ORIGINAL ARTICLE

Investigation of the antimicrobial and antifungal activities of some 1,2,4-triazole derivatives

Studium antimikrobiální a antimykotické aktivity některých derivátů 1,2,4-triazolu

Yuliia Frolova • Andrii Kaplaushenko • Sameliuk Yurii • Daria Romanina • Liubov Morozova

Received Februar 15, 2022 / Accepted August 1, 2022

Summary

This article presents the results of the study of the antimicrobial and antifungal properties among 1,2,4-triazole derivatives synthesized at the Department of Physical and Colloidal Chemistry of the Zaporizhzhia State Medical University. Previous studies have established the antimicrobial and antifungal activity of 1,2,4-triazole derivatives. Therefore, it was reasonable to investigate highly effective substances with antimicrobial and antifungal properties among synthesized compounds. In the first stage of our research, acute toxicity prediction was performed. The antimicrobial and antifungal properties were carried out by the method of "serial dilutions" on a liquid nutrient. Forty-seven compounds of the different classes were studied for these types of activities. According to our research, derivatives of 3-amino-1,2,4-triazole showed better performance than 3-thio-1,2.4-triazoles for *Staphylococcus aureus* and *Candida albicans*. 5-(1*H*-tetrazole-1-il)methyl-4*H*--1,2,4-triazole-3-yl-1-(5-nitrofuran-2-yl)methanimin (11.6) was showed the greatest antimicrobial and antifungal activity. Deeper research for compound 11.6 was done by diffusion in agar (method of "wells"). Studies have shown that molecule 11.6 showed antimicrobial and antifungal action to the studied test strains at a concentration of 2 μg/ml. Hence, this compound can be developed as a helpful therapeutic agent after establishing its safety pharmacology and toxicity.

Key words: 1,2,4-triazole • antimicrobial activity • antifungal activity

Souhrn

Článek představuje výsledky studia antimikrobiálních a antimykotických vlastností derivátů 1,2,4-triazolu syntetizovanými na Katedře fyzikální a koloidní chemie Záporožské státní lékařské univerzity. Předchozí studie stanovily antimikrobiální a antimykotickou aktivitu derivátů 1,2,4-triazolu. Proto bylo účelné zkoumat mezi syntetizovanými sloučeninami vysoce účinné látky s antimikrobiálními a antimykotickými vlastnostmi. V první fázi našeho výzkumu byla provedena predikce akutní toxicity. Antimikrobiální a antimykotické vlastnosti byly provedeny metodou sériového ředění na kapalné živné půdě. Na tyto typy aktivit bylo zkoumáno 47 sloučenin různých tříd. Podle našeho výzkumu vykazovaly deriváty 3-amino-1,2,4-triazolu lepší účinnost než 3-thio-1,2,4-triazoly na *Staphylococcus aureus* a *Candida albicans*. Největší antimikrobiální a antimykotickou aktivitu vykazoval 5-(1*H*-tetrazol-1-il)methyl-4*H*-1,2,4-triazol-3-yl-1-(5-nitrofuran-2-yl)methanimin (11.6). Hlubší výzkum sloučeniny 11.6 byl proveden difuzí v agaru (jamková metoda). Studie ukázaly, že molekula 11.6 vykazovala antimikrobiální a antimykotický účinek na studované testovací kmeny v koncentraci 2 μg/ml. Proto může být tato sloučenina po zjištění její farmakologické bezpečnosti a toxicity vyvinuta jako užitečná léčivá látka.

Klíčová slova: 1,2,4-triazol • antimikrobiální aktivita • antifungální aktivita

Yuliia Frolova, Ph.D. (✉) • A. Kaplaushenko • S. Yurii
Zaporizhzhia State Medical University
Department of Physical and Colloidal Chemistry,
Maiakovskyi Avenue 26, 69035 Zaporizhzhia, Ukraine
e-mail: yuliia_hulina@ukr.net

D. Romanina
Zaporizhzhia State Medical University
Department of Medicines Technology, Ukraine

L. Morozova
Vinnytsia National Agrarian University
Department Technologies
Processing of Livestock Products and Feeding, Ukraine

Introduction

Antimicrobials and antifungals are the most acquired drugs in the world. They are crucial treatments, notably

in the developing world, where infectious epidemics cause death. Antimicrobial resistance is shrinking the range of antimicrobial drugs and is a worldwide public health problem[1].

Typical bacterial pathogens such as *Escherichia coli, Streptococcus pneumoniae, Pseudomonas aeruginosa*, or *Staphylococcus aureus* are often multidrug-resistant in these settings. For example, *Mycobacterium tuberculosis* is common in low-income to middle-income countries, and highly drug-resistant strains have emerged. Based on this, antimicrobial resistance (AMR) is a worldwide problem[2].

AMR endangers the effective prevention and therapy of an ever-increasing number of infectious diseases caused by bacteria, microorganisms, and fungi. The World Health Organization (WHO) has recognized that AMR is a more serious danger to global public health[3].

