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Synthesis and antimicrobial evaluation of novel 1,2,4-triazole derivatives

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Derivatives of 1,2,4-triazole are widely recognized as promising scaffolds for the development of biologically active compounds, particularly those exhibiting antimicrobial properties. In the present study, the antimicrobial potential of a series of newly synthesized 1,2,4-triazole-based heterocyclic compounds was investigated using the *in vitro* disk diffusion method against 15 clinically significant bacterial strains, including both Gram-positive and Gram-negative species. The compounds were dissolved in 70% ethanol and tested at different concentrations to assess their spectrum and degree of antibacterial activity. One compound, ethyl 2-((3-mercapto-9-methylpyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazin-6-yl)thio)acetate, exhibited the most pronounced inhibitory effect, producing zones of bacterial growth inhibition exceeding 8 mm in diameter in 14 out of the 15 tested bacterial strains. Several other derivatives, particularly those bearing N-ethyl or N-phenyl substitutions within triazolothiadiazole frameworks, as well as dichlorophenyl-substituted triazolothiadiazines, exhibited moderate antibacterial activity, inhibiting the growth of five to seven bacterial strains depending on their structural features. The structure–activity relationship analysis suggests that the presence of electron-withdrawing groups, fused heterocyclic systems and sulfur-containing linkages may contribute to enhanced biological activity. Overall, the results underscore the importance of the 1,2,4-triazole core in the design of novel antimicrobial agents and provide a solid foundation for further structural optimization and pharmaceutical development of these compounds.

Keywords: properties; growth inhibition zone; bacterial colonies; multidrug-resistant strain.

Introduction

The significant level of interest in research related to the discovery of new useful properties of 1,2,4-triazole derivatives is due to their significant potential for the creation of biologically active substances. Every year, the use of 1,2,4-triazole derivatives is increasing both in medical practice (antifungal, antiviral, antitumour, anxiolytic and sedative drugs) and in agriculture as systemic fungicides and plant growth regulators. There is evidence in the literature that 1,2,4-triazole derivatives, along with antifungal properties, can also exhibit antibacterial activity and can be used both for humans and plants (Khomenko, 2024). Accordingly, the search for new compounds of this series is a promising direction. In most cases, 1,2,4-triazole derivatives are characterized by good solubility and high activity at low concentrations. A characteristic feature of the chemistry of substituted 1,2,4-triazoles is the wide variety of methods for their preparation, structures, and, accordingly, properties, including biological activity (Strzelecka & Świątek, 2021). In this regard, the development of convenient and efficient methods for the synthesis of both known 1,2,4-triazoles and their new derivatives and the establishment of the prospects for their use as antimicrobial agents is an urgent task. The uniqueness of these compounds is associated with the presence of several reaction centers in their molecules. This allows them to be involved in numerous transformations leading to a wide range of multifunctional products of the aliphatic and heterocyclic series (Banerjee et al., 2013). Derivatives of 1,2,4-triazole have become the subject of study by many research groups due to their wide range of applications. As a result, the number of publications on their synthesis and chemical transformations has increased significantly in recent years The main directions for the preparation of 1,2,4-triazole derivatives are synthesis from a number of acyclic precursors or functionalization of 1,2,4-triazole itself and further transformation of the resulting substances (Joshi et al., 2018; Kaproń et al., 2019; Shaykoon et al., 2020). Heterocyclic compounds and their derivatives are by far the most promising class of compounds for the development of new drugs due to their diverse electronic properties, geometric characteristics and wide range of biological activity (Qi et al., 2021; Youssef et al., 2022; Guan et al., 2024). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. The present work is a continuation of the research on the antibacterial effect of 1,2,4-triazole derivatives in connection with the spread of multidrug-resistant bacterial strains that are difficult to treat.

The aim of this article was to determine the antibacterial effect of new 1,2,4-triazole-containing compounds on 15 species of microorganisms. These molecules remain poorly understood in terms of antimicrobial activity and may have significant potential in modern medicine and veterinary medicine.

Materials and methods

The first stage of in silico studies involved the use of molecular docking, which allows a rational approach to the selection of compounds that are promising in terms of antimicrobial activity potential. Molecular docking included the preparation of ligand, enzyme and the docking process itself. In turn, the preparation of the ligand involved the use of MarvinSketch 6.3.0 to build structural formulas, HyperChem 8 to generate three-dimensional structures of substances, and AutoDock Tools-1.5.6 to convert structures to PDBQT file format. The process of enzyme preparation involved Discovery Studio 4.0 to remove water molecules and ligands from the model enzyme structure and AutoDock Tools-1.5.6, if necessary. Docking was performed in the Vina software package with further visualization of the results using Discovery Studio 4.0.

Peptide deformylase, which plays an important role in the process of protein synthesis in bacteria, was chosen as a model enzyme for docking studies. This enzyme belongs to the class of metalloenzymes and catalyses the removal of a formyl group from the N-terminus of newly formed peptides containing formylmethionine as the first aminoacid residue. This process is critical for the normal functioning of proteins. Inhibitors of peptide deformylase can block its activity, which leads to impaired protein synthesis and bacterial death. Since this enzyme is absent in humans, specific peptide deformylase inhibitors can be effective antibacterial agents with minimal impact on human cells. The analysis of the data allowed us to identify compounds that are promising in terms of antimicrobial activity (Fig. 1).

