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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SYNTHESIS, ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF YLIDENHYDRAZIDES OF 2-((4-R-5-R_1-*4H*-1,2,4-TRIAZOL-3-YL)THIO)ACETALDEHYDES

2-((4-R-5-R_1-4H-1,2,4-TRİAZOL-3-İL)TİYO)ASETALDEHİTLERİN İLİDENHİDRAZİDLERİNİN SENTEZİ, ANTİMİKROBİYAL VE ANTİFUNGAL AKTİVİTESİ

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ABSTRACT

Objective: *The aim of this work is the synthesis and studying of antimicrobial and antifungal properties of the ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes with the establishment of structure-activity relationships of the synthesized compounds.*

Material and Method: *Determination of antimicrobial and antifungal activity was performed by disc-diffusion method (DDM) on series of gram positive and gram negative bacteria such as Corynebacterium pseudodiphtheriticum, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus vulgaris, Escherichia coli, Salmonella spp., Staphylococcus saprophyticus, Staphylococcus aureus, Streptococcus pyogenes, Candida.*

Result and Discussion: *A new series of ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes were synthesized and their structures were confirmed by modern methods of instrumental analysis: IR and ^{1}H-spectroscopy, elemental analysis and GS\MS method. For 13 compounds, the antimicrobial and antifungal activities were studied on 10 strains of microorganisms in 3 different concentrations (0.1; 0.2; 0.5%). According to the results of the study, some regularities of the structure-activity relationship have been established. The obtained results can serve as a basis for the targeted search compounds with pronounced antibacterial action. An increasing the concentration of tested compounds predictably lead to increase on antimicrobial activity in almost all cases. The most active compound in our study is N'-(2-((5-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)-4-nitrobenzohydrazide **3i** (**ZOI** for **Sta.s**. and **Sp** in 0.5% concentration is 25 and 26 mm, respectively).*

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ÖZ

Amaç: *Bu çalışmanın amacı, 2-(4-R-5-R_1-4H-1,2,4-triazol-3-yl)tio)asetaldehitlerin bir dizi ilidenhidrazidinin sentezi ve antimikrobiyal ve antifungal özelliklerinin yapı-etki ilişkisi kurularak incelenmesidir.*

Gereç ve Yöntem: *Antimikrobiyal ve antifungal aktivitenin tespiti, aşağıdaki mikroorganizma türleri için disk-difüzyon yöntemi ile gerçekleştirilmiştir: Corynebacterium pseudodiphtheriticum, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus vulgaris, Escherichia coli, Salmonella spp., Staphylococcus saprophyticus, Staphylococcus aureus, Streptococcus pyogenes, Candida*.

Sonuç ve Tartışma: *Yeni 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)tio)asetaldehitlerin ilidenhidrazidleri sentezlenmiştir. Yapıları modern analiz yöntemleri olan IR, ^{1}H spektroskopisi, element analizi ve GS\MS yöntemi ile doğrulanmıştır. Sentezlenen 13 bileşik için antimikrobiyal aktivite, 3 farklı konsantrasyonda (0.1; 0.2; % 0.5) 10 mikroorganizma suşu üzerinde çalışılmıştır. Çalışmanın sonuçlarına göre, bazı yapı-etki ilişkileri kurulmuştur. Elde edilen sonuçlar, belirgin antibakteriyel etkiye sahip bileşikler için hedeflenen araştırmanın temelini oluşturabilir. Test maddelerinin konsantrasyonunun artırılmasının, neredeyse tüm durumlarda antimikrobiyal etkiyi artıracağı tahmin edilmektedir. Çalışmamızdaki en aktif bileşik N'-(2-((5-metil-4H-1,2,4-triazol-3-il)tiyo)etiliden)-4-nitrobenzohidrazid **3i**'dir (**ZOI** Sta.s. ve Sp. - 25 ve 26 mm % 0,5 konsantrasyonda).*

Anahtar Kelimeler: *Antimikrobiyal aktivite, sentez, 1,2,4-triazol*

INTRODUCTION

From ancient times humanity deals with the enormous amount of diseases [1]. From the report of World Health Organization (WHO) lethal cases, which are caused by pathogenic microorganisms occupy one of the leading places [2]. This problem is especially acute in countries, where economic opportunities do not allow to keep the flashes of infectious diseases under full control. Therefore, the need for new, affordable, and highly active bactericidal drugs is appropriate and justified [2].