Over several decades, the resistance to medicines to treat infections caused by *Staphylococcus aureus* has been widespread. People with MRSA (methicillin-resistant *Staphylococcus aureus*) are estimated to be 64% more likely to die than those with a non-resistant infection. Based on this, it becomes necessary to create new drugs that had no resistance to certain strains of microorganisms, viruses, and fungi.

In new antimicrobial drug development studies, 1,2,4-triazole and its derivatives design an important class of compounds. Also, a large number of drugs with 1,2,4-triazole core are showed that the core of this heterocyclic system is a structural fragment of antifungal (fluconazole, itraconazole), antidepressant (trazodone, alprazolam), antiviral (ribavirin), antimigraine (rizatriptan) drugs, etc.[4, 5].

Synthesis of 1,2,4-triazole derivatives is carried out by scientists from Poland, Russia, China, Turkey, and other countries worldwide; Ukraine is no exception[6–10]. The synthetics of Ukraine had created a huge bank of potentially active molecules that contain this heterocyclic system. Scientists at Zaporizhzhia State Medical University also search for biologically active substances which contained 1,2,4-triazole[11–14]. We decided to investigate the compounds obtained at the Department of Physical and Colloidal Chemistry of the Zaporizhzhia State Medical University for antimicrobial and antifungal activity. Thus, new substances and potential new original drugs can be found.

The purpose of our research is to investigate highly effective substances with antimicrobial and antifungal properties among 1,2,4-triazole derivatives, which were synthesized at the Department of Physical and Colloidal Chemistry of the Zaporizhzhia State Medical University.

Experimental part

Materials and methods

Tested compounds

All steps of 1,2,4-triazole derivatives, which were studied for antimicrobial and antifungal activities, were described earlier in the articles of the scientists of the Department of Physical and Colloidal Chemistry ZSMU. There were: Compounds 1 – 1,2,4-triazole-3-thiones, Compounds 2 – 3-alkylthio-1,2,4-triazoles, Compounds 3 – 3-alkylsulfonyl-1,2,4-triazoles, Compound 4 – 1,2,4-triazole-3-ylthio-acetonitrile, Compounds 5 – 1,2,4-triazole-3-ylthio-ethane(benz)imidates, Compounds 6 – 1,2,4-triazole-3-ylthio-acetic(benzoic) acid, Compound 7 – salts of 1,2,4-triazole-3-ylthio-acetic(benzoic) acids, Compounds 8 – hydrazides of 4-R-1,2,4-triazole-3-ylthio-acetic acids, Compounds 9 – ylidene hydrazides of 4-R-1,2,4-triazole-3-ylthio-acetic acids, Compound 10 – 1,2,4-triazole-3-ylthio-pyridin-3-amine, Compounds 11 – 1,2,4-triazole-3-yl-1-(alkyl-, aryl)methane(ethane)imines, Compounds 12 - reduced of 1,2,4-triazole-3-yl-1-(alkyl-, aryl)methane(ethane) imines[10, 18–20].

Toxicity

At the first stage of the study of antimicrobial and antifungal activities 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 were carried out according to structural formulas of the compounds in the online version of GUSAR-online[15]. The online prognosis was performed for 47 compounds derived of the 1,2,4-triazole derivatives.

The acute toxicity of the most active molecule – 5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4-triazole-3-yl--1-(5-nitrofuran-2-yl)methanimin (11.6) has been researched. Acute toxicity of the most active compound was performed according to the express method of V. B. Prozorovsky[16] on non-linear white rats. Four groups of animals were used for two observations, each with the additional use of one previous and one subsequent dose to determine the LD_{50} of compound 11.6. The water-soluble compound 11.6 was dissolved in 1.5 ml of distilled water and administered using a syringe intraperitoneally in compliance with the rules of asepsis and antiseptics (conclusion on bioethics of ZSMU No. 1 dated January 12, 2022). Observations were carried out after 24 hours.

Microbiology

According to our analysis[17], it has been established that some classes of 1,2,4-triazole derivatives have exhibited antimicrobial and antifungal activity.

The antimicrobial and antifungal activities of the synthesized compounds were conducted at the Department of Microbiology, Virology, and Immunology of the Zaporizhzhya State Medical University. These types of activity were carried out by the method of "serial dilutions" on a liquid nutrient. Aminopeptide was used as a nutrient (pH = 7.2). The maximum of the studied concentrations is 400 μg/ml. Sabouraud agar (pH 6.5–6.7) was used for growing mushrooms (500,000 reproductive cells in 1 ml). The antibacterial activity was carried out by the method of minimum bacteriostatic con-