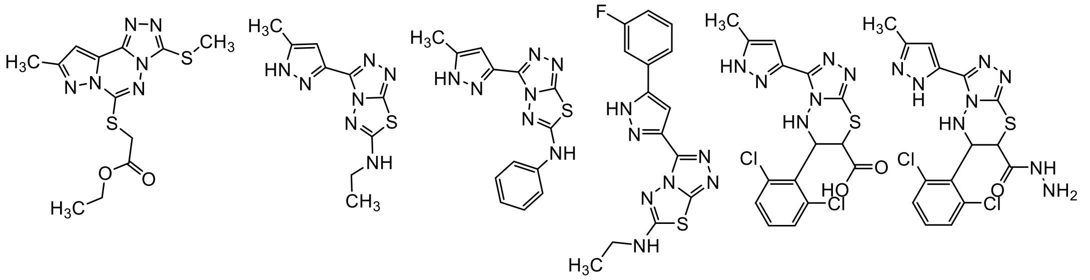
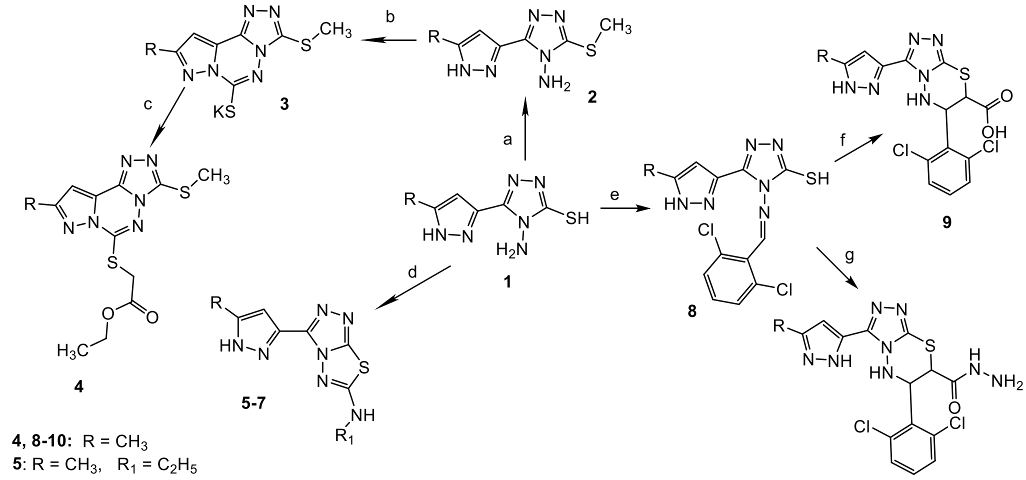


Fig. 1. Compounds selected for research based on the results of molecular docking

The synthesis of the selected compounds for further studies of antimicrobial properties was performed using well-known methods of organic chemistry (Fig. 2). The melting points of the studied substances were determined by the open capillary method using the OptiMelt Stanford Research Systems MPA100 device. Elemental analysis was performed using an Elementar Vario L cube analyser (sulfonamide as standard). 1H NMR spectra were obtained using a Varian VXR-400 spectrometer in DMSO-d6 solution (internal standard: tetramethylsilane). The purity of the synthesised reaction products was analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1260 Infinity gas-liquid chromatograph with an Agilent 6120 integrated mass spectrometer, ionization by electrospray: Zorbax SB-C18 column (1.8 µm, 30.0 mm × 4.60 mm), column temperature 40 °C; mobile phase – aqueous fraction (0.1% methanoic acid solution) and acetonitrile fraction (0.1 % methanoic acid solution) 84 : 16. The mobile phase velocity was 0.40 µl/min. Detection was performed at a wavelength of 272 nm. The sample volume was 5.00 µL.



4, 8-10: R = CH_3
5: R = CH_3, $R_1 = C_2H_5$
6: R = CH_3, $R_1 = C_6H_5$
7: R = 3-F-C_6H_4, $R_1 = C_2H_5$

a: CH_3I, b: CS_2, KOH, c: $ClCH_2COOC_2H_5$, d: C_2H_5NCS (5, 7), C_6H_5NCS (6),
e: 2,6-$Cl_2C_6H_3COH$, f: NaH, $ClCH_2COOH$, g: NaH, $ClCH_2CONHNH_2$

Fig. 2. Scheme of synthesis of target products of the study

The pharmacological profile was evaluated by ADME analysis based on the SwissADME online platform. The synthesis of the starting materials 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol and 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol was carried out according to the methods described in earlier scientific publications (Fedotov & Hotsulia, 2021, 2023).

Ethyl 2-((3-mercapto-9-methylpyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazin-6-yl)-thio)acetate (4). 0.005 mol of ethyl 2-chloroacetate was added to a solution of 0.005 mol of potassium 3-(ethylthio)-9-methylpyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazin-6-thiolate in 35 mL of propan-2-ol. This was heated for 1 hour. The excess alcohol was removed under vacuum. The resulting precipitate was filtered off and recrystallized from propan-1-ol.

N-ethyl-3-(3-methylpyrazol-5-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (5). To 0.01 mol of the starting 4-amino-5-(5-(methyl)pyrazol-3-yl)-1,2,4-triazol-3-thiol, previously dissolved in 40 mL of ethanoic acid, 0.01 mol of the corresponding ethyl isothiocyanate was added. The resulting reaction mixture was heated for 2 hours. The residue for analysis was crystallized from ethanol.

3-(3-Methylpyrazol-5-yl)-N-phenyl-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-amine (6). To 0.01 mol of the starting 4-amino-5-(5-(methyl)pyrazol-3-yl)-1,2,4-triazol-3-thiol, previously dissolved in 40 mL of ethanoic acid, 0.01 mol of the corresponding phenyl isothiocyanate was added. The resulting reaction mixture was heated for 2 hours. The residue for analysis was crystallized from ethanol.

N-ethyl-3-(3-(3-fluorophenyl)pyrazol-5-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (7). To 0.01 mol of the starting 4-amino-5-(5-(3-fluorophenyl)pyrazol-3-yl)-1,2,4-triazole-3-thiol, previously dissolved in 40 mL of ethanoic acid, 0.01 mol of the corresponding ethyl isothiocyanate was added. The resulting reaction mixture was heated for 2 hours. The reaction product was crystallised from ethanol for analysis.

6-(2,6-Dichlorophenyl)-3-(3-methylpyrazol-5-yl)-6,7-dihydro [1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazine-7-carboxylic acid (9). 0.002 mol of sodium hydride to 0.001 mol of 4-amino-5-(5-methylpyrazol-

3-yl)-1,2,4-triazole-3-thiol previously dissolved in 20 mL of dry tetrahydrofuran at room temperature was added. This was left to form a salt for 15 minutes. Then we added 0.0015 mol of chloroacetic acid and stirred the solution for 10 hours. The solvent was removed under vacuum. The product was recrystallized from ethanol.

