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Research Article

The investigation of antimicrobial activity of some s-substituted bis-1,2,4-triazole-3-thiones

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Abstract

New S-substituted 4-alkyl-5-((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol derivatives have been designed, synthesized and studied their antimicrobial activity on 11 standard Gram-positive and Gram-negative microorganism strains. Their spectral and physicochemical parameters were established using modern comprehensive methods of analysis, including ^{1}H NMR spectroscopy, GC-MS and elemental analysis.It has been found that compound 2a exhibits strong suppression of 5 test strains (MBC = 15.6 μg/mL). Compound 4a showed moderate inhibition of *Salmonella pullorum*, *Escherichia coli* O_2, *Salmonella enteritidis* strains (MBC = 31.25 μg/mL) Compound 6a was sensitive toward ten tested bacteria at 31.25 μg/mL concentration.

Keywords

1,2,4-triazole, organic synthesis, antimicrobial activity

Introduction

The currently rising numbers of drug-resistant bacterial pathogen strains disrupts the effectiveness of the existing treatments and amplifies the incidence of new bacterial infections. This circumstance urges researchers around the world to search for new effective and at the same time low-toxic medicines distinguished by innovative mechanisms of action, which is of the most significance for modern pharmaceutical science.

Organic compounds containing nitrogen atoms represent a large group of chemical structures, of both natural and synthetic origin, which may possess a broad range of biological activities. In this respect, 1,2,4-triazole system, a five-membered nitrogen-containing heterocycle, has arisen strong scientific interest (Shcherbyna et al. 2016; Jethwa 2016). Therefore, compounds that contain a 1,2,4-triazole cycle in their structure are of the most promising subjects of pharmacological research.

The derivatives of 1,2,4-triazole are widely used in pharmaceutical and medicinal chemistry as well as in organic synthesis (Gotsulya et al. 2015; Alrawashdeh 2018). For instance, such drugs as fluconazole, bevacizumab (Avestim), trifuzol-neo, itraconazole, thiotriazoline and many other substances containing 1,2,4-triazole ring have found applications in clinical and veterinary practices.

In our opinion, the investigation of the structures constructed using two bridged 1,2,4-triazole cycles belonging to various chemical classes may lead to the discovery of quite promising compounds that exhibit versatile

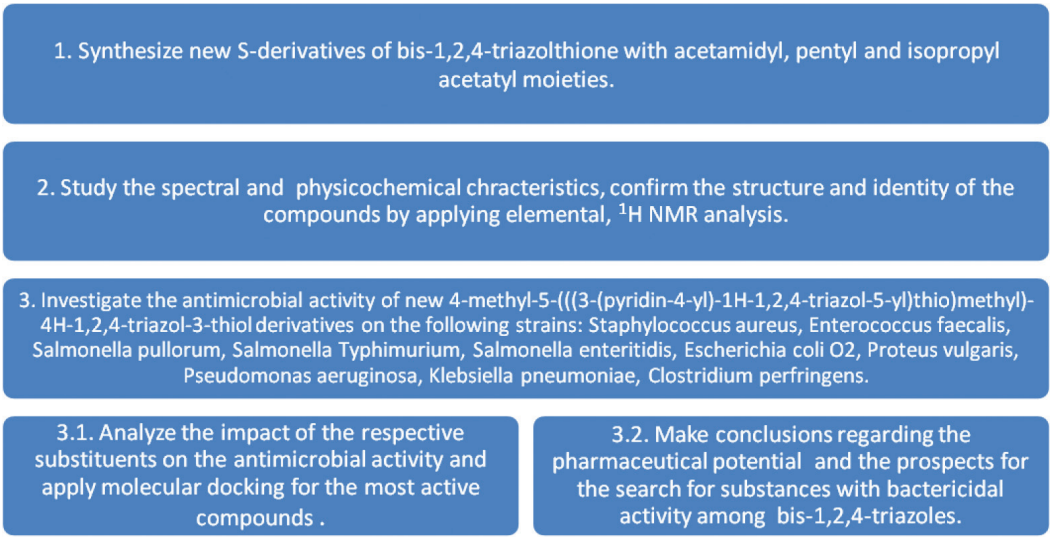


Figure 1. Algorithm of the research.

antibacterial effects. The increase in the number of the system's reaction sites enables sufficient variations of chemical modification of bis-1,2,4-triazole, which multiples the probability of finding active derivatives. During the recent years, there have been numerous publications on the syntheses of 1,2,4-triazole derivatives containing various pharmacophore frameworks that exhibit antimicrobial activity (Düğdü et al. 2014; Saadeh et al. 2010).

