodium (I) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.1).* Green 78 %, m.p. = 146–148 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.55 (2H, s, S-CH_2); 3.80 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.22 (1H, s, CH); 6.64–7.49 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}N_3NaO_4S$ %: C, 45.43; H, 3.81; N, 13.24; S, 10.10. Found %: C, 45.49; H, 3.75; N, 13.30; S, 10.04.

• *Potassium (I) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}acetate (3.2).* Green 74 %, m.p. = 123–125 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.60 (2H, s, S-CH_2); 3.83 (6H, d, O-CH_3); 3.87 (6H, d, O-CH_3); 4.18 (1H, s, CH); 6.62–7.78 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}KN_3O_4S$ %: C, 43.23; H, 3.63; N, 12.60; S, 9.62. Found %: C, 43.17; H, 3.69; N, 12.54; S, 9.69.

• *Ammonium (I) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}acetate (3.3). Orange* 85 %, m.p. = 116–118 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.55 (2H, s, S-CH_2); 3.85 (3H, s, O-CH_3); 3.91 (3H, s, O-CH_3); 4.22 (1H, s, CH); 6.64–7.82 (3H, m, C_6H_3); 7.20 (4H, s, NH_4). Calcd for $C_{12}H_{16}N_4O_4S$ %: C, 46.15; H, 5.16; N, 17.94; S, 10.26. Found %: C, 46.10; H, 5.12; N, 17.99; S, 10.31.

• *Sodium (I) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.4).* Yield 96 %, m.p. = 142–144 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.50 (2H, s, S-CH_2); 3.82 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.24 (1H, s, CH); 7.00–7.51 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}N_3NaO_4S$ %: C, 45.43; H, 3.81; N, 13.24; S, 10.10. Found %: C, 45.40; H, 3.85; N, 13.20; S, 10.15.

• *Potassium (I) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}acetate (3.5).* Yield 85 %, m.p. = 153–155 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.62 (2H, s, S-CH_2); 3.83 (3H, s, O-CH_3); 3.85 (3H, s, O-CH_3); 4.21 (1H, s, CH); 6.98–7.49 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}KN_3O_4S$ %: C, 43.23; H, 3.63; N, 12.60; S, 9.62. Found %: C, 43.27; H, 3.67; N, 12.57; S, 9.59.

• *Ammonium (I) 2-{5-[(3,4-dimethoxyphenyl)-3H--1,2,4-triazole-3-yl]thio}acetate (3.6).* Yield 73 %, m.p. = 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.61 (2H, s, S-CH_2); 3.81 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.23 (1H, s, CH); 6.96–7.47 (3H, m, C_6H_3); 7.22 (4H, s, NH_4). Calcd for $C_{12}H_{16}N_4O_4S$ %: C, 46.15; H, 5.16; N, 17.94; S, 10.26. Found %: C, 46.23; H, 5.10; N, 17.90; S, 10.32.

• *Sodium (I) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}benzoate (3.7).* Yield 91 %, m.p. = 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.82 (3H, s, O-CH_3); 3.89 (3H, s, O-CH_3); 4.47 (1H, s, CH); 6.63–7.79 (3H, m, C_6H_3); 7.57–8.31 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}$-N_3NaO_4S %: C, 53.82; H, 3.72; N, 11.08; S, 8.45. Found %: C, 53.85; H, 3.77; N, 11.04; S, 8.41.

• *Potassium (I) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}benzoate (3.8).* Yield 89 %, m.p. = 156–158 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.81 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.52 (1H, s, CH); 6.69–7.83 (3H, m, C_6H_3); 7.56–8.27 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}KN_3O_4S$ %: C, 51.63; H, 3.57; N, 10.63; S, 8.11. Found %: C, 51.66; H, 3.51; N, 10.60; S, 8.16.

• *Ammonium (I) 2-{5-[(2,4-dimethoxyphenyl)-3H--1,2,4-triazole-3-yl]thio}benzoate (3.9).* Yield 83 %, m.p. = 148-150 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.86 (3H, s, O-CH_3); 3.93 (3H, s, O-CH_3); 4.48 (1H, s, CH); 6.61–7.76 (3H, m, C_6H_3); 7.62–8.31 (4H, m, C_6H_4). Calcd for $C_{17}H_{18}N_4O_4S$ %: C, 54.53; H, 4.85; N, 14.96; S, 8.56. Found %: C, 54.59; H, 4.88; N, 14.92; S, 8.51.