This issue was partially solved by the drugs discovery, which capable of destroying pathogenic microflora (antibiotics). This discovery made a real revolution in medicine, and the creation of new drugs provided to doctors the opportunity, in almost 100 percent of cases, to successfully overcome any infectious disease. However, the important fact remains that most popular antibiotics are constantly losing their effectiveness. The reason is - antibiotic resistance of bacteria (ARB) [3]. Improper, irrational, and uncontrolled use of antimicrobial drugs leads to the formation of favorable conditions for the emergence, spread and storage of resistant microorganisms [3]. Balanced and appropriate use of antibiotics usually reduces the risk of ARB, but does not rule out the need to replace obsolete drugs with new, more effective and less toxic. In this aspect, nitrogen-containing systems are very promising class of compounds [4-7]. Triazole derivatives have long been included in world medical practice as highly active antibacterial, neuroleptic, antihypertensive and antispasmodic drugs [8, 9]. Derivatives of 1,2,4-triazole deserve special attention in this issue [10, 11]. A perfect example is posaconazole, voriconazole, itraconazole, which have already established themselves as quite effective antifungal agents [12]. Therefore, given the above information which based on previously obtained results, shows us the priority of drugs design among thio derivatives of 1,2,4-triazole.

MATERIAL AND METHOD

Equipment

The melting points of synthesized compounds were established by the open capillary method on the OptiMelt MPA100 device with platinum RTD sensor (the range of temperature measurements is 30-400°C with 1°C resolution). The elemental analysis was provided by the analyzer Elementar Vario L cube for Carbon, Hydrogen, Nitrogen ad Sulfur (II) with standard - 4-aminobenzenesulfonamide.

The 1H NMR spectra (400 MHz) were obtained from Varian MR-400 spectrometer (DMSO-d6 as a solvent) analyzed by ADVASP Analyzer program. IR-spectra (4000-400 cm-1) were taken off on the module of ALPHA-T (KBr) of spectrometer of Bruker ALPHA FT-IR. The individuality and purity of the targeting compounds were established by the gas chromatograph system Agilent 7890B with a 5977B mass spectrometry detector. The column is DB-5ms 30 m x 250 μm x 0.25 μm with length.

Chemical part

The initial compounds 2-((4R-5R1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes 2a-c were synthesized at the Department Of Natural Sciences For Foreign Students And Toxicological Chemistry of the Zaporizhzhya State Medical University (Ukraine) and purified by recrystallization with content of the main component ≥ 98%. All chemicals were obtained from UKRORGSYNTEZ Ltd (Kyiv, Ukraine) with documental approving of its purity and quality.

Biological Part

The determination of antimicrobial and antifungal activities was conducted by disc-diffusion method (DDM) on different strains of microorganisms: Corynebacterium of pseudodiphtheriticum ATCC 10700, Pseudomonas of aeruginosa ATCC 27853, Enterococcus of faecalis ATCC 29212, Proteus of vulgaris ATCC 13315, Escherichia of coli ATCC 25922, Salmonella of spp. ATCC 35664 Staphylococcus saprophyticus ATCC 15305, Staphylococcus of aureus ATCC 25923, Streptococcus of pyogenes ATCC 19615, Candida (Table 1). The studies were performed according to the current instructions for medical use of discs with antibiotics to determine the sensitivity of microorganisms to drugs [13-16]. Standard disks, previously sterilized and impregnated with solutions of test substances (in DMSO), were used for the experiment. Mueller-Hinton nutrient agar was used to determine sensitivity. The agar was poured into 15 ml sterile Petri dishes with a diameter of 100 mm, located on a horizontal surface. Before infection, the surface of the solidified agar was dried for 30-40 minutes.

The inoculum was prepared from pure 18-20-hour cultures of bacteria grown on the surface of a dense nutrient medium. Next, 5-10 isolated colonies were suspended in a liquid nutrient medium or in isotonic sodium chloride solution. The suspension or broth culture was diluted with isotonic sodium chloride solution to a turbidity of the optical standard by 10 units, and then the resulting mixture was diluted again 20 times.