6-(2,6-Dichlorophenyl)-3-(3-methylpyrazol-5-yl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazine-7-carbohydrazide (10). 0.002 mol of sodium hydride was added to 0.001 mol of 4-amino-5-(5-methylpyrazol-3-yl)-1,2,4-triazole-3-thiol previously dissolved in 20 mL of dry tetrahydrofuran at room temperature. This was left to form a salt for 15 minutes. Then we added 0.0015 mol of chloroacetic acid hydrazide and stirred the solution for 8 hours. The solvent was removed under vacuum. The residue for analysis was crystallized from propan-1-ol.

The studied compounds were prepared in the form of ethanolic solutions at a dilution of 0.1 %, 0.5 % and 1.0 %. Discs with a diameter of 6 mm were prepared from filter paper, saturated with extracts and dried in a laminar flow oven (LAM 2 kl, PORSA-Ukraine). The antibacterial activity of different concentrations of the compounds was determined by the disc diffusion method with epizootic (*Enterobacter cloacae*, *Klebsiella pneumoniae* ssp. *rhinoscleromatis*, *Klebsiella pneumoniae* ssp. *ozaenae*, *Klebsiella aerogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, *Proteus vulgaris*, *Morganella morganii*) and reference (*Salmonella typhimurium* UNCSM-014, *Escherichia coli* ATCC 25923, *Proteus vulgaris* UNCSM-011, *Staphylococcus aureus* UNCSM-017, *Bacillus subtilis* ATCC 6633) strains of microorganisms. A suspension was prepared from the daily cultures of these microorganisms, in which the number of bacteria (1.5×10^8 CFU (colony forming units) by the turbidity standard) was determined using a DEN-1 densitometer (Latvia). At the next stage of the study, the suspension was inoculated evenly into Petri dishes with Mueller Hinton Agar (Himedia). Discs (6 mm in diameter, with a minimum distance of 24 mm between discs, 15 mm from the edges of the Petri dish) were placed on the surface of the medium with inoculum using sterile tweezers, impregnated with the prepared extracts and cultured in a TSO-80/1 thermostat, in an upside-down position, at 37 °C for 24 hours. Discs with the standard antibiotic streptomycin at a concentration of 10 µg were used as a positive control. The result was evaluated using a ruler (Antibiotic Zone Scale-C, model PW297, India) by determining the area of inhibition of microbial growth around the discs and was expressed in millimetres. Statistical processing of the quantitative results of the study was performed using Statistica 8.0 software (StatSoft Inc., USA), calculating the mean ± standard deviation (x ± SD). Differences between the values in the control and experimental groups were determined using the Tukey test, where differences are considered reliable at $P < 0.05$ (taking into account the Bonferroni correction). Discs ($n = 8$) impregnated with the corresponding alcohol solutions of the studied compounds were applied to the microbial crops. Discs with 10 µg of streptomycin, an antibiotic produced by the radiant fungi of the genus *Streptomyces*, were used as a positive control. After 24 hours, the culture growth was measured using a zone scale to read the size of the zones of microbial growth inhibition (Antibiotic Zone Scale-C, model PW297, India) and TpsDig2 software. Data in the tables are presented as x ± SD (standard deviation).

Results

Molecular docking. The results of the molecular docking allowed us to focus on the most promising substances for research related to the identification of compounds with antimicrobial activity. Docking to peptide deformylase of *E. coli* and *S. aureus* was chosen as a preliminary demonstration of the ability of the compounds to exhibit this type of activity. Six compounds were selected for *in vitro* studies. Each of the compounds selected for the study is characterized by certain features regarding the type of interactions and the nature of amino acid residues that may be involved in this process. For example, ethyl 2-((9-methyl-3-(methylthio)pyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazin-6-yl)thio)acetate (4) is capable of forming exclusively alkyl and π-alkyl interactions with the active site of *E. coli* and *S. aureus* peptide deformylase. The first type of interactions is realized with the participation of leucine residues (LEU A: 105 and 112), the second – with the participation of histidine residues (HIS A: 154 and 158). The docking results of compound 6 also revealed the predominance of hydrophobic interactions (Fig. 3).

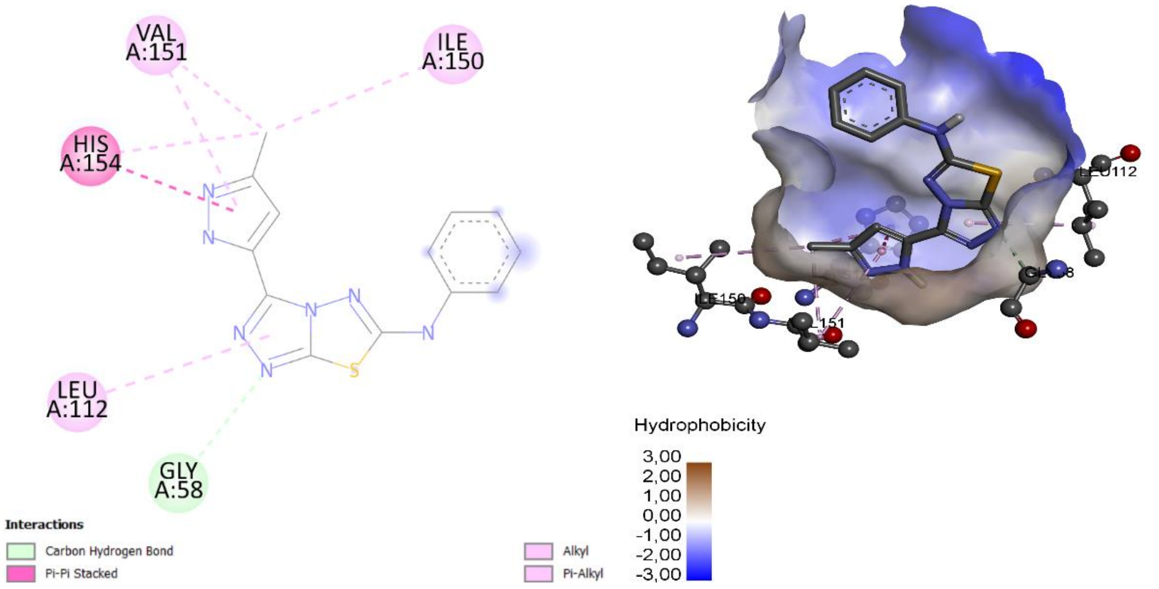


Fig. 3. Results of docking of compound 6 to the active site of *S. aureus* peptide deformylase