Purpose

The purpose of this work was to synthesize new compounds based on 4-alkyl-5-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5-yl)thio)methyl)-4*H*-1,2,4-triazole-3-thiol, in which isopropyl 2-chloroacetate, 1-bromopentane and 2-chloroacetamid were used as alkylation agents, study the ammonolysis of the previously obtained isopropyl ester with methylamine, ethylamine, dimethylamine, diethylamine, morpholine and 2-methylpiperidine, study their physicochemical properties and confirm the structure of these derivatives using modern multidimensional methods of identification. Along with that, we attempted to investigate the antimicrobial activities of the compounds on standard Gram-positive and Gram-negative microorganism strains to evaluate the pharmacological potential of the substances. The ultimate purpose was to conduct the comparative analysis of the relationships between the antimicrobial activity and functional groups of these molecules.

Planning (methodology) of research

To achieve this aim and find the answers to the queries, the experiment was designed in the way illustrated in Fig. 1.

Materials and methods

Generalities

Melting points were determined on OptiMelt MPA100 apparatus (USA) equipped with platinum RTD sensor and temperature measurement possibility of up to 400 °C and 0.1 °C resolution. Elemental analysis was performed on a Elementar Vario L cube multipurpose elemental analyzer (CHNS) produced by Analysen systeme GmbH (Germany) using sulfanilamide as the standard. The mass-to-charge ratio of *S*-derivatives of bis-1,2,4-triazole was been determined by gas chromatography via Agilent 7890B GC system equipped with Agilent 5977B mass spectrometry detector (USA). The column used for separation was DB-5ms. Type of ionization: electron impact (EI) with electron energy of 70 eV. ^{1}H NMR spectra were recorded at 400 MHz and 100 MHz using Varian MR-400 spectrometer with DMSO-d_6 as the solvent. Spectra were processed via ADVASP Analyzer software (Umatek International Inc.). Chemical shifts are reported in ppm (δ scale) down field with residual protons of the solvent (DMSO-d6) present at δ=2.49 ppm and using a common internal standard. Molecular docking was performed using Autodock 4.2.6. The screening was performed on the crystallographic structure of the enzyme "beta-lactamase cTEM-19m" (4R4S) (Gobeil et al. 2019). We applied automatic docking: the 4R4S structure was utilized in the subsequent docking experiments with other parameters established by default in the software.

Serial dilutions method was used to assess the sensitivity of the isolated microorganism strains to the experimental samples. The activity was studied according to the methodological recommendations (Volyansky et al. 2004), using standard diffusion method in Mueller-Hinton agar in media optimized for growing the test cultures at the concentration of 10^6 cells/mL. The compounds of interest were dissolved in dimethyl sulfoxide (1 mg/mL) prior to the experiment. A minimum inhibitory concentration (MIC) was assessed by observing the growth in a test tube, where the absence of growth represented the minimum concentration of the studied substance required to suppress the culture. In addition, control tests of the growth media and solvent were conducted by following common procedures.

The subject of the research was represented by isopropyl 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)

thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetate (**2a**), isopropyl 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetate (**2b**), 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetamide (**3a**), 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetamide (**3b**), (Fig. 2). A previously synthesized 4-(5-(((4-methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (**4a**) was also chosen as a potential antimicrobial compound, since in previous studies it showed good activity against some strains.

Amines were also synthesized from compounds 2a 2b by amonolysis with the appropriate reagents (methylamine, ethylamine, dimethylamine, diethylamine, morpholine and 2-methylpiperidine). N-methyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**5a**), 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-methylacetamide (**5b**), N,N-dimethyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**6a**), 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-N,N-dimethylacetamide (**6b**), N-ethyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**7a**), N-ethyl-2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**7b**), N,N-diethyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**8a**), N,N-diethyl-2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**8b**), 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-1-(2-methylpiperidin-1-yl)ethan-1-one (**9b**), 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-1-morpholinoethan-1-one (**10a**) (Fig. 3).

The structures were synthesized at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry of Zaporizhzhia State Medical University, according to a commonly known procedure; the compounds were further used in the investigation of their antimicrobial activity.

The synthesis of 4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol (**1a,b**) was performed using common and known procedures (Safonov 2020); these compounds appear as yellow crystals, which are freely soluble in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) and are insoluble in water.