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

Number 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	429.1 out of AD	353.0 in AD	346.0 in AD	268.3 in AD
1.2	897.4 in AD	215.3 in AD	1368.0 in AD	689.8 in AD
1.3	255.1 in AD	134.8 in AD	1087.0 in AD	294.8 in AD
1.4	365.7 in AD	131.6 in AD	804.3 in AD	1027.0 in AD
1.5	1077.0 in AD	215.2 in AD	844.7 in AD	1562.0 in AD
2.1	364.5 in AD	85.7 in AD	984.9 in AD	1079.0out of AD
2.2	516.6 in AD	84.7 in AD	2929.0 in AD	1268.0 in AD
2.3	516.6 in AD	84.7 in AD	2929.0 in AD	1268.0 in AD
3.1	339.7 in AD	76.3 in AD	925.9 in AD	1085.0out of AD
3.2	534.0 out of AD	76.590 in AD	1650.0 in AD	1330.0out of AD
3.3	547.4 in AD	147.1 in AD	1033.0 in AD	1438.0 in AD
3.4	682.6 in AD	111.0 in AD	2547.0out of AD	1635.0 in AD
4.1	863.5 out of AD	595.5 in AD	489.7 in AD	420.3 out of AD
5.1	873.9 in AD	239.3 in AD	467.4 in AD	1220.0out of AD
5.2	1710.0out of AD	134.3 in AD	483.4 in AD	483.1 out of AD
6.1	1321.0 in AD	677.9 in AD	593.8 in AD	2067.0out of AD
6.2	829.8 out of AD	382.3 in AD	905.6 in AD	514.7 out of AD
6.3	381.5 in AD	261.2 in AD	1244.0 in AD	907.3 in AD
7.1	751.7 out of AD	441.5 in AD	762.6 in AD	779.7 in AD
7.2	1538.0out of AD	321.8 in AD	790.4 out of AD	1435.0out of AD
7.3	408.5	408.5	408.5	408.5
7.4	408.5	408.5	408.5	408.5
7.5	564.3 out of AD	204.6 in AD	1061.0 in AD	632.6 out of AD
7.6	368.4	368.4	368.4	368.4
8.1	682.6 in AD	111.0 in AD	2547.0out of AD	1635.0 in AD
8.2	816.1 in AD	196.9 in AD	1338.0 in AD	2135.0 in AD
9.1	776.4 in AD	209.9 in AD	1828.0out of AD	2239.0out of AD
9.2	900.9 in AD	250.3 in AD	1676.0 in AD	4067.0 in AD
9.3	987.0 in AD	220.2 in AD	1551.0 in AD	2221.0 in AD
9.4	1184.0 in AD	179.9 in AD	1400.0 in AD	1246.0 in AD
10.1	889.8 out of AD	703.7 in AD	931.9 out of AD	651.9 out of AD
11.1	919.5 out of AD	429.8 in AD	592.6 out of AD	633.3 out of AD
11.2	1027.0out of AD	341.3 in AD	1427.0out of AD	949.2out of AD
11.3	997.5 out of AD	345.2 in AD	758.3 in AD	2108.0out of AD
11.4	319.0 in AD	214.6 in AD	293.4 in AD	217.7 in AD
11.5	655.7 out of AD	162.3 in AD	913.6 out of AD	1171.0 in AD
11.6	607.0 out of AD	233.5 in AD	1061.0 in AD	460.1 out of AD
11.7	479.4 out of AD	389.6 in AD	612.1 out of AD	495.5 out of AD
11.8	462.6 in AD	178.2 in AD	1708.0 in AD	606.4 out of AD
11.9	675.8 in AD	133.9 in AD	1297.0out of AD	636.3 out of AD
11.10	306.5 in AD	113.9 in AD	1062.0 in AD	520.9 out of AD
11.11	469.2 in AD	174.9 in AD	1572.0 in AD	392.8 out of AD
11.12	680.0 in AD	83.8 in AD	1179.0 in AD	323.3 out of AD
11.13	310.9 in AD	280.6 in AD	1147.0 in AD	283.9 out of AD
12.1	639.7 out of AD	206.8 in AD	1103.0out of AD	660.4 out of AD
12.2	725.1 in AD	130.0 in AD	947.9 in AD	530.2 out of AD
12.3	494.4 in AD	187.4 in AD	1537.0 in AD	302.7 out of AD

AD – applicability domain

centration (MBC) of a chemical in μg/ml. Ethacridine lactate was used as a comparison standard. The study of antimicrobial and activity was carried out on separate test cultures of microorganisms, representatives of both gram-positive and gram-negative microflora.

The antimicrobial and antifungal estimation of synthesized compounds were carried out against Gram-positive and Gram-negative bacteria: *Staphylococcus aureus 209-P*, *Escherichia coli 675*, *Candida albicans*, *Pseudomonas Aeruginosa 165*.

An in-depth study of the antimicrobial activity of the most active substance 5-(1*H*-tetrazole-1-yl)methyl-4*H*--1,2,4-triazole-3-yl-1-(5-nitrofuran-2-yl)methanimine (11.6) was determined by diffusion in agar (method of "wells"). The method is based on comparing the degree of inhibition of growth of the test microbe with certain concentrations of antibiotic or another agent in the test material with the inhibition of its growth by known concentrations of the standard. Suppression of the growth of the test microbe is carried out by diffusion of the substance or antibiotic from the test material in a dense medium. The working standards are specially made purified samples of antibiotics, the activity of which is established from international standard drugs. Standards are stored in sealed ampoules at a temperature of 4–10 °C. The labels of ampoules indicate the content of units or micrograms in 1 mg of the drug.

The test strains included: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Candida famata*, *Aspergillus niger*.

Diffusion in agar can determine the concentration of all antibiotics contained in fluids (blood, cerebrospinal fluid, urine, bile, ascitic fluid, etc.) and body tissues (lungs, liver, kidneys, brain, muscles, etc.).