Instead, 6-(2,6-dichlorophenyl)-3-(3-methylpyrazol-5-yl)-6,7-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-7-carbohydrazide (10) is characterized by the absence of the ability to form alkyl and π-alkyl interactions when analyzed by the model enzyme *E. coli*. At the same time, this substance actively demonstrates the ability to form intermolecular hydrogen bonds with glutamic acid residues (GLU B: 41 and 95), bonds with cysteine sulfur (CYS B: 90), π-cationic and π-anionic interactions with arginine (ARG B: 97) and glutamic acid residues (GLU B: 95), respectively. The nature of the interactions of compound 10 with the active site of *S. aureus* peptide deformylase is fundamentally different: in this case, alkyl interactions (ILE A: 150, VAL A: 150), π-alkyl interactions (HIS A: 154, LEU A: 112, VAL A: 150), and π-π-stacking (HIS A: 154) were recorded. Thus, the same compound can form both similar and different chemical interactions with the active site of the same enzyme, but of different microorganisms (Table 1). A general analysis of interactions with the enzyme allows us to identify N-ethyl-3-(5-(3-fluorophenyl)pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (7) as the substance with the highest potential for antimicrobial activity, which is determined by a relatively large number of contacts with the active site of the enzyme (Fig. 4).

Thus, this substance shows the ability to form π-alkyl interactions with histidine (HIS B: 132), leucine (LEU B: 91) and isoleucine (ILE B: 44) residues, as well as proline (PRO B: 94). Additionally, the possibility of forming π-cationic, π-anionic and π-σ interactions can also be noted. The first type can involve arginine residues (ARG B: 97), the second – glutamic acid residues (GLU B: 41 and 95), and the third – leucine residues (LEU B: 91). These types of contacts can also be strengthened by intermolecular hydrogen bonding (GLU B: 95) and C-H bonding (GLY B: 89). Less promising is the possibility of this compound affecting the studied *S. aureus* enzyme: in this case, the nature of interactions is limited to alkyl and π-alkyl contacts.

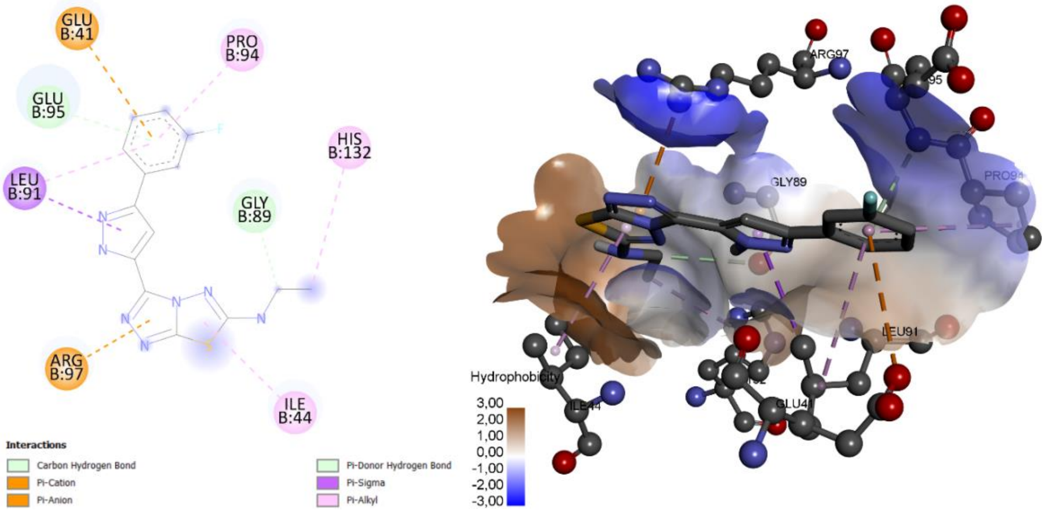


Fig. 4. Results of docking of compound 7 to the active site of *E. coli* peptide deformylase

Table 1
Results of molecular docking of the studied compounds

Compound	Type of interaction with peptide deformylase	
	E. coli	*S. aureus*
4	LEU A: 105 (alkyl), LEU A: 112 (alkyl), HIS A: 154 (π-alkyl), HIS A: 158 (π-alkyl)	LEU A: 105 (alkyl), LEU A: 112 (alkyl), HIS A: 154 (π-alkyl), HIS A: 158 (π-alkyl)
5	ARG B: 97 (π-cation), ILE B: 44 (alkyl, π-alkyl), ILE B: 86 (alkyl), LEU B: 91 (alkyl), LEU B: 125 (alkyl)	GLU A: 185 (π-anion), HIS A: 154 (π-alkyl, π-S), LEU A: 112 (alkyl), VAL A: 59 (π-alkyl), VAL A: 151 (π-alkyl)
6	ARG B: 97 (π-anion), CYS B: 90 (H-bond), LEU B: 91 (π-alkyl), PRO B: 94 (π-alkyl)	HIS A: 154 (π-π stacked, π-alkyl), LEU A: 112 (π-alkyl), VAL A: 151 (π-σ), ILE A: 150 (π-alkyl), GLY A: 58 (C-H bond)
7	ARG B: 97 (π-cation), GLU B: 41 (π-anion), GLU B: 95 (π-anion, H-bond), GLY B: 89 (C-H bond), ILE B: 44 (π-alkyl), HIS B: 132 (π-alkyl), LEU B: 91 (π-alkyl, π-σ), PRO B: 94 (π-alkyl)	HIS A: 154 (π-alkyl), ILE A: 150 (alkyl), LEU A: 112 (π-alkyl), VAL A: 59 (π-alkyl), VAL A: 151 (π-alkyl)
9	ARG B: 97 (π-cation), ILE B: 44 (alkyl), LEU B: 91 (π-σ)	ILE A: 150 (alkyl), LEU A: 112 (π-alkyl), VAL A: 59 (π-alkyl), VAL A: 151 (π-alkyl)
10	ARG B: 97 (π-cation), CYS B: 90 (Sulfur-X), GLU B: 41 (H-bond), GLU B: 95 (π-anion, H-bond)	HIS A: 154 (π-alkyl, π-π-stacked), ILE A: 150 (alkyl), LEU A: 112 (π-alkyl), VAL A: 150 (alkyl, π-alkyl)
Streptomycin	ARG B: 66 (H-bond), ARG B: 97 (π-cation), GLU B: 41 (attractive charge), GLU B: 42 (H-bond)	ASN A: 117 (H-bond), GLU A: 185 (H-bond), GLY A: 110 (C-H bond), SER A: 57 (H-bond), TYR A: 147 (C-H bond)