Isopropyl 2-chloroacetate, 2-chloroacetamid and 1-bromopentane were used as alkylation agents (Fig. 2.). The reaction was held according to a common method described in the previous works (Shcherbyna 2019). A mixture of sodium hydroxide (0.01 mol, 0.40 g) and 4-alkyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol (0.01 mol, 3.05 g / 3.19 g) in methanol was heated until the dissolution of the solid material, after which 2-chloroacetamid (0.01 mol, 0.93 g) / isopropyl 2-chloroacetate (0.01 mol, 1.36 g) / 1-bromopentane (0.01 mol, 1.51 g) was added. After cooling, the residue was filtered, dried and recrystallized from water-methanol (1:1). Next, the obtained isopropyl ester (**2a**, **2b**) (0.01 mol) was transferred into a beaker, to which methanol (50 mL) and primary or secondary amines (0.01 mol) were added. The mixture was stirred for 8 h, during which the temperature was monitored. Further the residue was filtered, dried and recrystallized from water-methanol (1:2).

Based on the previous research, it was approved empirically that the introduction of functional groups at Sulfur atom of bis-1,2,4-triazole-3-thione leads to the increase of solubility in more polar substances due to the charge redistribution in the molecule and formation of intermolecular hydrogen bonds. The derivatives containing one 1,2,4-triazole cycle are practically insoluble in water and poorly soluble in highly polar solvents. However, a number of bis-1,2,4-triazoles are soluble in water, acetic acid, DMF and alcohols. This system is of great interest with regard to its biological activity since its reactivity is increased meaning a broader range of possible chemical modifications in various directions.

The structure of the synthesized bis-derivatives of 1,2,4-triazole was confirmed by a set of physical and instrumental analysis methods such as elemental analysis, ^{1}H NMR spectrometry. The relative time and mass to charge ratio of the compounds was studied using GC-MS.

Isopropyl 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetate (**2a**). Yellow powder in 81% yield, m.p. 151–153 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ: 1.66 (br. s., 7 H), 2.00–2.07 (m, 3 H), 3.19 (br. s., 2 H), 3.65 (s, 2 H), 4.36 (s, 1 H), 7.77 (d, J=4.88 Hz, 2 H), 8.40 (d, J=5.19 Hz, 1 H). MS (m/z): 404 (M+). Anal. calcd for $C_{16}H_{19}N_7O_2S_2$, % C 47.38; H 4.74; N 26.11, S 15.90. Found, % C, 47.39; H, 4.72; N, 24.18, S 15.82.

Isopropyl 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetate (**2b**). Yellow powder in 68% yield, m.p. 118–120 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ: 1.52 (br. s., 7 H), 1.89–1.95 (m, 3 H), 3.19–3.25 (br. s., 4 H), 3.85 (s, 2 H), 4.36 (s, 1 H), 7.77 (d, J=4.88 Hz, 2 H), 8.40 (d, J=5.19 Hz, 2 H). MS (m/z): 420 (M+). Anal. calcd for $C_{17}H_{21}N_7O_2S_2$, % C 48.65; H 5.04; N 23.44, S 15.20. Found, % C 48.67; H 5.05; N 23.37, S 15.29.

2-((4-Methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetamide (**3a**). Yellow powder in 76% yield, m.p. 118–120 °C. 1H NMR (400 MHz, DMSO-d6) δ: 3.70–3.79 (s, 3 H); 4.36–4.39 (m, 2 H); 4.77 (s, 2 H); 7.77–7.82 (d, J=5.80 Hz, 2 H); 8.38–8.43 (m, 1 H). (M+). Anal. calcd for $C_{13}H_{14}N_8OS_2$, % C 43.18; H 3.84; N 30.91, S 17.78. Found, % C 43.08; H 3.89; N 30.92, S 17.69%.

2-((4-Ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio)acetamide (**3b**). Yellow powder in 75% yield, m.p. 118–120 °C.