Results and discussion

As predicted by GUSAR-online for tested compounds (Table 1), the average lethal dose of LD_{50} was when administered: intraperitoneally – from 255.1 to 1710.0 mg/kg, intravenously – from 76.3 to 703.7 mg/kg, orally – from 346.0 to 2929.0 mg/kg and subcutaneously – from 217.7 to 2239.0 mg/kg. Results of GUSAR-prognosis indicates that the compounds probably belong to class 4 and 5 of toxicity (low-toxic and practically non-toxic substances)[21].

It was established that the LD_{50} of 5-(1*H*-tetrazole-1--yl)methyl-4*H*-1,2,4-triazole-3-yl-1-(5-nitrofuran-2-yl) methanimin (11.6) was 1060 ± 179 mg\mg, which fully corresponded to the on-line prediction.

Forty-seven compounds of the different classes were studied for antimicrobial and antifungal activities. Our antimicrobial and antifungal activity screening results are presented in Table 2.

In the study of antifungal and antimicrobial activity among 1,2,4-triazole derivatives, the influence of various substituents on C3 and C4 carbon atoms was analyzed, and it was interesting to trace the effect of compounds when replacing a thio-group with an amino-group.

Replacing the thio-group for the amino-group in the third position of 1,2,4-triazole, an increase (in some cases insignificant) in antimicrobial and antifungal activity was observed. If the results obtained were analyzed, it can conclude that the compounds with the thio-group in the third position have almost no antimicrobial and antifungal activity, except for compounds 11 and their analogs with a reduced double bond. Derivatives of 3-amino-1,2,4-triazole showed better performance than *Staphylococcus aureus* and *Candida albicans* but generally did not exceed the reference drug.

According to 1,2,4-triazole-3-thions (compounds 1) and their derivatives, as acetonitrile (compound 4.1), acetic and benzoic acids (compounds 6.1–6.3), iminoesters (compounds 5.1–5.2), the antimicrobial and antifungal activity was below the reference standard. For salts of 2-, 4- (5-R-4-R_1-1,2,4-triazole-3-ylthio) acetic(benzoic) acids (compounds 7), sodium (compound 7.2), ammonium (7.5) and diethylammonium (7.4 and 7.6) salts showed better results than ethacridine lactate in relation to *Staphyllococcus aureus*, other indicators of bacteriostatic action were worse.

As for the class 5-(phenoxymethylene)-4-R-3-alkylsulfonyl-1,2,4-triazoles (compounds 3), they showed a high performance against the *Staphylococcus aureus*, which in turn was due to the high performance of two oxygens. The most active substance was 5-(phenoxymethylene)-4-ethyl-3-propylsulfonyl-1,2,4-triazole (compound 3.1) which contained a hexavalent sulfur atom.

Particular attention should be paid to substances containing halogen atoms, namely hydrazides and ylidene hydrazides of 2-(5-(phenoxymethylene)-4-R--1,2,4-triazole-3-ylthio) acetic acids (compounds 8 and 9). Among the latter, compounds with aromatic fluorine or chlorine atoms were selected. Analyzing the obtained data, it should be noted that the most effective of them to the *Staphylococcus aureus* were benzylidene hydrazides 9.2 and 9.4, in the structure of which, in the fourth position of the phenyl substituent, a chlorine atom was present. The best results of fungicidal and fungiostatic action against *Candida albicans* among these classes of compounds were shown by substances 8.1 and 9.3, which were hydrazide 2-(5-(phenoxymethylene)-4-ethyl-1,2,4-triazole-3-ylthio) acetic acid (8.1) and N'-(4-(fluorobenzylidene)-2-((5-(phenoxymethylene)-4-phenyl-1,2,4-triazole-3-yl)thio)acetohydrazide (9.3).

The addition of an aminochloropyridine fragment in the molecule significantly increased the indicators of antimicrobial and antifungal activity, so we synthesized 5-R-4-R_1-1,2,4-triazole-3-yl-1-(alkyl, aryl) methane (ethane) imines (compounds 11.1-11.3, 11.8-11.13).

Considering the amino derivatives of 1,2,4-triazole, we can identify 5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4--triazole-3-yl-1-(alkyl-, aryl) methanimines (11.4-11.7), which exceeded the antimicrobial and antifungal