ADME analysis. Visualization of the key properties of the investigated molecules using the bioavailability radar, which affect oral bioavailability, helps to recognize the fact that the most favorable nature for the manifestation of the relevant properties is that of ethyl 2-((9-methyl-3-(methylthio)pyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazin-6-yl)thio)acetate (4) and N-ethyl-3-(5-methylpyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (5). However, all other substances may have limitations in this process due to their water solubility. The number of heavy atoms (nHA) does not exceed 30, which has a positive effect on the overall pharmacokinetics and indicates a predictably satisfactory nature of the processes associated with bioavailability (Table 2). The proportion of Csp3-hybrid atoms, which is associated with a positive prognostic character of pharmacokinetic parameters, should be at least 0.25, which was recorded for compounds 4 and 5 (Table 2). The number of rotating bonds (nRB) does not exceed 6, which preliminarily implies optimal permeability through biomembranes and the necessary conformational flexibility in the active site of the enzyme (Table 2). The number of atoms that can act as a hydrogen bond donor (nHBD) and acceptor forms (nHBA) an interval of values that have an acceptable level for a preliminary satisfactory assessment of oral bioavailability (Table 2). According to the results of the study, it was found that the investigated compounds have molecular refraction (MR) values ranging from 65.32 to 105.18, which demonstrate the favorable overall pharmacological nature of this indicator. Instead, the value of the topological polar surface area (TPSA) has a positive component for all the studied compounds, except for substance 10, which is important for assessing the ability of molecules to penetrate cell membranes (Table 2).

Table 2
Physicochemical properties of the investigated compounds

Indicator	4	5	6	7	9	10
nHA	22	17	21	23	26	27
nHA aromatic	12	13	19	19	16	16
Csp^3 fraction	0.42	0.33	0.08	0.14	0.20	0.20
nRB	6	3	3	4	3	4
nHBA	6	4	4	5	5	5
nHBD	0	2	2	2	3	4
MR	83.65	65.32	80.75	85.75	101.24	105.18
TPSA. Å^2	137.28	112.03	112.03	112.03	134.02	151.84

The solubility calculated using the ESOL, Ali and SILICOS-IT methods characterizes the studied compounds as soluble or moderately soluble (Table 3). Compound 5 is predicted to be the most soluble.

The consensus value of Log Po/w forms a range of 1.60–2.50 (Table 4). This interval indicates moderate lipophilicity, which is favorable for oral drugs and other types of molecules that need to penetrate cell membranes to reach their biological targets.

Table 3
Predicted water solubility of the studied compounds

Indicator	4	5	6	7	9	10
Log S (ESOL)	–3.00	–2.60	–3.75	–3.93	–4.71	–4.08
Solubility, mg/mL; mol/L	3.35×10^{-1}; 9.90×10^{-4}	6.30×10^{-1}; 2.53×10^{-3}	5.30×10^{-2}; 1.78×10^{-4}	8.77×10^{-3}; 1.17×10^{-4}	8.07×10^{-3}; 1.96×10^{-5}	3.53×10^{-2}; 8.29×10^{-5}
Class	S	S	P	P	MS	MS
Log S (Ali)	–4.18	–3.29	–4.53	–4.71	–5.76	–5.10
Solubility, mg/mL; mol/L	2.25×10^{-2}; 6.65×10^{-5}	1.27×10^{-1}; 5.08×10^{-4}	8.79×10^{-3}; 2.96×10^{-5}	6.49×10^{-3}; 1.97×10^{-5}	7.17×10^{-4}; 1.74×10^{-6}	3.41×10^{-3}; 8.03×10^{-6}
Class	MS	S	MS	MS	MS	MS
Log S (SILICOS-IT)	–2.88	–3.20	–4.91	–5.58	–5.07	–5.32
Solubility, mg/mL; mol/L	4.46×10^{-1}; 1.32×10^{-3}	1.57×10^{-1}; 6.28×10^{-4}	3.64×10^{-3}; 1.22×10^{-5}	8.68×10^{-4}; 2.64×10^{-6}	3.52×10^{-3}; 8.57×10^{-6}	2.03×10^{-3}; 4.76×10^{-6}
Class	S	S	MS	MS	MS	MS

Note: MS – moderately soluble, S – soluble.

Table 4
Lipophilicity of the studied compounds

Indicator	4	5	6	7	9	10
Log $P_{o/w}$ (iLogP)	2.27	1.70	1.90	2.31	1.90	1.13
Log $P_{o/w}$ (XLogP3)	1.68	1.34	2.53	2.70	3.27	2.27
Log $P_{o/w}$ (WLogP)	1.46	1.13	2.63	3.04	2.23	1.14
Log $P_{o/w}$ (MLogP)	2.04	0.68	2.27	2.26	2.98	2.60
Log $P_{o/w}$ (SILICOS-IT)	0.58	1.63	2.23	2.99	2.13	1.12
Consensus Log $P_{o/w}$	1.60	1.29	2.31	2.66	2.50	1.65

Further studies of pharmacokinetic parameters allow us to identify compounds 5–7, 9 as substances with a pronounced ability to gastrointestinal adsorption (GIA) (Table 5). A slightly lower ability to manifest this property was found in compound 4 and the lowest ability to GIA should be in compound 10 (Table 5). The possibility of overcoming the blood-brain barrier (BBB) is considered extremely unlikely for all analyzed compounds (Table 5). The probability of active participation in P-glycoprotein transporter activity looks diverse: for compounds 4, 6, 7 and 9, this phenomenon is considered unlikely. On the contrary, compounds 5 and 10 may be actively involved in this process, which preliminarily indicates some potential limitations in bioavailability for these compounds. The majority of cytochromes listed in Table 6 should not be affected by the test compounds, which reduces to zero the possible effect on the metabolism of other substances.