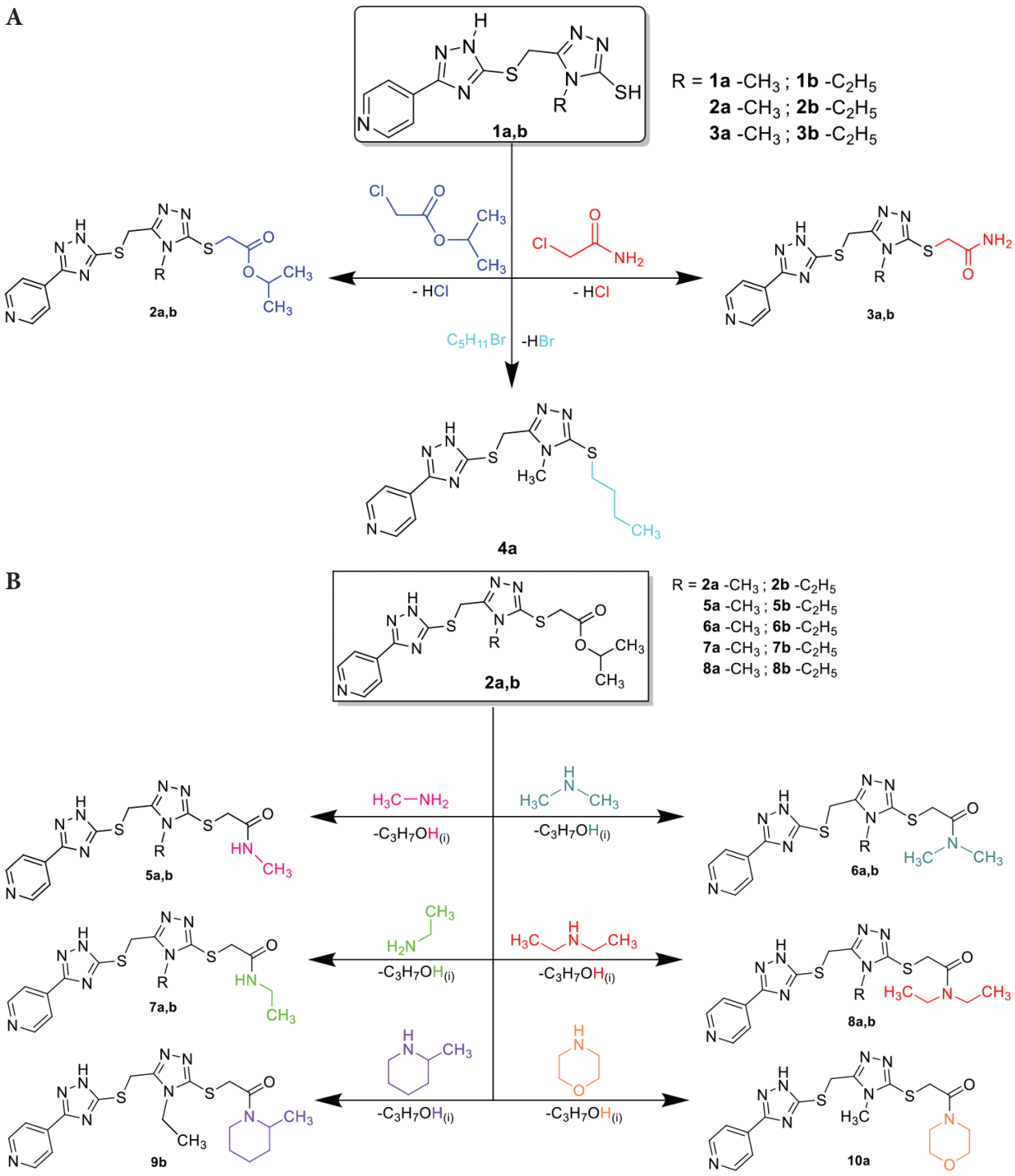


Figure 2. (**A**) Scheme of synthesizing the S-derivatives of bis-1,2,4-triazoles; (**B**) Scheme of synthesizing the S-amides of bis-1,2,4-triazoles.

^{1}H-NMR (400 MHz, DMSO-d6) δ: 1.63–1.69 (s, 3 H), 3.49–3.58 (br. s., 2 H), 4.47–4.52 (s, 2 H), 4.77–4.85 (s, 2 H), 7.73–7.83 (m, 2 H), 8.41–8.49 (t, J=4.43 Hz, 1 H). MS (m/z): 376 (M+). Anal. calcd for $C_{14}H_{16}N_8OS_2$, % C 44.78; H 4.60; N 29.71, S 17.01%. Found, % C 44.67; H 4.28; N 29.77, S 17.04.

4-(5-(((4-Methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl) methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (**4a**). Yellow powder in 85% yield, m.p. 118–120 °C. ^{1}H-NMR (400 MHz, DMSO-d6) δ: 0.67–0.85 (m, 3H), 1.10–1.32 (m, 4H), 1.79–1.95 (m, 2H), 2.63 (br.s., 1 H), 3.82 (br.s., 3H), 4.07 (s, 2H), 4.49 (t, J=7.03, 2H), 7.83 (d, J=4.52, 2H); 8.65 (d, J=4.50 , 2H). MS (m/z): 375 (M+). Anal. calcd for $C_{16}H_{21}N_7S_2$, C, 51.18; H, 5.64; N, 26.11, S 17.08%. Found: C, 51.30; H, 5.60; N, 26.06, S 17.01%.

N-methyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**5a**). Yellow powder in 85% yield, m.p. 139–141 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.29 (q, *J*=6.10 Hz, 3 H), 3.54–3.61 (m, 3 H), 4.05–4.11 (m, 2 H), 4.34 (s, 2 H),

7.40 (s, 1 H), 8.14 (d, *J*=5.87 Hz, 2 H), 8.79 (d, *J*=5.62 Hz, 2 H). MS (m/z): 376 (M+). Anal. calcd for $C_{14}H_{16}N_8OS_2$, C, 44.67; H, 4.28; N, 29.77; S, 17.04%. Found: C, 44.58; H, 4.32; N, 29.86, S 16.96%.