Table 1. Antibacterial and antifungal activity ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes (**3a-3m)**

Zone of inhibition around the disks, mm	Compound / Strains	*Concentration*	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l	3m	Tobr[y]	Nyst[y]
	Corynebacterium pseudodiphtheriticum **ATCC 10700**[x]	**0.1** %	–[z]	**14**	–	–	–	–	–	–	**12**	–	–	**5**	–	**23**	–
		0.2 %	–	**19**	–	**5**		–	–	–	**15**	–	–	**7**	–		
		0.5 %	**7**	**24**	–	**9**	**7**	–	–	–	**18**	–	**6**	**12**	–		
	Pseudomonas aeruginosa **ATCC 27853**[x]	**0.1** %	**6**	–	–	–	–	–	–	–		–	–	–	–	**26**	–
		0.2 %	**15**	–	**7**	–	**9**	–	**5**	–	**8**	–	–	**7**	–		
		0.5 %	**19**	–	**16**	**5**	**14**	**9**	**13**	–	**11**	–	–	**10**	–		
	Enterococcus faecalis **ATCC 29212**[x]	**0.1** %	**5**	–	–	–	–	–	–	–	**5**	–	–	–	–	**19**	–
		0.2 %	**7**	–	–	–	–	–	–	–	**6**	–	–	–	–		
		0.5 %	**10**	–	–	–	–	–	**8**	–	**10**	–	–	–	–		
	Proteus vulgaris **ATCC 13315**[x]	**0.1** %	**9**	–	–	–	–	–	–	–	**5**	–	**6**	–	–	**14**	–
		0.2 %	**17**	–	–	–	–	–	–	–	**8**	–	**8**	–	–		
		0.5 %	**23**	–	–	–	–	–	–	–	**13**	–	**9**	–	–		
	Escherichia coli **ATCC 25922**[x]	**0.1** %	–	–	–	–	–	–	–	**12**	–	–	–	–	–	**25**	–
		0.2 %	–	–	–	–	**5**	–	–	**17**	**6**	–	–	–	–		
		0.5 %	**12**	–	**9**	–	**8**	–	–	**26**	**9**	–	**6**	–	**5**		
	Salmonella spp. **ATCC 35664**[x]	**0.1** %	–	–	–	–	–	–	–	–	–	–	–	–	–	**12**	–
		0.2 %	–	–	–	–	–	–	–	–	–	–	–	–	–		
		0.5 %	**6**	–	–	–	–	–	–	–	–	–	–	–	–		
	Staphylococcus saprophyticus **ATCC 15305**[x]	**0.1** %	**9**	**12**	–	**7**	–	–	–	–	**12**	–	–	**5**	–	**21**	–
		0.2 %	**13**	**16**	**8**	**11**	**10**	–	**10**	**7**	**17**	**10**	–	**9**	–		
		0.5 %	**19**	**19**	**13**	**14**	**11**	–	**15**	**9**	**25**	**14**	–	**13**	**7**		
	Staphylococcus aureus **ATCC 25923**[x]	**0.1** %	**5**	**10**	–	**7**	–	–	–	–	**7**	–	–	–	–	**29**	–
		0.2 %	**10**	**16**	–	**12**	–	–	–	–	**10**	–	–	–	–		
		0.5 %	**13**	**18**	**7**	**16**	–	–	**5**	–	**10**	**8**	–	**5**	–		
	Streptococcus pyogenes **ATCC 19615**[x]	**0.1** %	–	**9**	–	–	**5**	–	**7**	–	**10**	–	–	–	–	**20**	–
		0.2 %	**9**	**16**	**9**	**6**	**8**	–	**10**	–	**19**	–	–	–	–		
		0.5 %	**20**	**22**	**14**	**12**	**8**	–	**13**	–	**26**	**6**	–	–	**9**		
	Candida albicans **ATCC 2091**[x]	**0.1** %	–	–	–	–	–	–	–	–	–	**14**	–	–	–	–	**29**
		0.2 %	–	–	–	–	–	–	–	–	–	–	–	–	–		
		0.5 %	**9**	–	**11**	–	–	–	–	–	–	–	–	–	–		

[x] **C.p.** - *Corynebacterium pseudodiphtheriticum* **ATCC 10700, P.a.** - *Pseudomonas aeruginosa* **ATCC 27853, E.f.** - *Enterococcus faecalis* **ATCC 29212, P.v.** - *Proteus vulgaris* **ATCC 13315, E.c.** - *Escherichia coli* **ATCC 25922, Sal.s.**- Salmonella spp. **ATCC 35664, Sta.s.** - *Staphylococcus saprophyticus* **ATCC 15305, S.a.** - *Staphylococcus aureus* **ATCC 25923, S.p.** - *Streptococcus pyogenes* **ATCC 19615, C.** – *Candida albicans* **ATCC 2091**.