The data of the logarithm of the skin penetration coefficient (Log Kp) of the studied compounds allow us to predict a rather low rate of overcoming the skin barrier (Table 5). The absence of PAINS (pan assay interference compounds) warnings suggests that the structure of the tested compounds does not contain fragments that can cause false-positive results in biological studies. There are no Brenk filter warnings for compounds 4, 6, 7 and 9, which allows us to preliminarily state that these substances do not contain functional groups or other structural fragments that can cause or aggravate adverse reactions or provoke undesirable pharmacokinetic phenomena. Compounds 4, 6 and 7 meet the leadership criteria. Instead, compounds 5, 9 and 10 have a discrepancy related to molar mass. The Lipinski and Ghose filters are overcome without disturbances, indicating that the investigated substances have optimal properties for absorption, penetration through biological membranes and distribution in the body, making them suitable for further research and development as a potential therapeutic agent. Likewise, compounds 5–7 pass the Weber, Egan and molecular weight filters without incident, lipophilicity, number of hydrogen bonds required to interact with the active site of the enzyme and flexibility for membrane penetration, good absorption and pharmacokinetic properties. Substance 10 demonstrates the lowest ability to overcome drug-like filters.

Table 5
Pharmacokinetic capacity of the test substances

Indicator	4	5	6	7	9	10
GIA	+	++	++	++	++	–
Overcome BBB	–	–	–	–	–	–
P-gp substrate	–	+	–	–	–	+
CYP1A2 inhibitor	+	+	+	+	–	–
CYP2C19 inhibitor	+	–	+	+	–	–
CYP2C9 inhibitor	–	–	–	–	–	–
CYP2D6 inhibitor	–	–	–	–	–	–
CYP3A4 inhibitor	–	–	–	–	–	–
Log Kp, cm/s	–7.17	–6.87	–6.32	–6.39	–6.49	–7.28

Synthesis. The resulting compounds (4–7, 9, 10) are white substances that dissolve in alcohols when heated and are slightly soluble in water.

Ethyl 2-((3-mercapto-9-methylpyrazolo[1,5-d][1,2,4]triazolo[3,4-f][1,2,4]triazin-6-yl)-thio)acetate (4). Yield: 70 %. M.p.: 201–203 °C; 1H NMR, δ (ppm): 13.82 (s, 1H, SH), 11.40 (s, 1H, NH, pyrazole), 4.16–4.10 (m, 2H, COO-CH_2-CH_3), 4.08 (s, 2H, S-CH_2), 2.54 (s, 3H, CH_3-pyrazole), 1.23 (t, J = 6.6 Hz, 3H, COO-CH_2-CH_3). ESI-MS: m/z = 339 [M+H]+. Elemental analysis ($C_{11}H_{12}N_6O_2S_2$): calculated (%) – C, 40.73; H, 3.73; N, 25.91; S, 19.77; found (%) – C, 40.62; H, 3.74; N, 25.84; S, 19.82.

N-ethyl-3-(3-methylpyrazol-5-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (5). Yield: 67%. M.p.: 222 – 224 °C; 1H NMR, δ (ppm): 12.73 (s, 1H, NH, pyrazole), 6.82 (s, 1H, CH, pyrazole), 6.57 (t, J = 3.4 Hz, 1H, NH-CH_2-CH_3), 3.55–3.51 (m, 2H, NH-CH_2-CH_3), 2.34 (s, 3H, CH_3), 1.19 (t, J = 5.6 Hz, 3H, NH-CH_2-CH_3). ESI-MS: m/z = 337 [M+H]+. Elemental analysis ($C_9H_{11}N_7S$): calculated (%) – C, 43.36; H, 4.45; N, 39.33; S, 12.86; found (%) – C, 43.25; H, 4.46; N, 39.42; S, 12.89.

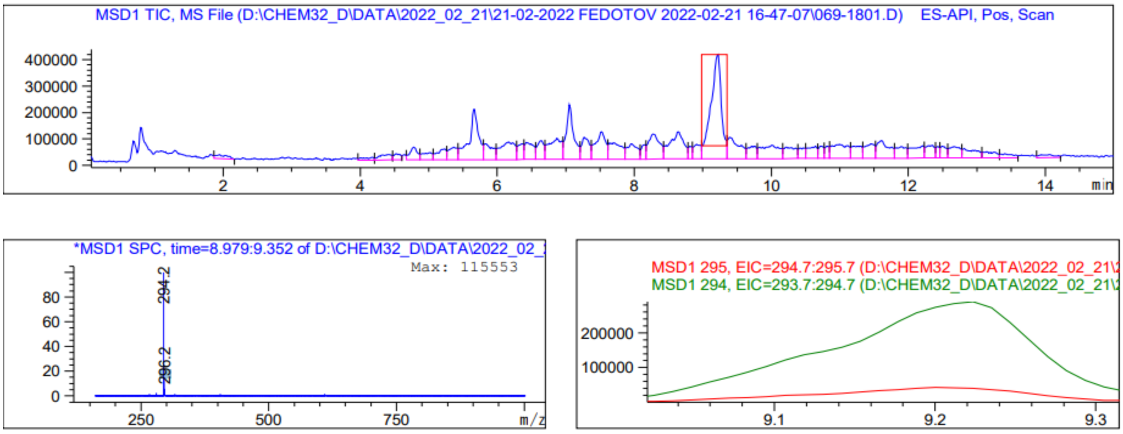


Fig. 5. GC-MS spectrum of compound 6

3-(3-Methylpyrazol-5-yl)-N-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (6). Yield: 75%. M.p.: 176–178 °C; 1H NMR, δ (ppm): 12.85 (s, 1H, NH, pyrazole), 10.06 (s, 1H, NH), 7.56–7.52 (m, 2H, H-2, 6, C_6H_5), 7.36–7.31 (m, 2H, H-3, 5, C_6H_5), 6.88 (t, J = 6.7 Hz, 1H, H-4, C_6H_5), 6.81 (s, 1H, CH, pyrazole), 2.33 (s, 3H, CH_3). ESI-MS: m/z = 294, 296 [M+H]+ (Fig. 5). Elemental analysis ($C_{13}H_{11}N_7S$): calculated (%) – C, 52.51; H, 3.73; N, 32.98; S, 10.78; found (%) – C, 52.38; H, 3.74; N, 32.89; S, 10.81.