2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl) thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-methylacetamide **(5b).** Orange powder in 78% yield, m.p. 145–147 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.13 (t, *J*=6.10 Hz, 4 H), 3.88 (s, 2 H), 3.98 (q, *J*=6.20 Hz, 3 H), 4.28 (s, 2 H), 4.87 (q, *J*=4.80 Hz, 1 H), 7.84 (s, 3 H), 8.59–8.72 (m, 3 H). MS (m/z): 390 (M+). Anal. calcd for $C_{15}H_{18}N_8OS_2$, C, 46.14; H, 4.65; N, 28.70; S, 16.42%. Found: C, 46.20; H, 4.60; N, 28.76, S 16.38%.

N,N-dimethyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio) acetamide **(6a).** Yellow powder in 81% yield, m.p. 116–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.82 (s, 6 H), 3.57 (s, 3 H), 4.05 (s, 2 H), 4.60 (s, 2 H), 7.82–7.88 (m, 3 H), 8.64–8.70 (m, 3 H). MS (m/z): 390 (M+). Anal. calcd for $C_{15}H_{18}N_8OS_2$, C, 46.14; H, 4.65; N, 28.70; S, 16.42%. Found: C, 46.24; H, 4.69; N, 28.66, S 16.31%.

2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl) thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-N,N-dimethylacetamide (**6b**). Orange powder in 77% yield, m.p. 142–144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.81 (t, *J*=6.10 Hz, 3 H), 3.04 (s, 5 H), 3.88–4.09 (m, 5 H), 4.82 (s, 2 H), 7.76–7.93 (m, 2 H), 8.56–8.70 (m, 2 H). MS (m/z): 404 (M+). Anal. calcd for $C_{16}H_{20}N_8OS_2$, C, 47.51; H, 4.98; N, 27.70; S, 15.85%. Found: C, 47.40; H, 4.99; N, 26.84, S 15.80%.

N-ethyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**7a**). Yellow powder in 70% yield, m.p. 154–157 °C. ^{1}H-NMR (400 MHz, DMSO-d6) δ ppm 1.19 (t, *J*=6.5 Hz, 3H), 3.27 (qd, *J*=6.4, 3.8 Hz, 2H), 3.57 (s, 2H), 3.83–3.87 (s, 2H), 4.57–4.63 (s, 2H), 7.40 (t, *J*=3.8 Hz, 1H), 7.89–7.94 (m, 2 H), 8.52–8.63 (m, 2 H).. MS (m/z): 390 (M+). Anal. calcd for $C_{15}H_{18}N_8OS_2$, C, 46.14; H, 4.65; N, 28.70; S, 16.42%. Found: C, 46.02; H, 4.69; N, 28.77, S 14.53%.

N-ethyl-2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**7b**). Orange powder in 65% yield, m.p. 161–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (br. t, *J*=6.30, 6.30 Hz, 3 H), 1.12 (t, *J*=6.20 Hz, 3 H), 3.96 (qd, *J*=6.20, 5.40 Hz, 2 H), 4.10 (s, 2 H), 4.34 (q, *J*=6.00 Hz, 2 H), 5.13 (s, 2 H), 7.53 (t, *J*=4.20 Hz, 1 H), 8.14–8.34 (m, 2 H), 8.77–8.90 (m, 2 H). MS (m/z): 404 (M+). Anal. calcd for $C_{16}H_{20}N_8OS_2$, C, 47.51; H, 4.98; N, 27.70; S, 15.85%. Found: C, 47.56; H, 4.92; N, 27.76, S 15.81%.

N,N-diethyl-2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio) acetamide (**8a**). Yellow powder in 72% yield, m.p. 163–165 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (t, *J*=7.21 Hz, 7 H), 2.83 (q, *J*=7.09 Hz, 4 H), 3.58 (s, 2 H), 4.24 (s, 2 H), 4.60 (s, 2 H), 7.84–7.91 (m, 2 H), 8.61–8.72 (m, 2 H). MS (m/z): 418 (M+). Anal. calcd for $C_{17}H_{22}N_8OS_2$, C, 48.79; H, 5.30; N, 26.77; S, 15.32%. Found: C, 48.85; H, 5.40; N, 26.68, S 15.24%.