[z] – means «not active»

[y] **Tobramycin** disks 10 μg

[y] **Nystatin** NS 100 Units

The inoculum, with a total volume of 1 ml, was applied to the surface of the agar medium and evenly distributed by shaking it. The remaining liquid was removed with a pipette. Semi-open Petri dishes were dried at room temperature for 10-15 minutes.

The discs were placed with tweezers on the surface of the contaminated nutrient medium at equal distances from each other and at approximately distance of 2 cm from the edge of the cup. The plates were incubated in a thermostat for 18-20 hours at t 35-37 0C, with the bottom upside down.

Using a ruler or meter (caliper) the zone of inhibition around the disks, including the diameter of the disks to the nearest 1 mm was measured. At not sharply delineated edges of zones, or zones with

double contours measurements of a zone on the clearest contour were carried out.

The discs with Tobramycin (SD044 Tobramycin 10 µg) and Nystatin (SD025 Nystatin NS 100 Units) manufactured by HiMedia Laboratories Pvt. Ltd. were used as a reference drug.

RESULT AND DISCUSSION

Chemical Part

For the synthesis of ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes (**3a-m)** in quality of an initial matter 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes **2a-c** were used. The reaction of producing products **3a-m** were conducted by condensation of **2a-c** compounds with acetohydrazide **(3a-c)**, benzohydrazide **(3d, 3e)**, 3-nitrobenzohydrazide **(3f-h)**, 4-nitrobenzohydrazide **(3i, 3j)**, nicotinohydrazide **(3k)** and isonicotinohydrazide **(3l, 3m).** The structure confirmation of the synthesized compounds (**3a-m)** was released by: **IR** (Infrared of spectroscopy), **^{1}H-NMR** (Proton Nuclear Magnetic Resonance Spectroscopy), **EA** (Elemental of analyses). Individuality and purity of compounds were confirmed by the method of **GS/MS** (gas chromatography-mass spectrometry) (Figure 1).

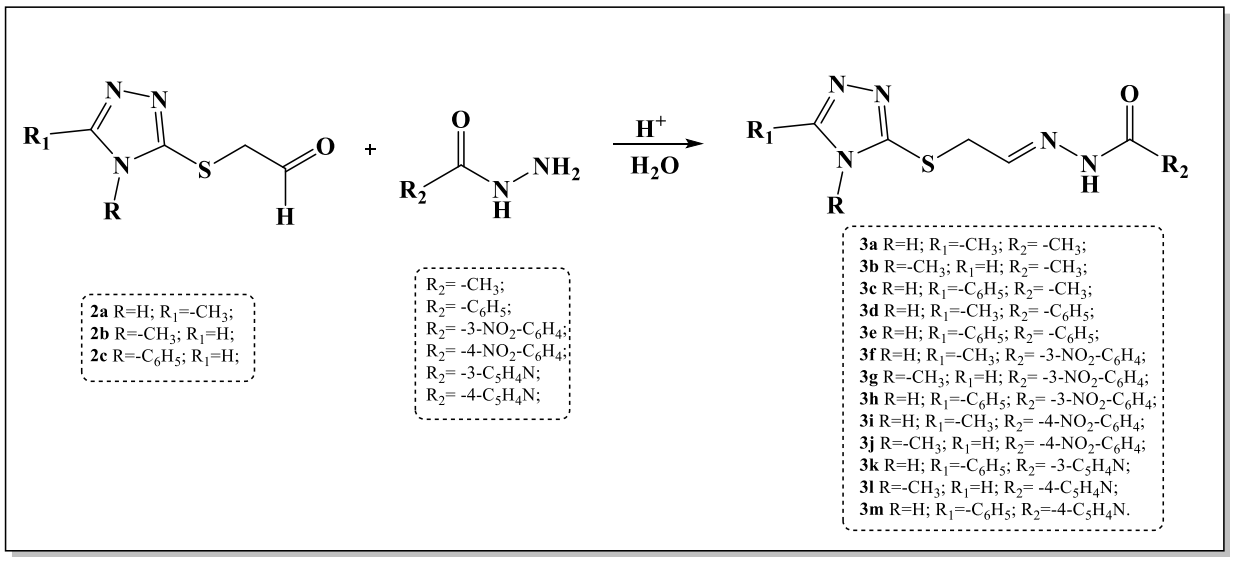

Figure 1. The synthesis of ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes (**3a-3m)**