Table 2. The results of antimicrobial activity screening for the compounds

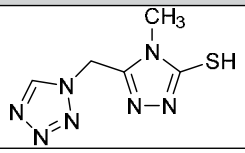
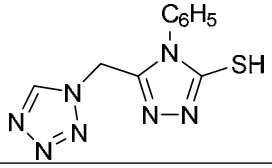
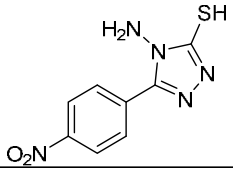
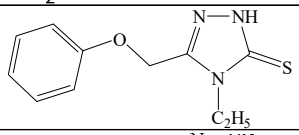
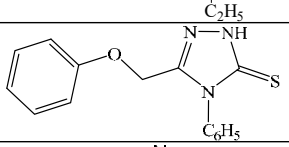
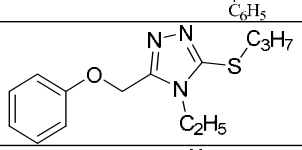
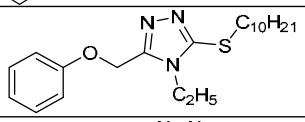
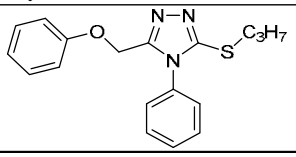
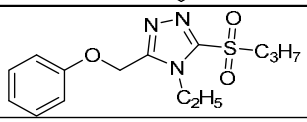
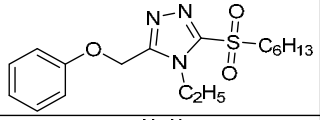
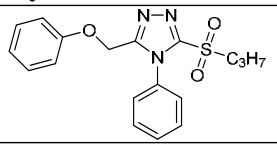
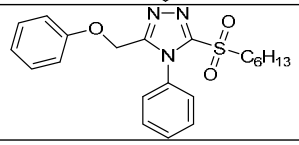
Compound	Formula of compound	Cultures of microorganisms			
		Staphylococcus aureus **Bacteriostatic/ Bactericidal action (μg/ml)**	***Escherichia*** **colli Bacteriostatic/ Bactericidal action (μg/ml)**	***Candida albicans*** **Fungistatic/ Fungicidal action (μg/ml)**	***Pseudomonas aeruginosa*** **Bacteriostatic/ Bactericidal action (μg/ml)**
Ethacridine Lactate		50/400	50/50	25/–	50/50
1.1		50/50	50/100	50/100	100/200
1.2		25/50	50/100	50/100	100/200
1.3		25/50	50/100	50/100	100/200
1.4		50/50	50/100	50/100	100/200
1.5		50/100	100/100	50/100	100/200
2.1		50/100	100/200	100/100	100/200
2.2		50/100	100/100	100/200	100/200
2.3		50/50	100/200	50/100	100/200
3.1		25/50	50/100	100/100	100/200
3.2		50/100	100/200	50/100	100/200
3.3		50/50	100/100	100/50	100/200
3.4		50/100	100/100	100/50	100/100

Table 2. The results of antimicrobial activity screening for the compounds – table continuation

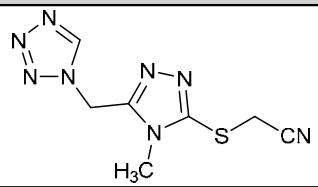
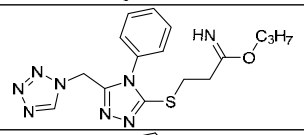
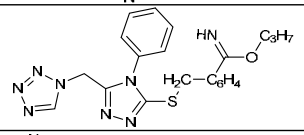
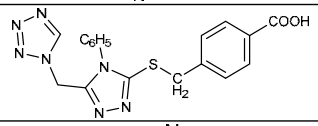
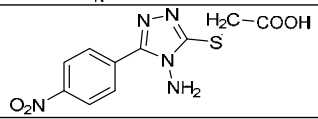
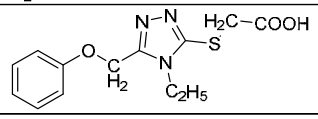
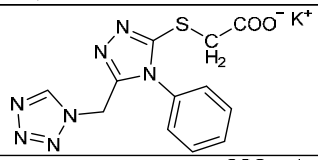
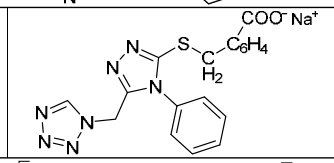
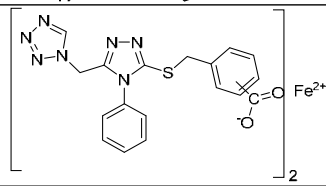
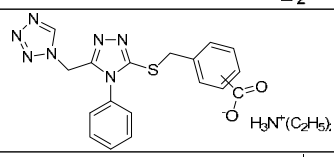
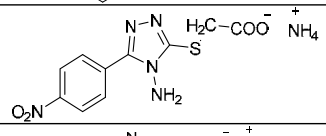
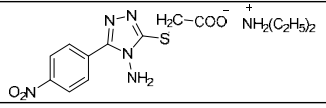
Compound	Formula of compound	Cultures of microorganisms			
		Staphylococcus aureus **Bacteriostatic/ Bactericidal action (μg/ml)**	***Escherichia* colli Bacteriostatic/ Bactericidal action (μg/ml)**	***Candida albicans*** **Fungistatic/ Fungicidal action (μg/ml)**	***Pseudomonas aeruginosa*** **Bacteriostatic/ Bactericidal action (μg/ml)**
Ethacridine Lactate		50/400	50/50	25/–	50/50
4.1		50 /50	100/100	100/100	50/200
5.1		100/200	100/200	50/50	50 /100
5.2		100/200	100/200	50/100	50/50
6.1		50/100	100/200	50/100	50/50
6.2		25/50	50/100	100/100	100/200
6.3		50/50	100/100	50/100	100/200
7.1		50/100	50/100	50/100	50/200
7.2		25/50	50/50	50/100	200/200
7.3		50/50	50/100	50/100	100/200
7.4		25/50	50/100	50/100	100/200
7.5		25 /25	100/200	50/50	100/100
7.6		25/50	100/200	50/100	100/200