N,N-diethyl-2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**8b**). Orange powder in 80% yield, m.p. 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.14 (t, *J*=7.34 Hz, 6 H), 1.81 (t, *J*=6.20 Hz, 1 H), 2.83 (q, *J*=7.17 Hz, 4 H), 3.94–4.02 (m, 4 H), 4.78 (s, 4 H), 7.79–7.90 (m, 2 H), 8.59–8.69 (m, 2 H). MS (m/z): 432 (M+). Anal. calcd for $C_{18}H_{24}N_8OS_2$, C, 49.98; H, 5.59; N, 25.91; S, 14.82%. Found: C, 49.87; H, 5.61; N, 26.02, S 14.84%.

2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl) thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-1-(2-methylpiperidin-1-yl)ethan-1-one (**9b**). Orange powder in 66% yield, m.p. 104–106 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.02 (dd, *J*=5.10, 4.20 Hz, 4 H), 3.56 (s, 3 H), 3.77 (dd, *J*=5.30, 4.40 Hz, 4 H), 4.29 (s, 2 H), 4.59–4.69 (m, 2 H), 7.78–7.89 (m, 2 H), 8.58–8.68 (m, 2 H). MS (m/z): 458 (M+). Anal. calcd for $C_{20}H_{26}N_8OS_2$, C, 52.38; H, 5.71; N, 24.43; S, 13.98%. Found: C, 52.30; H, 5.82; N, 24.40, S 14.00%.

2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-1-morpholinoethan-1-one (**10a**). Yellow powder in 69% yield, m.p. 151–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.16 (d, *J*=6.36 Hz, 3 H), 1.39 (t, *J*=6.10 Hz, 3 H), 1.51–1.73 (m, 5 H), 1.80 (m, *J*=5.40 Hz, 1 H), 3.11–3.15 (m, 1 H), 3.76–3.83 (m, 1 H), 3.88 (br. dtd, *J*=6.60, 6.40, 6.40, 5.80 Hz, 1 H), 3.98 (d, *J*=15.60 Hz, 1 H), 4.22–4.36 (m, 3 H), 4.57–4.67 (m, 2 H), 7.75–7.91 (m, 2 H), 8.55–8.68 (m, 2 H). MS (m/z): 432 (M+). Anal. calcd for $C_{17}H_{20}N_8O_2S_2$, C, 47.21; H, 4.66; N, 25.91; S, 14.82%. Found: C, 47.24; H, 4.61; N, 26 .06, S 17.01%.

Results

The results of antimicrobial and antifungal activity of the alkylated bis-derivatives of 1,2,4-triazole with regard to the test cultures are presented in Table 1. A solution without the addition of the compounds was used as a control substance in the trials of antimicrobial activity.

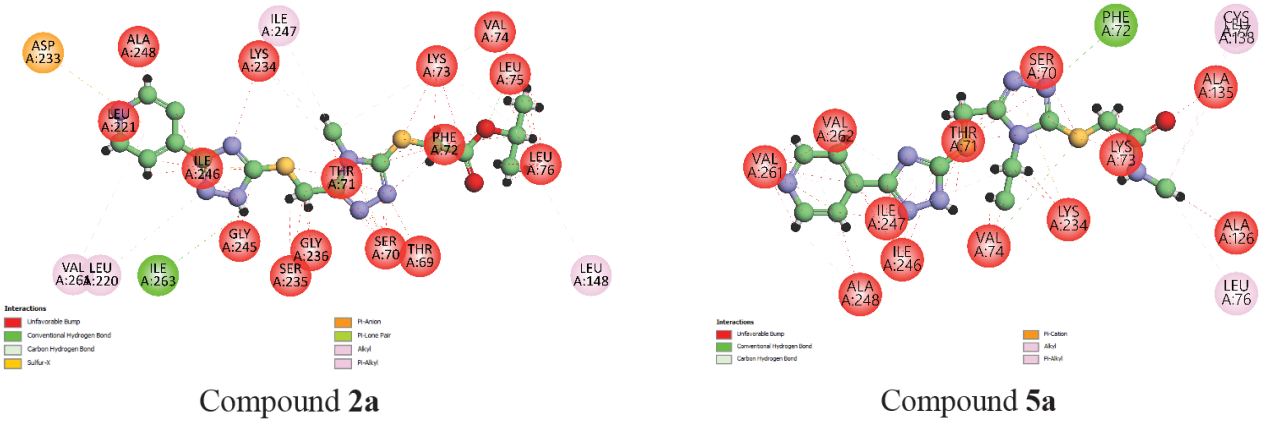
According to the results of the preliminary microbiological screening, only five of the 4-alkyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol derivatives (**2a**, **4a, 5b, 6a, 10a**) demonstrate the antimicrobial effect. Unfortunately, other compounds did not exhibit any growth inhibition of the studied strains even at the concentration of 250 μg/cm^3.