The obtained data of elemental analysis values are corresponding to a theoretical data. Moreover, the individuality and molecular weight of compounds **3a-3m** are confirmed by GS/MS. In obtained chromatograms, individual peaks of the synthesized compounds are registered, and the mass spectra of these compounds showed a molecular peak matching to the theoretical mass.

In the IR-spectra of **3a-3m** compounds are present peaks of groups absorption in range 1644-1598 cm^{-1} and 1685-1663 cm^{-1}, which belong to –C=N– and >C=O groups accordingly. The absorption peaks

of –C–S– groups are also present at 706-682 cm^{-1} and the bars of absorption are absent within the limits of 2600-2550 cm^{-1} that can testify to the absence in the molecule of – SH groups.

In the IR-spectra of 3f-3j compounds found out the characteristic vibrations of aromatic O2N-groups within the range of 1351-1343 cm^{-1}. Compounds of 3k- 3m own the characteristic valency vibrations of pyridine cycle within the limits of 3041-3048 cm^{-1}.

In ^{1}H NMR spectra of **3a, 3b, 3d, 3f, 3g, 3h, 3i, 3j, 3l** compounds proton –CH= group resonate by triplet within 7.50-7.59 ppm, in compounds **3c, 3e, 3m** in the kind of multiplets displacement is determined within 7.50-7.53 ppm. It is important to note that signals of proton-NH- group in the remnants of hydrazides of carbonic acids are specific to synthesized compounds and resonate by monoproton singlets within 7.0-6.89 ppm. The exception is **3j** compound**,** where there is the shielding of proton-NH- group by multiplet of protons of aromatic cycle on the 7.26 ppm.

Methylene protons of -S-CH_2-group are displayed as a singlet 1.75 ppm. (**3j**) and doublets within 1.91, 1.81, 0.87, 3.16, 1.60 ppm. (**3e**, **3g, 3k, 3l, 3m**). Analysing **3b** and **3c** compounds we noted that singlets of protons of methyl group in the remnants of hydrazide of acetic acid resonate within 0.86 and 1.16 ppm accordingly. In **3g** and **3l** compounds are the displacement of protons of methyl group of 1,2,4-triazole cycle in as a singlet with the meaning of 3.60 and 2.77 ppm. However, in **3j** compound the given protons resonate as a doublet with the meaning 3.60 ppm.

The protons of 4-pyridine cycle show up as doublets: 8.59, 7.30 ppm (compound **3l)** and 8.59, 7.31 ppm (compound **3m)**. In ^{1}H of NMR spectrum of compound **3k** the signals of pyridine-3-cycle protons are present as a triplet (8.49 ppm), multiplet (7.75 ppm) and doublet (7.48 ppm).

Biological Part

Analysing the obtained data from Table 1 we can consider that new derivatives of 1,2,4-triazole shows selective antimicrobial activity. The compounds **3a** and **3i** deserves special attention. Thus, **3a** compound can inhibit considerably the growth of 4 species of microorganisms (**P.a., Sta.s., S.a. S.p.)** starting from 0,2% concentration. It is necessary to note that namely above compound in 0,5% concentration is active against ***P.v*** with zone of inhibition (**ZOI**) around the disks 23 mm. Paying attention to the substance **3i**, it can be stated that it is in maximum concentration quite active against **Sta.s**. and **S.p**. (**ZOI** 25 and 26 mm). Such results of the antimicrobial activity of compounds **3a** and **3i** are most likely caused by the positive effect of the starting aldehyde molecule (**2a**). Replacement of the methyl radical (**3a**) by the 4-nitrophenyl (**3i**) at the R_2 position leads to an increasing of the strength of antimicrobial activity against **Sta.s**. and **S.p.,** but the breadth of the spectrum of antibacterial action is reduced. Changing the location of the methyl radical from C_5 (**3a**) to N_4 (**3b**) of the 1,2,4-triazole ring is also accompanied by a narrowing of the spectrum of antimicrobial action. Instead, there is a pronounced antimicrobial effect against **C.p.** (**3b**). Replacement of the methyl radical (**3a, 3c**) with phenyl (**3d, 3e**) in acid hydrazides leads to a decreasing of antimicrobial activity.