Table 2. The results of antimicrobial activity screening for the compounds – table continuation

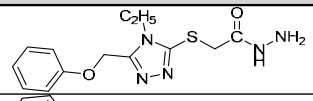
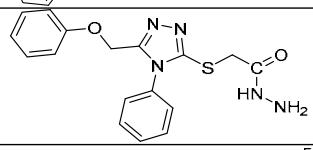
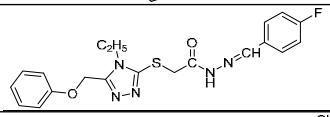
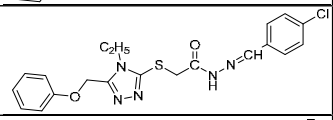
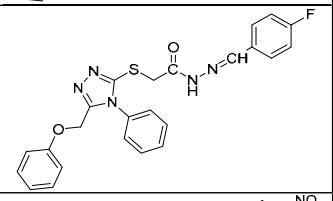
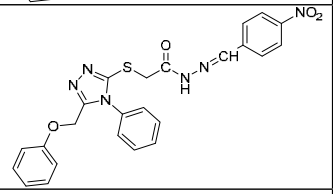
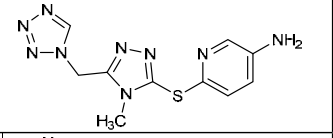
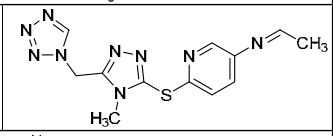
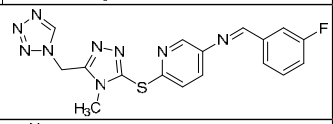
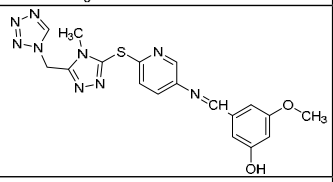
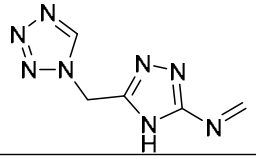
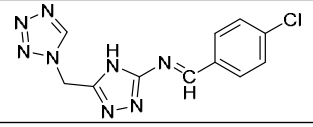
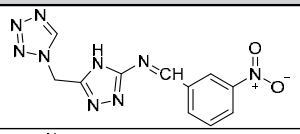
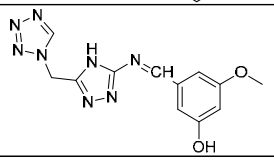
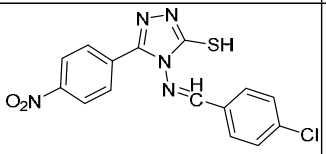
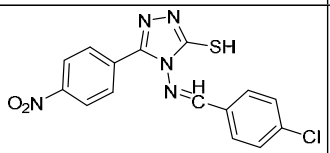
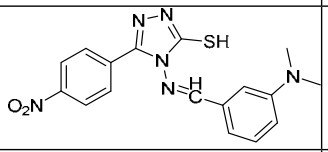
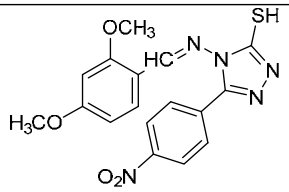
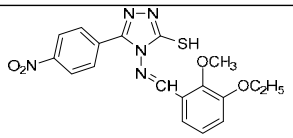
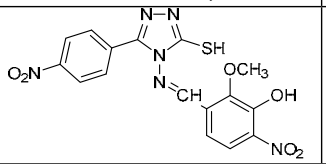
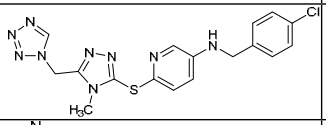
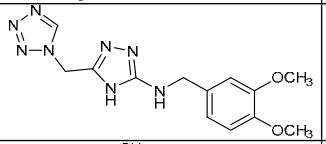
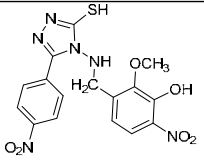
Compound	Formula of compound	Cultures of microorganisms			
		Staphylococcus aureus **Bacteriostatic/ Bactericidal action (μg/ml)**	***Escherichia*** **colli Bacteriostatic/ Bactericidal action (μg/ml)**	***Candida albicans*** **Fungistatic/ Fungicidal action (μg/ml)**	***Pseudomonas aeruginosa*** **Bacteriostatic/ Bactericidal action (μg/ml)**
Ethacridine Lactate		50/400	50/50	25/–	50/50
8.1		25/50	25/50	50/100	25/25
8.2		25/50	100/100	50/100	200/200
9.1		25/50	100/100	50/100	100/200
9.2		25/50	100/100	50/100	100/200
9.3		50/50	100/100	50/50	100/200
9.4		25/50	50/100	50/100	100/200
10.1		25/50	50/50	25/100	100/200
11.1		12,5/50	50/200	50/50	100/200
11.2		25/50	50/100	50/100	100/200
11.3		100/200	100/200	50/100	100/200
11.4		100/200	50/100	100/100	100/200
11.5		50/100	100/100	25/50	50/100