Molecular docking

For molecular docking, the cTEM-19m beta-lactamase enzyme (PDB ID: 4R4S, 1.1 Å) was chosen, which is synthesized by the bacteria *E. coli* O_2 and *K. pneumoniae*, due to which the strains acquire resistance to many antibiotics. Compounds **2a** and **5b** were selected based on analysis of data of antimicrobial activity as potential inhibitors of beta-lactamase. The interaction of the ligand with the active center of the enzyme is complex and is provided mainly

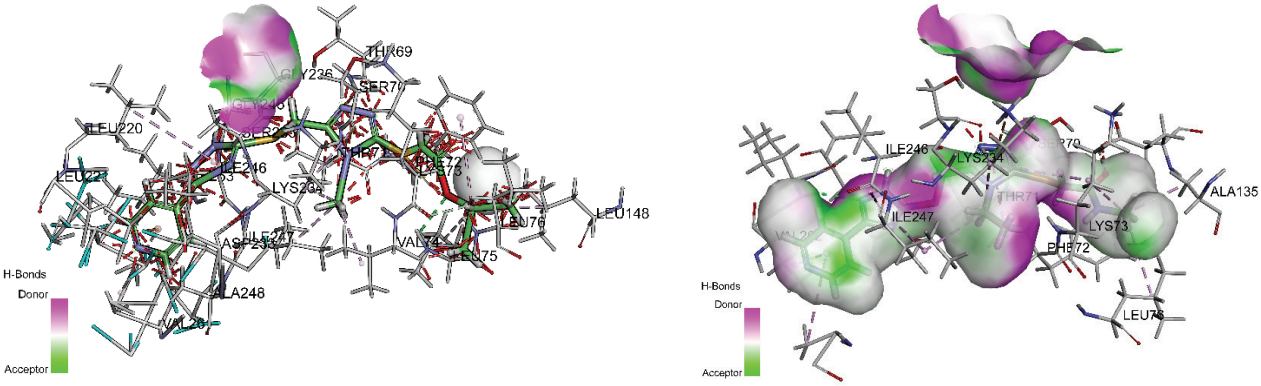
Table 1. The results of antimicrobial activity screening for the compounds.

Microorganism culture	Minimum bactericidal concentration (MBC), μg/cm³															
	control	2a	2b	3a	3b	4a	5a	5b	6a	6b	7a	7b	8a	8b	9b	10a
S. aureus	>250	31.25	>250	>250	>250	125	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	125
S. fecalis	>250	15.6	>250	>250	>250	125	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	125
S. pullorum	>250	15.6	>250	>250	>250	62.5	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	62.5
S. typhimurium	>250	15.6	>250	>250	>250	125	>250	31.25	62.5	>250	>250	>250	>250	>250	>250	125
S. enteritidis	>250	31.25	>250	>250	>250	62.5	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	62.5
E. coli O_2	>250	31.25	>250	>250	>250	62.5	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	62.5
P. vulgaris	>250	31.25	>250	>250	>250	125	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	62.5
P. aeruginosa	>250	31.25	>250	>250	>250	62.5	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	62.5
K. pneumoniae	>250	15.6	>250	>250	>250	31.25	>250	31.25	31.25	>250	>250	>250	>250	>250	>250	62.5
C. perfringens	>250	31.25	>250	>250	>250	125	>250	31.25	62.5	>250	>250	>250	>250	>250	>250	125



Compound **2a** Compound **5a**

Figure 3. Interaction network between the enzyme 4R4S and selected compounds.



Compound **2a** Compound **5a**

Figure 4. Visualization of 3D structure of the "ligand-enzyme" complex.

by alkyl bonds, ionic and conventional hydrogen bonds with water molecules and amino acid residues of the enzyme. An important point for binding to the enzyme is the presence of a sulfur atom in the molecules of the test compounds (Fig. 3).

Visualization of the 3D structure of the "ligand-enzyme" complex is shown in (Fig. 4.)

In Fig. 4 shows the surface drawn around the active center, indicating the region of the donor H bond, and the acceptor region of the hydrogen bond. As can be seen in Figure 4, the main structural element of the inhibitor that is complementary to the ligand is the 1,2,4-triazole ring and the side ether and amide radical.