A rather interesting picture emerges in the detailed analysis of compounds **3f, 3g** and **3h**. From a chemical point of view, these condensed systems differ only in the substituents at the N_4 and C_5 atoms of the 1,2,4-triazole ring. In contrast to compounds **3a** and **3b**, the change of methyl radical from R position (**3g**) to R_1 position (**3f**) in ylidenehydrazide molecules leads to a weakening of the antimicrobial action, but the replacement of the same radical (**3f**) by phenyl fragment (**3h**) gives a selective antimicrobial activity against **E.c.**. It should be noted that this effect of the phenyl substituent at the C_5 atom of 1,2,4-triazole is not unambiguous. Thus, comparing compounds **3c** and **3h**, it can be concluded that the selective bacteriostatic effect is manifested precisely by the introduction of the 3-nitrophenyl radical in R_2 position.

During interpretation of the obtained results, attention should be paid to the influence of the position of the aromatic nitro group on the results of biological screening. It is interesting to note that the transition from the meta- (**3g**) to para- (**3j**) position of the nitro group has almost no effect on the intensity of antimicrobial action, but in case with compounds **3f** and **3i** such a transition is accompanied by a wide range of antimicrobial action. Also, it is possible to state that the introduction of 3-, 4-pyridyl substituents into synthesized molecules, unfortunately, does not lead to the manifestation of high results of antimicrobial action. The biological screening did not reveal compounds that are able to sufficiently inhibit the growth of the **C.**

General Produce of the Ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes (3a-3m)

To 0.01 mol of compound initial aldehydes **2a-2c** in 20 ml of concentrated acetic acid the 0.01 mol of corresponding hydrazide were added and heated to boiling. Then, solution set at room temperature for 12 hours to form a precipitate, which filtered, washed with diethyl ester and dried at air. For further studies, the obtained substances for analysis are recrystallized from acetic acid.

N'-(2-((5-methyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)acetohydrazide **3a*:*** Yellow solid with 81% yield, m.p. 189-191 ^oC; IR (KBr) cm^{-1}: 3084 (aromatic CH); 1687 (C=O); 1618 (-C=N-); 1594 (N-H+C-N); 688 (-C-S-); ^1H NMR (400 Mz, $DMSOd_6$) δ ppm: 7.79 (s, 1H, 1,2,4-triazole-H), 7.51 (t, 1H, -CH=N-), 6.90 (s, 1H, =N-NH-), 3.78 (s, 3H, $-CH_3$), 3.25 (d, 2H, $-CH_2$), 1.71 (s, 3H, $-C(O)CH_3$); GS/MS: 213 (m/z); Anal. Calcd. for $C_7H_{11}N_5OS$: C, 39.42; H, 5.20; N, 32.84; S, 15.05. Found: C, 39.76; H, 5.24; N, 32.95; S, 15.09.

N'-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)acetohydrazide **3b***:* Yellow solid with 65% yield, m.p. 210-212 ^oC; IR (KBr) cm^{-1}: 3089 (aromatic CH); 1685 (C=O); 1620 (-C=N-); 1591 (N-H+C-N); 682 (-C-S-); ^1H NMR (400 Mz, $DMSOd_6$) δ ppm: 7.71 (s, 1H, 1,2,4-triazole-H), 7.53(t, 1H, -CH=N-), 6.94 (s, 1H, =N-NH-), 3.72 (s, 3H, $-CH_3$), 3.29 (d, 2H, $-CH_2$), 1.74 (s, 3H, $-C(O)CH_3$); GS/MS: 213 (m/z); Anal. Calcd. for $C_7H_{11}N_5OS$: C, 39.42; H, 5.20; N, 32.84; S, 15.05. Found: C, 39.55; H, 5.18; N, 32.74; S, 15.08.