Table 2. The results of antimicrobial activity screening for the compounds – table continuation

Compound	Formula of compound	Cultures of microorganisms			
		Staphylococcus aureus **Bacteriostatic/ Bactericidal action (μg/ml)**	***Escherichia* colli Bacteriostatic/ Bactericidal action (μg/ml)**	***Candida albicans*** **Fungistatic/ Fungicidal action (μg/ml)**	***Pseudomonas aeruginosa*** **Bacteriostatic/ Bactericidal action (μg/ml)**
Ethacridine Lactate		50/400	50/50	25/–	50/50
11.6		12,5/25	50/100	50/50	100/200
11.7		100/200	50/100	25/50	50/100
11.8		25/50	50/50	50/100	200/200
11.9		50/100	50/100	50/100	50/200
11.10		50/50	50/100	50/100	100/200
11.11		25/50	50/100	50/100	100/200
11.12		25/50	50/100	50/100	100/200
11.13		25/50	25/50	50/100	100/200
12.1		25/50	100/100	50/50	200/200
12.2		50/50	100/200	100/100	100/200
12.3		25/50	50/100	50/100	50/200

"–" is lack of activity at a concentration of 400 μg/ml or less

activity of the comparison drug against *Staphyllococcus aureus* and *Candida albicans*. Other synthesized amino-derivatives of 1,2,4-triazole did not exceed ethacridine lactate, and in some cases were even lower.

Among the studied compounds, the most noteworthy was 5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4-triazole--3-yl-1-(5-nitrofuran-2-yl)methanimine (11.6), which exceeded the activity of ethacridine lactate against *Staphylococcus aureus* (12.5/25 μg/ml) and *Escherichia coli* (50/100 μg/ml). According to the results of studies, this substance was recommended for in-depth study.

5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4-triazole-3-yl--1-(5-nitrofuran-2-yl)methanimin (11.6) showed the greatest antimicrobial and antifungal activity. A deeper research for compound 11.6 was made. It was carried out at the Sumy National Agrarian University under the auspices of Doctor of Veterinary Sciences, Professor Fotina Tetyana Ivanivna.

In-depth study of the antimicrobial activity of 5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4-triazole-3-yl-1-(5--nitrofuran-2-yl)methanimine (11.6) was determined by diffusion in agar (method of "wells").

The studies have shown that the molecule of 5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4-triazole-3-yl-1-(5--nitrofuran-2-yl)methanimine (11.6) showed antimicrobial and antifungal action to the studied test strains at a concentration of 2 μg/ml of inhibition zone. The research results are shown in Table 3.

Table 3. Antimicrobial activity of 5-(1H-tetrazole-1-yl)methyl--4H-1,2,4-triazole-3-yl-1-(5-nitrofuran-2-yl)methanimine (11.6)

Test strains	Growth retardation zone, mm
Staphylococcus aureus	18
Escherichia coli	15
Bacillus subtilis	22
Candida famata	14

Conclusion

A novel group of 47 derivatives of 1,2,4-triazoles was successfully tested for antimicrobial and antifungal activities. According to our research, derivatives of 3-amino-1,2,4-triazole showed better performance as compared with 3-thio-1,2.4-triazoles for *Staphylococcus aureus* and *Candida albicans.*

Some classes of 3-thio-1,2,4-triazole, namely salts of 2-, 4- (5-R-4-R_1-1,2,4-triazole-3-ylthio) acetic(benzoic) acids (compounds 7) showed better results than ethacridine lactate in relation to *Staphyllococcus aureus*, other indicators of bacteriostatic action were worse. The best results of fungicidal action against the *Candida albicans* among 3-thio-1,2,4-triazole were shown by substances 8.1 and 9.3.

Considering the amino derivatives of 1,2,4-triazole, we can identify 5-(1*H*-tetrazole-1-yl)methyl-4*H*-1,2,4--triazole-3-yl-1-(alkyl-, aryl) methanimines (11.4-11.7), which exceeded the antimicrobial and antifungal activity of the comparison drug against *Staphyllococcus aureus* and *Candida albicans*.

The present research work revealed that the 5-(1*H*-tetrazole-1-yl-methyl)-4*H*-(1,2,4-triazole-3-yl)-1--(5-nitrofuran-2-yl)methanimine showed the greatest antimicrobial and antifungal activity. Hence, this compound can be developed as useful therapeutic agents after establishing their safety pharmacology and toxicity.

Conflict of interest: none.