• *Sodium (I) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.10).* Yield 96 %, m.p. = 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.82 (3H, s, O-CH_3); 3.84 (3H, s, O-CH_3); 4.52 (1H, s, CH); 6.99–7.50 (3H, m, C_6H_3); 7.62–8.33 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}N_3NaO_4S$ %: C, 53.82; H, 3.72; N, 11.08; S, 8.45. Found %: C, 53.80; H, 3.78; N, 11.2; S, 8.49.

• *Potassium (I) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4--triazole-3-yl]thio}benzoate (3.11).* Yield 93 %, m.p. = 156–158 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.85 (3H, s, O-CH_3); 3.88 (3H, s, O-CH_3); 4.54 (1H, s, CH); 6.96–7.46 (3H, m, C_6H_3); 7.54–8.27 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}KN_3O_4S$ %: C, 51.63; H, 3.57; N, 10.63; S, 8.11. Found %: C, 51.68; H, 3.52; N, 10.66; S, 8.08.

• *Ammonium (I) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.12).* Yield 86 %, m.p. = 141–143 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.80 (3H, s, O-CH_3); 3.82 (3H, s, O-CH_3); 4.48 (1H, s, CH); 6.93–7.44 (3H, m, C_6H_3); 7.59–8.31 (4H, m, C_6H_4). Calcd for $C_{17}H_{18}N_4O_4S$ %: C, 54.53; H, 4.85; N, 14.96; S, 8.56. Found %: C, 54.55; H, 4.81; N, 14.99; S, 8.53.

• *Iron (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.13)*. Black 71 %, m.p. = 193–195 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.58 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.90 (3H, s, O-CH_3); 4.20 (1H, s, CH); 6.62–7.80 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}FeN_3O_4S$ %: C, 41.16; H, 3.45; N, 12.00; S, 9.16. Found %: C, 41.21; H, 3.50; N, 11.93; S, 9.10.

• *Copper (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.14)*. Green 66 %, m.p. = 169–171 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.49 (2H, s, S-CH_2); 3.81 (3H, s, O-CH_3); 3.88 (3H, s, O-CH_3); 4.24 (1H, s, CH); 6.67–7.81 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}CuN_3O_4S$ %: C, 40.28; H, 3.38; N, 11.74; S, 8.96. Found %: C, 40.22; H, 3.43; N, 11.78; S, 8.91.

• *Zinc (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.15)*. *Brown* 73 %, m.p. = 172–174 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.52 (2H, s, S-CH_2); 3.83 (3H, s, O-CH_3); 3.90 (3H, s, O-CH_3); 4.22 (1H, s, CH); 6.64–7.79 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}N_3O_4SZn$ %: C, 40.07; H, 3.36; N, 11.68; S, 8.91. Found %: C, 40.02; H, 3.39; N, 11.75; S, 8.88.

• *Iron (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.16)*. *Brown* 63 %, m.p. = 197–199 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.63 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.89 (3H, s, O-CH_3); 4.20 (1H, s, CH); 6.94–7.43 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}FeN_3O_4S$ %: C, 41.16; H, 3.45; N, 12.00; S, 9.16. Found %: C, 41.19; H, 3.42; N, 12.07, S, 9.12.

• *Copper (II) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.17)*. Green 75 %, m.p. = 146–148 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.51 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.89 (3H, s, O-CH_3); 4.21 (1H, s, CH); 6.69–7.80 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}CuN_3O_4S$ %: C, 40.28; H, 3.38; N, 11.74; S, 8.96. Found %: C, 40.22; H, 3.39; N, 11.79; S, 8.94.

• *Zinc (II) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.18)*. *Brown* 70 %, m.p. = 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.55 (2H, s, S-CH_2); 3.83 (3H, s, O-CH_3); 3.85 (3H, s, O-CH_3); 4.20 (1H, s, CH); 6.65–7.49 (3H, m, C_6H_3). Calcd for $C_{12}H_{12}N_3O_4SZn$ %: C, 40.07; H, 3.36; N, 11.68; S, 8.91. Found %: C, 40.00; H, 3.33; N, 11.74; S, 8.98.