Discussion

Taking into account the previous research, it is safe to say that a great number of 1,2,4-triazole derivatives show considerable capabilities for suppressing the growth of pathogenic bacteria (Onkol et al. 2008). In 2020, the antimicrobial activity of morpholine 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazole-3-yl)thio)acetate has been assessed using the experimental pancreatitis model on rats (Bigdan et al. 2016). In its turn, a research team from India has studied the antimicrobial properties of new asymmetric bis-1,2,4-triazoles, demonstrating the moderate bacteriostatic activity of the synthesized

compounds against the strains of *B. subtilis*, *S. aureus*, *E. coli* та *P. aeruginosa* (Singh et al. 2013). Feng Gao et al. have shown (Gao et al. 2019) that hybridization of 1,2,4-triazole moiety with other antibacterial pharmacophore groups may yield even more effective substances in this regard. A study conducted in China describes the latest findings in targeted hybridization of 1,2,4-triazole with quinoline/quinone, which holds promise for treating infections caused by drug-resistant bacterial strains (Zhang et al. 2019). Finally, Zahid H. Chohan and Muhammad Hanif from Bahauddin Zakariya University have researched the antibacterial and antifungal activities of 1,2,4-triazole derivatives and illustrated that bivalent metal complexes can present as more potent antibacterial and antifungal drugs than the precursor Schiff bases (Chohan and Hanif 2013).

The investigation of the antimicrobial activity of the obtained compounds showed that at the concentration of 10 mg/mL five S-derivatives of bis-1,2,4-triazole **(2a, 4a, 5b, 6a, 10a)** exhibit bactericidal activity against all bacterial strains used in this study, and specifically *Staphylococcus* aureus, *Enterococcus faecalis*, *Salmonella pullorum*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Escherichia coli* O_2, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Clostridium perfringens*.

Based on the obtained data, it is correct to submit that isopropyl 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio) acetate (**2a**) exhibits strong antimicrobial activity against the strains of *Enterococcus faecalis*, *Salmonella pullorum*, *Salmonella typhimurium* (MBC = 15.6 μg/mL). The change of ethyl substituent into methyl at the 4-position of the second 1,2,4-triazole ring in isopropyl ether of bis-1,2,4-triazole acetic acid leads to the appearance of strong bactericidal activity against all of the studied strains.

It was established that isopropyl 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl)thio) acetate (**2a**) exhibits strong suppression of *Klebsiella pneumoniae* test strain (MBC = 31.25 μg/mL). Compound 4-(5-(((4-methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl) pyridine **(4a)** showed moderate inhibition of *Salmonella pullorum*, *Escherichia coli* O_2, *Salmonella enteritidis* strains (MBC = 31.25 μg/mL). Novel 2-((4-ethyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)thio)methyl)-4H-1,2,4-triazol-3-yl)thio)-N-methylacetamide (**5b**) slightly active at 31.25 μg/mL concentrations toward all strains. Compounds **6a** and **10a** were sensitive toward ten tested bacteria at 31.25 μg/mL to 125 μg/mL concentrations. On the other hand, the remaining amides (**6a, 6b, 7a, 7b, 8a, 8b, 9b**) did not show activity at 250 μg/mL concentrations.

Study limitations

The main disadvantages that may be pointed out from the serial dilution include the time restriction for conducting the research and expansion of the error during the consecutive dilutions. In the first case, time limitation is specific to the serial dilution method, due to which the prepared media must be used immediately, without the possibility of storage. As for the second case, the most considerable error is assigned to the most concentrated solution. In order to compensate for the error, longer mixing times are required, which makes the serial dilution more time-consuming.

Prospects for further research

The results of the preliminary research of antimicrobial activity of the synthesized S-substituted bis-1,2,4-triazoles demonstrate the promise of further studies of their biological properties, while also the targeted synthesis of new 1,2,4-triazoles possessing bactericidal activity represents great scientific interest.

Conclusions

1. New S-substituted 4-alkyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiols containing isopropyl acetate, pentane and acetamide moieties were synthesized. Their spectral and physicochemical parameters were established using modern comprehensive methods of analysis, including ^{1}H NMR spectroscopy, GC-MS and elemental analysis.
2. Bis-1,2,4-triazole derivatives (**2a**, **4a**) have shown biostatic activity with regard to eleven microorganism strains. Minimum bactericidal concentration for isopropyl 2-((4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-yl) thio)acetate (**2a**) constituted 15.6 μg/cm^3 in relation to *Enterococcus faecalis*, *Salmonella pullorum*, *Salmonella typhimurium*, *S. Enteritidis*, *Klebsiella pneumoniae* strains. In its turn, compound 4-(5-(((4-methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (**4a**) inhibited the growth of *Klebsiella pneumoniae* at the concentration of 31.25 μg/cm^3. 1,2,4-Triazole amides derivatives compounds (**5b, 6a, 10a**) which have been obtained from isopropyl ester (2a, 2b) showed a good antimicrobial effect against all selected strains of microorganisms within the limits of at 31.25 μg/mL to 125 μg/mL.
3. Structure-activity relationship (SAR) has been determined for the synthesized compounds. The conducted research and molecular docking underlines the reasonability of further searches for biologically active S-derivatives of bis-1,2,4-triazole.

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