N-ethyl-3-(3-(3-fluorophenyl)pyrazol-5-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-amine (7). Yield: 67 %. M.p.: 251 – 253 °C; 1H NMR, δ (ppm): 12.21 (s, 1H, NH, pyrazole), 8.68 (s, 1H, CH, pyrazole), 7.69–7.49 (m, 2H, H-6 3-F-C_6H_4), 7.43–7.38 (m, 4H, H-2, 5, 3-F-C_6H_4), 7.22–7.11 (m, 3H, H-4 3-F-C_6H_4, NH-CH_2-CH_3), 3.53–3.43 (m, 2H, NH-CH_2-CH_3), 1.20 (t, J = 5.6 Hz, 3H, NH-CH_2-CH_3). ESI-MS: m/z = 337 [M+H]+. Elemental analysis ($C_{14}H_{12}FN_7S$): calculated (%) – C, 51.06; H, 3.67; N, 29.77; S, 9.73; found (%) – C, 51.19; H, 3.66; N, 29.67; S, 9.70.

6-(2,6-Dichlorophenyl)-3-(3-methylpyrazol-5-yl)-6,7-dihydro[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazine-7-carboxylic acid (9). Yield: 80%. M.p.: 183–185 °C. 1H NMR, δ (ppm): 12.32 (s, 1H, NH, pyrazole), 10.45 (s, 1H, COOH), 7.63 (s, 1H, H-4, pyrazole), 7.42–7.34 (m, 3H, H-3,4,5 2,6-Cl-C_6H_3), 6.63 (d, J = 6.7 Hz, 1H, N-NH), 5.85 (dd, J = 9.8, 5.2 Hz, 1H, CH, thiadiazine), 5.08 (d, J = 5.3 Hz, 1H, SCH, thiadiazine), 2.53 (s, 3H, CH_3-pyrazole). ESI-MS: m/z = 413, 415 [M+H]+ (Fig. 5). Elemental analysis ($C_{15}H_{12}Cl_2N_6O_2S$): calculated (%) – C, 43.81; H, 2.94; N, 20.44; S, 7.80; found (%) – C, 43.70; H, 2.95; N, 20.38; S, 7.82.

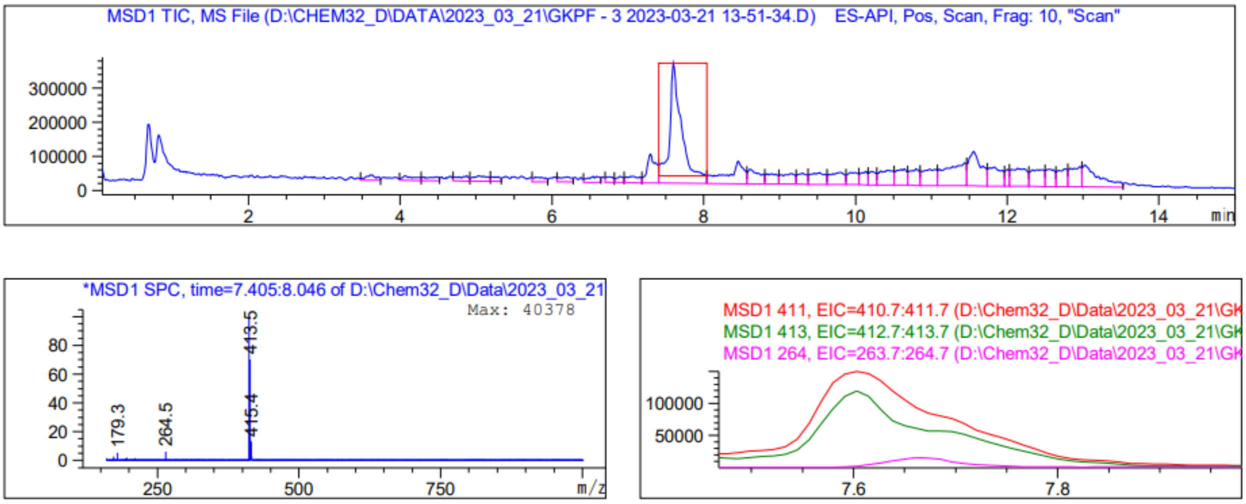


Fig. 6. GC-MS spectrum of compound 9

6-(2,6-Dichlorophenyl)-3-(3-methylpyrazol-5-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-7-carbohydrazide (10). Yield: 69%. M.p.: 245–247 °C. 1H NMR, δ (ppm): 11.77 (s, 1H, NH, pyrazole), 7.72 (s, 1H, H-4 pyrazole), 7.48–7.32 (m, 4H, NH-NH_2, H-3,4,5 2,6-Cl-C_6H_3), 6.48 (d, J = 5.3 Hz, 1H, SCH, thiadiazine), 4.13 (d, J = 4.0 Hz, 2H, NH-NH_2), 2.49 (s, 3H, CH_3-pyrazole). ESI-MS: m/z = 426 [M+H]+. Elemental analysis ($C_{15}H_{14}Cl_2N_8OS$): calculated (%) – C, 42.36; H, 3.32; N, 26.35; S, 7.54; found (%) – C, 42.47; H, 3.33; N, 26.28; S, 7.57.

Microbiology. The results of the study of antibacterial activity showed high potential.