• *Iron (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.19)*. *Brown* 88 %, m.p. = 189–191 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.85 (3H, s, O-CH_3); 3.91 (3H, s, O-CH_3); 4.52 (1H, s, CH); 6.6907.84 (3H, m, C_6H_3); 7.62–8.31 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}FeN_3O_4S$ %: C, 49.53; H, 3.42; N, 10.19; S, 7.78. Found %: C, 49.56; H, 3.38; N, 10.11; S, 7.85.

• *Copper (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.20)*. Green 69 %, m.p. = 143–145 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.81 (3H, s, O-CH_3); 3.88 (3H, s, O-CH_3); 4.53 (1H, s, CH); 6.69–7.82 (3H, m, C_6H_3); 7.61–8.33 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}CuN_3O_4S$ %: C, 48.63; H, 3.36; N, 10.01; S, 7.63. Found %: C, 48.67; H, 3.29; N, 10.09; S, 7.60.

• *Zinc (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.21)*. *Brown* 78 %, m.p. = 183–185 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.80 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.54 (1H, s, CH); 6.68–7.82 (3H, m, C_6H_3); 7.55–8.27 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}N_3O_4SZn$ %: C, 48.41; H, 3.35; N, 9.96; S, 7.60. Found %: C, 48.46; H, 3.32; N, 9.91; S, 7.69.

• *Iron (II) 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.22)*. *Brown* 82 %, m.p. = 182–194 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.86 (3H, s, O-CH_3); 3.88 (3H, s, O-CH_3); 4.53 (1H, s, CH); 6.99–7.52 (3H, m, C_6H_3); 7.57–8.29 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}FeN_3O_4S$ %: C, 49.53; H, 3.42; N, 10.19; S, 7.78. Found %: C, 49.57; H, 3.45; N, 10.15; S, 7.76.

• *Copper (II) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.23)*. Green 77 %, m.p. = 146–148 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.84 (3H, s, O-CH_3); 3.86 (3H, s, O-CH_3); 4.48 (1H, s, CH); 6.96–7.46 (3H, m, C_6H_3); 7.57–8.28 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}CuN_3O_4S$ %: C, 48.63; H, 3.36; N, 10.01; S, 7.63. Found %: C, 48.67; H, 3.34; N, 9.92; S, 7.67.

• *Zinc (II) 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.24)*. *Brown* 71 %, m.p. = 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.79 (3H, s, O-CH_3); 3.82 (3H, s, O-CH_3); 4.54 (1H, s, CH); 6.97–7.47 (3H, m, C_6H_3); 7.57–8.28 (4H, m, C_6H_4). Calcd for $C_{17}H_{14}N_3O_4SZn$ %: C, 48.41; H, 3.35; N, 9.96; S, 7.60. Found %: C, 48.46; H, 3.32; N, 9.92; S, 7.66.

• *Ethanaminium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.25)*. Yield 58 %, m.p. = 111–113 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.50 (2H, s, S-CH_2); 3.78 (3H, s, O-CH_3); 3.85 (3H, s, O-CH_3); 4.17 (1H, s, CH); 6.69–7.83 (3H, m, C_6H_3). Calcd for $C_{14}H_{20}N_4O_4S$ %: C, 49.40; H, 5.92; N, 16.46; S, 9.42. Found %: C, 49.36; H, 5.99; N, 16.40; S, 9.48.

• *Dimethylammonium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.26)*. *Orange* 85 %, m.p. = 87–89 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.53 (2H, s, S-CH_2); 3.75 (3H, s, O-CH_3); 3.82 (3H, s, O-CH_3); 4.22 (1H, s, CH); 6.71–7.85 (3H, m, C_6H_3). Calcd for $C_{14}H_{20}N_4O_4S$ %: C, 49.40; H, 5.92; N, 16.46; S, 9.42. Found %: C, 49.32; H, 5.87; N, 16.53; S, 9.49.

• *Diethylammonium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.27)*. *Brown* 75 %, m.p. = 194–196 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.55 (2H, s, S-CH_2); 3.82 (3H, s, O-CH_3); 3.89 (3H, s, O-CH_3); 4.20 (1H, s, CH); 6.68–7.80 (3H, m, C_6H_3). Calcd for $C_{16}H_{24}N_4O_4S$ %: C, 52.16; H, 6.57; N, 15.21; S, 8.70. Found %: C, 52.22; H, 6.63; N, 15.28; S, 8.74.