References

1. **Shcherbyna R., Panasenko O., Polonets, O., et al.** Synthesis, antimicrobial and antifungal activity of ylidenhydrazides of 2-((4-R-5-R_1-4*H*-1,2,4-triazol-3-yl)thio)acetaldehydes. J. Fac. Pharm. Ankara 2021; 45(3), 504–514.
2. **Morgan D., Okeke I., Laxminarayan R., et al.** Non-prescription antimicrobial use worldwide: a systematic review. The Lancet Infectious Diseases 2011; 11(9), 692–701.
3. World Health Organization. Antimicrobial resistance. Web site. (2018). Retrieved Febriary 15, 2018, from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
4. **Lindberg E., Hammarstrom H., Ataollahy N.** Fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, micafungin, and caspofungin: Species distribution and antifungal drug susceptibilities of yeasts isolated from the blood samples of patients with candidemia. Sci. Rep. 2019; 9(1), 3838–3850.
5. **El-Houssaini H. H., Elnabawy O. M., Nasser, H. A.** Correlation between antifungal resistance and virulence factors in Candida albicans recovered from vaginal specimens. Microb. Pathog. 2019; 128, 13–19.
6. **Rybak V., Kerimova G., Korol V.** Investigation of the anabolic activity of dry extracts of *Iris hungarica* leaves and rhizomes on the model of hydrocortisone-induced protein catabolism. Čes. slov. Farm. 2021; 70, 59–65.
7. **Shcherbyna R., Parchenko V., Varynskyi B., Kaplaushenko A.** The development of HPLC-DAD method for determination of active pharmaceutical ingredient in the potassium 2-((4-amino-5-(morpholinomethyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate substance. Curr. Issues Pharm. Med. Sci. 2019; 32(1), 5–9.
8. **Ihnatova T., Kaplaushenko A., Frolova Yu., Pryhlo E.** Synthesis and antioxidant properties of some new 5-phenethyl-3-thio-1,2,4-triazoles. Pharmacia 2020; 68(1): 129–133.
9. **Varynskyi B., Kaplaushenko A.** The force degradation study of the morpholinium 2-((4-(2-methoxyphenyl)-5--(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)thio) acetate. Indones. J. Pharm. 2019; 30(1), 25–34.
10. **Hulina Y. S., Kaplaushenko A. G.** Synthesis, physical and chemical properties of 5-((1*H*-tetrazole-1-yl)methyl)-4-R--4*H*-1,2,4-triazole-3-thiols and their chemical transformations. Russ. J. Biopharm. 2018; 10(1), 26–30.
11. **Samelyuk Y. G., Kaplaushenko A. G.** Synthesis of 3-alkylthio(sulfo)-1,2,4-triazoles, containing methoxyphenyl

substituents at c5 atoms, their antipyretic activity, propensity to adsorption and acute toxicity. J. Chem. Pharm. Res. 2014; 6(5), 1117–1121.

12. **Shcherbyna R.** An investigation of the pharmacokinetics and potential metabolites of potassium 2-((4-amino--5-(morfolinometyl)-4*H*-1,2,4-triazol-3-yl)thio) acetate on rats. Ankara Universitesi Eczacilik Fakultesi Dergisi 2020; 44(2), 233–241.
13. **Safonov A.** Derivatives of 3-(alkylthio)-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-4-amines as increasing efficiency substances. Indonesian Journal of Pharmacy 2018; 29(3), 167.
14. **Safonov A**. Method of synthesis novel N'-substituted 2-((5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)thio) acetohydrazides. Indones. J. Pharm. 2020; 44 (2), 242-252.
15. Web-service GUSAR on-line, http://www.way2drug.com/gusar/acutoxpredict.html
16. **Prozorovsky V. B.** On the choice of method of construction of the lethality curve and determination of the average lethal dose. J. General Biology 1960; 21(3), 221–228.
17. **Frolova, Y., Kaplaushenko, A., Nagornaya, N.** Design, synthesis, antimicrobial and antifungal activities of new 1,2,4-triazole derivatives containing 1H-tetrazole moiety. Ankara Univ. Eczacilik Fak. Derg. 2020; 44(1), 70–88.
18. **Kucheryavyi Yu. N., Kaplaushenko A. G., Pruhlo E. S.** Synthesis and diuretic activity of 2-(5-(phenoxymethyl)--4-R_1-1,2,4-triazole-3-ylthio)acetic acids and their salts. Zaporozhye Med. J. 2014; 6, 101–104.
19. **Hulina Yu. S., Kaplaushenko A. G.** Synthesis and physical-chemical properties of 6-(5-(1*H*-tetrazole-1--ylmethyl)-4-R-1,2,4-triazole-3-ylthio)pyridin-3-amines and 6-((5-(1H-tetrazole-1-yl)methil-4-R-1,2,4-triazole-3--ylthio)pyridin-3-yl)-(alk,ar,heter)ylmethanimines. Zaporozhye Med. J. 2017; 1, 100–104.
20. **Shcherbak M. A., Kaplaushenko A. G., Maletskiy N. N., Sharaya Ye. A.** The research on creation the dosage form based on 3-(4-nitrophenyl)-5-(nonylsulfonyl)-1,2,4-triazol-4-amine. Zaporozhye Med. J. 2014; 4, 82–85.
21. **Sidorov, K. K.** About the poison toxicity classification in parenteral administration methods. Toksikologiya novykh promyshlennykh veshchestv 1973; 13, 45–71 [in Russian].