N'-(2-((5-phenyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)acetohydrazide **3c***:* Yellow solid with 80% yield, m.p. 240-242 °C; IR (KBr) cm^{-1}: 3090 (aromatic CH); 1680 (C=O); 1644 (-C=N-); 1578 (N-H+C-N); 693 (-C-S-); ^{1}H NMR(400 Mz, $DMSOd_6$) δ ppm: 13.49 (s, 1H, NH-triazole), 8.09 (d, 2H, Ar), 7.54 (m, 3H, Ar), 7.01(t, 1H, -CH=N-), 6.69 (s, 1H, =N-NH-), 3.97 (d, 2H, $-CH_2$), 2.01 (s, 3H, $-CH_3$); GS/MS: 275 (m/z); Anal. Calcd. for $C_{12}H_{13}N_5OS$: C, 52.35; H, 4.76; N, 25.44; S, 11.65. Found: C, 52.28; H, 4.75; N, 25.49; S, 11.61.

N'-(2-((5-methyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)benzohydrazide **3d***:* Yellow solid with 79% yield, m.p. 202-204 °C; IR (KBr) cm^{-1}: 3079 (aromatic CH); 1684 (C=O); 1614 (-C=N-); 1571 (N-H+C-N); 681 (-C-S-); ^{1}H NMR(400 Mz, $DMSOd_6$) δ ppm: 13.57 (s, 1H, NH-triazole), 8.31 (d, 2H, Ar), 8.19 (d, 2H, Ar), 7.65 (t, 1H, Ar), 7.20 (t, 1H, -CH=N-), 7.04 (s, 1H, =N-NH-), 3.75 (s, 3H, $-CH_3$), 3.21 (d, 2H, $-CH_2$); GS/MS: 275 (m/z); Anal. Calcd. for $C_{12}H_{13}N_5OS$: C, 52.35; H, 4.76; N, 25.44; S, 11.65. Found: C, 52.29; H, 4.81; N, 25.48; S, 11.60.

N'-(2-((5-phenyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)benzohydrazide **3e***:* Orange solid with 79% yield, m.p. 179-181 °C; IR (KBr) cm^{-1}: 3075 (aromatic CH); 1686 (C=O); 1613 (-C=N-); 1573 (N-H+C-N); 689 (-C-S-); ^{1}H NMR(400 Mz, $DMSOd_6$) δ ppm: 13.52 (s, 1H, NH-triazole), 8.43 (d, 2H, Ar), 8.15 (d, 2H, Ar), 7.74 (m, 6H, Ar), 7.22 (t, 1H, -CH=N-), 7.00 (s, 1H, =N-NH-), 3.19 (d, 2H, $-CH_2$); GS/MS: 337 (m/z); Anal. Calcd. for $C_{17}H_{15}N_5OS$: C, 60.52; H, 4.48; N, 20.76; S, 9.50. Found: C, 60.38; H, 4.50; N, 20.82; S, 9.48.

N'-(2-((5-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)-3-nitrobenzohydrazide **3f***:* Brown solid with 68% yield, m.p. 156-158 °C; IR (KBr) cm^{-1}: 3504 (aromatic NH); 3089 (aromatic CH); 1684 (C=O); 1612 (-C=N-); 1574 (N-H+C-N); 1524, 1356 (aromatic NO_2) 701 (-C-S-); ^{1}H NMR (400 Mz, $DMSOd_6$) δ ppm: 8.17 (s, 1H, 1,2,4-triazole-H), 7.65 (m, 4H, Ar), 7.33 (t, 1H, -CH=N-), 7.01 (s, 1H, =N-NH-), 3.50 (s, 3H, $-CH_3$), 3.04 (d, 2H, $-CH_2$); GS/MS: 320 (m/z); Anal. Calcd. for $C_{12}H_{12}N_6O_3S$: C, 44.99; H, 3.78; N, 26.24; S, 10.01. Found: C, 45.13; H, 3.74; N, 26.19; S, 10.06.