• *Hexane-1,6-diaminium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.28)*. Yield 67 %, m.p. = 173–175 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.52 (2H, s, S-CH_2); 3.80 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.24 (1H, s, CH); 6.69–7.81 (3H, m, C_6H_3). Calcd for $C_{18}H_{30}N_5O_4S$ %: C, 52.41; H, 7.33; N, 16.98; S, 7.77. Found %: C, 52.47; H, 7.39; N, 16.92; S, 7.73.

• *Ethanaminium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.29)*. Yield 64 %, m.p. = 102–104 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.54 (2H, s, S-CH_2); 3.80 (3H, s, O-CH_3); 3.83 (3H, s, O-CH_3); 4.19 (^{1}H, s, CH); 6.99–7.51 (3H, m, C_6H_3). Calcd for $C_{14}H_{20}N_4O_4S$ %: C, 49.40; H, 5.92; N, 16.46; S, 9.42. Found %: C, 49.47; H, 5.95; N, 16.40; S, 9.38.

• *Dimethylammonium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.30).* Yield 78 %, m.p. = 144–146 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.57 (2H, s, S-CH_2); 3.81 (3H, s, O-CH_3); 3.83 (3H, s, O-CH_3); 4.19 (1H, s, CH); 6.97–7.45 (3H, m, C_6H_3). Calcd for $C_{14}H_{20}N_4O_4S$ %: C, 49.40; H, 5.92; N, 16.46; S, 9.42. Found %: C, 49.48; H, 5.99; N, 16.39; S, 9.36.

• *Diethylammonium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.31). Orange* 63 %, m.p. = 187–189 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.51 (2H, s, S-CH_2); 3.84 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.22 (1H, s, CH); 6.98–7.50 (3H, m, C_6H_3). Calcd for $C_{16}H_{24}N_4O_4S$ %: C, 52.16; H, 6.57; N, 15.21; S, 8.70. Found %: C, 52.19; H, 6.62; N, 15.19; S, 8.67.

• *Hexane-1,6-diaminium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}acetate (3.32). Orange* 72 %, m.p. = 94–96 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.54 (2H, s, S-CH_2); 3.82 (3H, s, O-CH_3); 3.84 (3H, s, O-CH_3); 4.21 (1H, s, CH); 6.99–7.50 (3H, m, C_6H_3). Calcd for $C_{18}H_{30}N_5O_4S$ %: C, 52.41; H, 7.33; N, 16.98; S, 7.77. Found %: C, 52.47; H, 7.27; N, 16.91; S, 7.85.

• *Ethanaminium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.33).* Yield 63 %, m.p. = 106–108 °C. ^{1}H NMR (400 MHz, DMSO-d6) d d 3.82 (3H, s, O-CH_3); 3.98 (3H, s, O-CH_3); 4.51 (1H, s, CH); 6.65–7.78 (3H, m, C_6H_3); 7.57–8.31 (4H, m, C_6H_4). Calcd for $C_{19}H_{22}N_4O_4S$ %: C, 56.70; H, 5.51; N, 13.92; S, 7.97. Found %: C, 56.76; H, 5.57; N, 13.82; S, 7.95.

• *Dimethylammonium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.34).* Yield 69 %, m.p. = 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.87 (3H, s, O-CH_3); 3.93 (3H, s, O-CH_3); 4.53 (1H, s, CH); 6.68–7.81 (3H, m, C_6H_3); 7.56–8.26 (4H, m, C_6H_4). Calcd for $C_{19}H_{22}N_4O_4S$ %: C, 56.70; H, 5.51; N, 13.92; S, 7.97. Found %: C, 56.75; H, 5.52; N, 13.95; S, 7.90.

• *Diethylammonium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.35). Orange* 73 %, m.p. = 184–186 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.81 (3H, s, O-CH_3); 3.87 (3H, s, O-CH_3); 4.44 (1H, s, CH); 6.62–7.77 (3H, m, C_6H_3); 7.56–8.29 (4H, m, C_6H_4). Calcd for $C_{21}H_{26}N_4O_4S$ %: C, 58.59; H, 6.09; N, 13.01; S, 7.45. Found %: C, 58.56; H, 6.16; N, 13.04; S, 7.40.