The bactericidal effect of only 1.0% solution of compound 10 (10.08 mm; $P < 0.05$) on *E. cloacae* 1, as well as 0.5% (19.88 mm; $P < 0.05$) and 1.0% (19.92 mm; $P < 0.05$) solutions of compound 4 on *E. cloacae* 2 were found compared to lower concentrations. Moderate sensitivity of *E. cloacae* 1 was observed to 0.5% and 1.0% solutions of compound 4 (8.18, 9.16 mm); to 0.1% and 0.5% solutions of compound 10 (8.88, 9.20 mm); 1.0% solution of compound 6 (8.44 mm); up to 0.5% and 1.0% solutions of compound 5 (8.88, 9.14 mm); up to 1.0% solution of compound 7 (8.18 mm) and solutions of all concentrations of compound 9 (8.13, 8.02, 9.36 mm), while on *E. cloacae* 2 bacteriostatic effect was detected under the influence of 1.0% solutions of compounds 5 and 10 (8.12 mm). We note the inhibitory effect of 0.5% and 1.0% solutions of compound 4 on two field strains of *K. pneumonia* ssp. *ozaenae* 1 (12.90 and 13.82 mm; $P < 0.05$) and *K. pneumonia* ssp. *ozaenae* 3 (20.80 and 23.96 mm; $P < 0.05$). The bactericidal effect of compound 4 on *K. pneumonia* ssp. *ozaenae* 3 was 10.15 mm higher than the control; $P < 0.01$. Solutions of compound 9 with high concentration had an inhibitory effect against *K. pneumonia* ssp. *ozaenae* 1 (10.28 mm; $P < 0.05$), and similar solutions of compounds 5 and 6 – against *K. pneumonia* ssp. *ozaenae* 2 (11.98 and 10.84 mm; $P < 0.05$). Moderate susceptibility of *K. pneumonia* ssp. *ozaenae* 3 was found when using 1.0% solutions of compounds 6 and 10 (8.32, 8.06 mm). High sensitivity of *E. coli* to 1.0% solution of compound 4 (12.18 mm; $P < 0.01$); 0.5% and 1.0% solutions of compound 6 (10.18, 10.90 mm; $P < 0.05$) was found. The inhibition of growth and development of *K. aerogenes* was detected under the influence of 1.0% solutions of compounds 7 and 9 (8.22 and 8.20 mm). A similar phenomenon was also observed in the case of *S. enterica* under the influence of 1.0% solutions of compounds 4 and 6 (8.84 and 8.08 mm; $P < 0.05$).

Discussion

Recent trends in the Ukrainian pharmaceutical industry underscore the urgent need for the development of novel therapeutics that are both highly effective and exhibit low toxicity. Among the broad spectrum of biologically active compounds, 1,2,4-triazole derivatives continue to hold a prominent place. For decades, this class of heterocyclic structures has attracted considerable interest not only within the pharmaceutical sector but also across various scientific disciplines. It is well established that the introduction of diverse functional groups into the 1,2,4-triazole core significantly enhances the biological potential, reduces toxicity, and increases the reactivity of the resulting compounds (Gao et al., 2019; Wen et al., 2020; Pachuta-Stec, 2022).

The study of tuberculocidal and tuberculostatic properties of 1,2,4-triazole derivatives, as well as the determination of MIC (minimum inhibitory concentration) is a promising area of modern scientific research (Berida et al., 2023). Studies have revealed the potential tuberculocidal and tuberculostatic activity of the studied 1,2,4-triazole derivatives against *M. fortuitum* and *M. bovis* (Gotsulya et al., 2022). The results of scientific research, which are actively covered in specialized publications, indicate a high degree of safety of these compounds (Zazharskyi et al., 2024).

For the first time, a team of domestic researchers has investigated the antimicrobial and antifungal properties of novel fluorophenyl- and furan-containing 1,2,4-triazole derivatives (Bihdan, 2021; Zazharskyi et al., 2021). The majority of the compounds demonstrated moderate antimicrobial activity (Zazharskyi et al., 2021) and relatively high antifungal efficacy (Bihdan, 2021). The *Staphylococcus aureus* strain was found to be the most susceptible to 5-(2-fluorophenyl)-4-(((5-nitrofuran-2-yl)methylene)amino)-4H-1,2,4-triazole-3-thiol. Meanwhile, *Candida albicans* exhibited pronounced sensitivity to 5-(2-fluorophenyl)-4-((4-bromophenyl)methylene)amino-1,2,4-triazole-3-thiol and 5-(2-fluorophenyl)-4-((2,3-dimethoxyphenyl)methylene)amino-1,2,4-triazole-3-thiol.

The obtained results demonstrate that the bactericidal activity of the studied compounds depends on the concentration and type of bacteria. It was found that compounds 4 and 10 have the most pronounced bactericidal effect on various bacterial strains, in particular on *E. cloacae*, *K. pneumoniae*, *E. coli*, *P. vulgaris* and *M. morganii*, which indicates their potential as antibacterial agents. A particularly noticeable effect was observed when using a 1.0% concentration of the compounds, which provides significant inhibition of bacterial growth and development. Moderate susceptibility was observed in *K. pneumoniae* ssp. *ozaenae* 3, *E. coli*, *K. aerogenes*, *S. enterica*, *E. faecalis*, *E. faecium* and *S. aureus* strains with the use of most compounds, indicating the possibility of their use in the fight against these pathogens. At the same time, for *S. aureus* and *B. subtilis* strains, compound 4 was the most effective, providing a bactericidal effect at a concentration of 0.5% and 1.0%. Compound 9 also showed significant antibacterial activity against a number of tested bacteria, making it a promising candidate for further study and application in antibacterial therapy. At the same time, other compounds have shown limited activity only against certain strains, indicating the need for additional research. Overall, the study confirms the effectiveness of these compounds in inhibiting the growth and development of pathogenic microorganisms. Thus, according to our results, the investigated solutions of the studied compounds in ethanol inhibit the growth of colonies of many species of microorganisms of the families Enterobacteriaceae, Morganellaceae, Pseudomonadaceae, Enterococcaceae, Staphylococcaceae and Bacillaceae. It should be noted that the strains of *K. aerogenes*, *K. pneumoniae* ssp. *rhinoscleromatis*, *E. coli*, *P. vulgaris*, *M. morganii*, *E. faecalis* and *S. aureus* studied by us had a reduced susceptibility to streptomycin (11.08, 11.24, 11.83, 9.94, 11.14, 11.06 and 12.13 mm).

Conclusions

The complex inhibitory effect of promising 1,2,4-triazole derivatives on 15 bacterial strains was studied for the first time. The expressed antibacterial effect of 15 tested multidrug-resistant bacterial strains was established. We consider it possible to recommend ethanol solutions of compounds 4 and 9 for further studies on the control of multidrug-resistant strains of the above microorganisms.

The authors claim no conflict of interest.

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