• *Hexane-1,6-diaminium 2-{5-[(2,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.36). Orange* 71 %, m.p. = 96–98 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.87 (3H, s O-CH_3); 3.93 (3H, s O-CH_3); 4.54 (1H, s, CH); 6.71–7.85 (3H, m, C_6H_3); 7.64–8.35 (4H, m, C_6H_4). Calcd for $C_{17}H_{15}N_3O_4S$ %: C, 58.21; H, 6.80; N, 14.76; S, 6.76. Found %: C, 58.19; H, 6.86; N, 14.72; S, 6.78.

• *Ethanaminium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.37).* Yield 67 %, m.p. = 105–107 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.82 (3H, s,

Table 1. Prediction of acute toxicity of the 1,2,4-triazole derivatives using GUSAR-online prognosis

Number of compounds	Rat IP LD_{50} (mg/kg)	Rat IV LD_{50} (mg/kg)	Rat Oral LD_{50} (mg/kg)	Rat SC LD_{50} (mg/kg)
1.1	396.7 in AD	224.7 in AD	1243.0 in AD	1238.0 in AD
1.2	408.9 in AD	192.4 in AD	787.2 in AD	979.4 in AD
1.3	384.7 in AD	187.2 in AD	1420.0 out of AD	733.3 in AD
1.4	259.8 in AD	166.2 in AD	348.1 in AD	636.4 in AD
1.5	384.4 in AD	209.3 in AD	1650.0 out of AD	632.8 out of AD
1.6	391.0 out of AD	188.1 in AD	1348.0 out of AD	1377.0 in AD
1.7	446.3 in AD	164.8 in AD	1722.0 in AD	1742.0 out of AD
1.8	684.9 in AD	138.2 in AD	1268.0 in AD	1464.0 in AD
2.1	455.4 in AD	340.5 in AD	1235.0 in AD	1043.0 in AD
2.2	480.1 in AD	315.1 in AD	575.8 in AD	1150.0 in AD
2.3	345.6 out of AD	404.4 in AD	986.7 in AD	587.6 in AD
2.4	273.0 in AD	398.8 in AD	1014.0 in AD	586.0 in AD
2.5	820.7 in AD	375.3 in AD	1237.0 in AD	1998.0 in AD
2.6	840.3 in AD	335.8 in AD	1458.0 in AD	1929.0 in AD
3.1	589.9 in AD	318.0 in AD	1289.0 in AD	1384.0 in AD
3.2	589.9 in AD	318.0 in AD	1289.0 in AD	1384.0 in AD
3.3	589.9 in AD	318.0 in AD	1289.0 in AD	1384.0 in AD
3.4	662.1 in AD	257.0 in AD	763.5 in AD	1588.0 in AD
3.5	662.1 in AD	257.0 in AD	763.5 in AD	1588.0 in AD
3.6	662.1 in AD	257.0 in AD	763.5 in AD	1588.0 in AD
3.7	887.7 in AD	469.1 in AD	1299.0 in AD	1976.0 in AD
3.8	887.7 in AD	469.1 in AD	1299.0 in AD	1976.0 in AD
3.9	887.7 in AD	469.1 in AD	1299.0 in AD	1976.0 in AD

Table 1. – continuation

Number of compounds	Rat IP LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat Oral LD50 (mg/kg)	Rat SC LD50 (mg/kg)
3.10	760.9 in AD	265.7 in AD	1351.0 in AD	2032.0 in AD
3.11	760.9 in AD	265.7 in AD	1351.0 in AD	2032.0 in AD
3.12	760.9 in AD	265.7 in AD	1351.0 in AD	2032.0 in AD
3.13	589.9 in AD	318.0 in AD	1289.0 in AD	1384.0 in AD
3.14	589.9 in AD	318.0 in AD	1289.0 in AD	1384.0 in AD
3.15	589.9 in AD	318.0 in AD	1289.0 in AD	1384.0 in AD
3.16	662.1 in AD	257.0 in AD	763.5 in AD	1588.0 in AD
3.17	662.1 in AD	257.0 in AD	763.5 in AD	1588.0 in AD
3.18	662.1 in AD	257.0 in AD	763.5 in AD	1588.0 in AD
3.19	887.7 in AD	469.1 in AD	1299.0 in AD	1976.0 in AD
3.20	887.7 in AD	469.1 in AD	1299.0 in AD	1976.0 in AD
3.21	887.7 in AD	469.1 in AD	1299.0 in AD	1976.0 in AD
3.22	760.9 in AD	265.7 in AD	1351.0 in AD	2032.0 in AD
3.23	760.9 in AD	265.7 in AD	1351.0 in AD	2032.0 in AD
3.24	760.9 in AD	265.7 in AD	1351.0 in AD	2032.0 in AD
3.25	340.4	340.4	340.4	340.4
3.26	340.4	340.4	340.4	340.4
3.27	340.4	340.4	340.4	340.4
3.28	340.4	340.4	340.4	340.4
3.29	368.5	368.5	368.5	368.5
3.30	368.5	368.5	368.5	368.5
3.31	368.5	368.5	368.5	368.5
3.32	368.5	368.5	368.5	368.5
3.33	398.4	398.4	398.4	398.4
3.34	398.4	398.4	398.4	398.4
3.35	398.4	398.4	398.4	398.4
3.36	398.4	398.4	398.4	398.4
3.37	402.5	402.5	402.5	402.5
3.38	402.5	402.5	402.5	402.5
3.39	402.5	402.5	402.5	402.5
3.40	402.5	402.5	402.5	402.5

AD – applicability domain

O-CH_3); 3.86 (3H, s, O-CH_3); 4.52 (1H, s, CH); 6.96–7.45 (3H, m, C_6H_3); 7.54–8.26 (4H, m, C_6H_4); 12.68 (^{1}H, s, COOH). Calcd for $C_{19}H_{22}N_4O_4S$ %: C, 56.70; H, 5.51; N, 13.92; S, 7.97. Found %: C, 56.76; H, 5.47; N, 13.97; S, 7.90.

• *Dimethylammonium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.38)*. Yield 75 %, m.p. = 142–144 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.80 (3H, s, O-CH_3); 3.83 (3H, s, O-CH_3); 4.48 (1H, s, CH); 6.97–7.46 (3H, m, C_6H_3); 7.56–8.27 (4H, m, C_6H_4). Calcd for $C_{19}H_{22}N_4O_4S$ %: C, 56.70; H, 5.51; N, 13.92; S, 7.97. Found %: C, 56.67; H, 5.54; N, 13.99; S, 7.92.

• *Diethylammonium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.39)*. *Orange* 79 %, m.p. = 181–183 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.79 (3H, s, O-CH_3); 3.81 (3H, s, O-CH_3); 4.47 (1H, s, CH); 6.97–7.46 (3H, m, C_6H_3); 7.57–8.32 (4H, m, C_6H_4). Calcd for $C_{21}H_{26}N_4O_4S$ %: C, 58.59; H, 6.09; N, 13.01; S, 7.45. Found %: C, 58.54; H, 6.18; N, 13.07; S, 7.38.

• *Hexane-1,6-diaminium 2-{5-[(3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}benzoate (3.40)*. *Orange* 74 %, m.p. = 97–99 °C. ^{1}H NMR (400 MHz, DMSO-d6) d 3.80 (3H, s, O-CH_3); 3.84 (3H, s, O-CH_3); 4.56 (1H, s, CH); 6.97–7.48 (3H, m, C_6H_3); 7.57–8.30 (4H, m, C_6H_4). Calcd for $C_{17}H_{15}N_3O_4S$ %: C, 58.21; H, 6.80; N, 14.76; S, 6.76. Found %: C, 58.27; H, 6.88; N, 14.72; S, 6.66.

Toxicity indicators of synthesized compounds

According to the obtained results of the GUSAR-online forecast, for the tested synthesized compounds, the average lethal dose of LD_{50} for the corresponding acids was when administered: intraperitoneally – from

273.0 to 840.3 mg/kg, intravenously – from 315.5 to 404.4 mg/kg, orally – from 578.8 to 1458 mg/kg, and subcutaneously – from 586.0 to 1250.0 mg/kg. The average lethal dose of LD_{50} for the corresponding

Table 2. The results of hypoglycemic activity screening for the salts {5-[(2,4-, 3,4-dimethoxyphenyl)-3H-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids

Number of compounds	Compounds	Glucose, mmol/l	P	Δ% corresponds to the control
1	Control	4.61 ± 0.54	–	–
2	Glucose	6.71 ± 0.54	< 0.05	42.00
3	Metformin + glucose	3.61 ± 0.09	< 0.05	–20.00
4	3.1	5.45 ± 0.09	< 0.05	11.22
5	3.2	6.44 ± 0.47	< 0.05	38.65
6	3.3	5.70 ± 0.44	> 0.05	–15.93
7	3.4	4.29 ± 0.36	> 0.05	–6.93
8	3.5	5.94 ± 0.36	< 0.05	27.89
9	3.6	5.70 ± 0.39	> 0.05	22.71
10	3.7	4.93 ± 0.47	> 0.05	6.14
11	3.8	5.05 ± 0.44	> 0.05	8.76
12	3.9	4.83 ± 0.18	> 0.05	3.98
13	3.10	6.91 ± 0.13	> 0.05	–13.65
14	3.11	3.41 ± 0.25	< 0.05	–26.49
15	3.12	4.90 ± 0.18	> 0.05	5.58
16	3.13	6.91 ± 0.31	> 0.05	3.13
17	3.14	4.83 ± 0.43	> 0.05	4.08
18	3.15	4.41 ± 0.15	> 0.05	–13.53
19	3.16	4.07 ± 0.30	> 0.05	–12.27
20	3.17	4.83 ± 0.33	> 0.05	–17.92
21	3.18	2.43 ± 0.33	> 0.05	–47.25
22	3.19	4.53 ± 0.33	> 0.05	–2.38
23	3.20	4.97 ± 0.08	> 0.05	7.81
24	3.21	4.16 ± 0.45	> 0.05	–10.42
25	3.22	4.15 ± 0.46	> 0.05	–9.92
26	3.23	4.17 ± 0.49	> 0.05	7.81
27	3.24	5.84 ± 0.17	< 0.05	25.80
28	3.25	4.50 ± 0.14	> 0.05	–2.32
29	3.26	4.86 ± 0.34	> 0.05	4.59
30	3.27	4.20 ± 0.14	> 0.05	–8.83
31	3.28	4.54 ± 0.55	> 0.05	5.58
32	3.29	4.30 ± 0.07	> 0.05	–6.66
33	3.30	5.19 ± 0.19	> 0.05	11.75
34	3.31	5.16 ± 0.18	> 0.05	1.17
35	3.32	5.93 ± 0.0	> 0.05	10.84
36	3.33	5.44 ± 0.37	> 0.05	17.13
37	3.34	4.40 ± 0.10	> 0.05	–4.49
38	3.35	4.50 ± 0.25	> 0.05	–2.32
39	3.36	4.70 ± 0.04	> 0.05	2.02
40	3.37	5.03 ± 0.11	> 0.05	8.37
41	3.38	3.27 ± 0.13	> 0.05	–33.27
42	3.39	4.87 ± 0.13	> 0.05	5.64
41	3.40	4.87 ± 0.13	> 0.05	5.64

salts was when administered: intraperitoneally – from 340.4 to 760.9 mg/kg, intravenously – from 257.0 to 769.5 mg/kg, orally – from 340.4 to 1351.0 mg/kg, and subcutaneously – from 340.4 to 2032.0 mg/kg (Table 1)[19].

Hypoglycemic activity of the synthesized compounds

Thirty-eight compounds of the different classes were studied for hypoglycemic activity. Our hypoglycemic activity screening results are presented in Table 2.

According to ammonium, sodium, and potassium salts {5-[(2,4-, 3,4-dimethoxy phenyl)-3*H*-1,2,4--triazole-3-yl]thio}(acetic, propanoic, benzoic) acids (compounds 3.1–3.12), these compounds did not show higher results in hypoglycemic activity. Compounds 3.3 and 3.10 showed hypoglycemic activity but were below the reference standard. Potassium (I) 2-{5-[(3,4-dimethoxy phenyl)-3*H*-1,2,4--triazole-3-yl]thio}benzoate (3.11) showed better results than metformin but not the best.

Particular attention should be paid to substances of zinc (II), copper (II), and iron (II) salts {5-[(2,4-, 3,4-dimethoxyphenyl)-3*H*-1,2,4-triazole-3-yl]thio} (acetic, propanoic, benzoic) acids (compounds 3.13–3.24), compounds 3.15, 3.16, 3.17, 3.21 showed good results in hypoglycemic activity but were below the reference standard. Zinc (II) 2-{5-[(3,4-dimethoxyphenyl)--3*H*-1,2,4-triazole-3-yl]thio} acetate (3.18) showed the highest efficiency in terms of the ability to lower blood glucose levels.

As for the salts {5-[(2,4-, 3,4-dimethoxyphenyl)-3*H*--1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids with organic bases (ethylamine, dimethylamine, diethylamine, hexamethylenediamine dihydrochloride) (compounds 3.25–3.40), only compound 3.38 showed positive result. Other compounds did not exceed metformin, and in some cases were even lower.

Thus, based on the results of this study, it can be concluded that one of the synthesized compounds Zinc (II) 2-{5-[(3,4-dimethoxy phenyl)-3*H*-1,2,4-triazole-3-yl] thio} acetate (3.18) showed the highest efficiency in terms of the ability to lower blood glucose levels, namely, by 27.3% (approximately 1.3 times) in comparison with the group of laboratory animals that received the reference drug metformin. To date, there was no evidence that the zinc cation exhibits hypoglycemic activity. Also, scientific sources indicate a wide range of biological effects, including hypoglycemic activity, among 1,2,4-triazole and methoxyphenyl substituents[5–7, 11]. There was information that proves that in this substance, due to the coordination bonds, the zinc cation cannot exhibit any activity[20, 21].

The results of the studies are shown in Table 3.

Conclusion

Preparative methods for the synthesis of {5-[(2,4-, 3,4-dimethoxy phenyl)-3*H*-1,2,4-triazole-3-yl]thio} (acetic, propanoic, benzoic) acids were developed, for which the interaction reactions were studied with organic and inorganic bases. At the same time, the studied physical-chemical properties of the obtained substances and their structure were confirmed, the indicators of their toxicity were predicted, and the hypoglycemic activity of salts {5-[(2,4, 3,4-dimethoxy phenyl)-3*H*-1,2,4-triazole-3-yl]thio}(acetic, propanoic, benzoic) acids, among which the leader compound. Zinc (II) 2-{5-[(3,4-dimethoxy phenyl)-3*H*-1,2,4-triazole--3-yl]thio}acetate showed greater efficiency in terms of the ability to lower blood glucose levels, namely, by 27.3% (approximately 1.3 times) in comparison with the group of laboratory animals that received the reference drug metformin. The study of the potential pharmacological activities of the synthesized compounds in this series is ongoing.

Conflict of interest: none.

All manipulations were carried out following the accepted bioethical standards, observing the relevant rules of the ICH (International Conference on Harmonization), The Law of Ukraine "On the Protection of Animals from Cruel Treatment" (No. 2447-IV of 04.08.2017), the rules of the European Convention

Table 3. Results of the hypoglycemic activity of Zinc (II) 2-{5-[(3,4-dimethoxy phenyl)-3H-1,2,4-triazole-3-yl]thio} acetate (3.18)

Groups of animals (n = 10)	Glucose level before compound administration	Glucose level 60 min. after the administration of the compound	Glucose level 180 min. after the administration of the compound
Animals that were injected with 40% aqueous glucose solution and water	4.7 ± 0.150 mmol/L	6.9 ± 0.250 mmol/L	6.7 ± 0.050 mmol/L
Animals that were injected with 40% aqueous glucose solution and metformin	4.5 ± 0.250 mmol/L	4.1 ± 0.150 */** mmol/L	3.6 ± 0.050 */** mmol/L
Animals that were injected with a 40% aqueous solution of glucose with propane substance	4.55 ± 0.150 mmol/L	2.95 ± 0.150 */** mmol/L	2.4 ± 0.200 */** mmol/L

where, n is the number of animals in each study group;

* reliability results in relation to the control (40% aqueous glucose solution) ($P < 0.05$) according to Student's t-test

** a non-parametric statistical test that is used to estimate the difference between two samples – Whitney Mann U test

for the Protection of Vertebrate Animals used in experimental research and for different purposes dated March 18, 1986, and according to the statement of a committee approving animal experiments conclusion of ZSMU No. 1 dated January 12, 2022.

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