N'-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)-3-nitrobenzohydrazide **3g***:* Red solid with 81% yield, m.p. 222-224 °C; IR (KBr) cm^{-1}: 3500 (aromatic NH); 3087 (aromatic CH); 1680 (C=O); 1610 (-C=N-); 1569 (N-H+C-N); 1528, 1351 (aromatic NO_2) 706 (-C-S-); ^{1}H NMR (400 Mz, $DMSOd_6$+CCl_4) δ ppm: 8.14 (s, 1H, 1,2,4-triazole-H), 7.69 (m, 4H, Ar), 7.37 (t, 1H, -CH=N-), 7.06 (s, 1H, =N-NH-), 3.59 (s, 3H, $-CH_3$), 3.01 (d, 2H, $-CH_2$); GS/MS: 320 (m/z); Anal. Calcd. for $C_{12}H_{12}N_6O_3S$: C, 44.99; H, 3.78; N, 26.24; S, 10.01. Found: C, 44.80; H, 3.76; N, 26.20; S, 10.09.

N'-(2-((5-phenyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)-3-nitrobenzohydrazide **3h***:* Red solid with 83% yield, m.p. 185-187 °C; IR (KBr) cm^{-1}: 3505 (aromatic NH); 3089 (aromatic CH); 1681 (C=O); 1612 (-C=N-); 1563 (N-H+C-N); 1532, 1348 (aromatic NO_2) 702 (-C-S-); ^{1}H NMR (400 Mz, $DMSOd_6$) δ ppm: 8.49 (d, 2H, Ar), 8.19 (s, 1H, 1,2,4-triazole-H), 8.11 (d, 2H, Ar), 7.64 (m, 5H, Ar), 7.35 (t, 1H, -CH=N-),

7.04 (s, 1H, =N-NH-), 3.09 (d, 2H, -CH_2); GS/MS: 382 (m/z); Anal. Calcd. for $C_{17}H_{14}N_6O_3S$: C, 53.40; H, 3.69; N, 21.98; S, 8.39. Found: C, 53.17; H, 3.72; N, 21.81; S, 8.47.

N'-(2-((5-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)-4-nitrobenzohydrazide **3i*:*** Yellow solid with 74% yield, m.p. 194-196 ^oC; IR (KBr) cm^{-1}: 3331 (aromatic NH); 3270 (-CH_3); 1670 (C=O); 1604 (-C=N-); 1548 (N-H+C-N); 1508, 1341 (aromatic NO_2), 690 (-C-S-); ^1H NMR (400 Mz, $DMSOd_6$) δ ppm: 8.59 (s, 1H, 1,2,4-triazole-H), 8.21 (d, 1H, Ar), 8.01 (d, 1H, Ar), 7.52 (t, 1H, -CH=N-), 7.09 (s, 1H, =N-NH-), 3.84 (s, 3H, -CH_3), 3.60 (d, 2H, -CH_2); GS/MS: 320 (m/z); Anal. Calcd. for $C_{12}H_{12}N_6O_3S$: C, 44.99; H, 3.78; N, 26.24; S, 10.01. Found: C, 44.80; H, 3.82; N, 26.35; S, 10.05.

N'-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)-4-nitrobenzohydrazide **3j*:*** Orange solid with 71% yield, m.p. 119-121 ^oC; IR (KBr) cm^{-1}: 3327 (aromatic NH); 3274 (-CH_3); 1674 (C=O); 1599 (-C=N-); 1555 (N-H+C-N); 1515, 1343 (aromatic NO_2), 697 (-C-S-); ^1H NMR (400 Mz, $DMSOd_6$) δ ppm: 8.55 (s, 1H, 1,2,4-triazole-H), 8.28 (d, 1H, Ar), 8.17 (d, 1H, Ar), 7.53 (t, 1H, -CH=N-), 7.04 (s, 1H, =N-NH-), 3.80 (s, 3H, -CH_3), 3.62 (d, 2H, -CH_2); GS/MS: 320 (m/z); Anal. Calcd. for $C_{12}H_{12}N_6O_3S$: C, 44.99; H, 3.78; N, 26.24; S, 10.01. Found: C, 45.17; H, 3.76; N, 26.34; S, 9.94.

N'-(2-((5-phenyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)nicotinohydrazide **3k***:* Grey solid with 91% yield, m.p. 182-184 ^oC; IR (KBr) cm^{-1}: 3041 (pyridine); 1679 (C=O); 1645 (-C=N-); 1568 (N-H+C-N); 686 (-C-S-); ^1H NMR(400 Mz, $DMSOd_6$+CCl_4) δ ppm: 13.53 (s, 1H, NH-triazole), 8.64 (d, 2H, Ar), 8.51 (s, 1H, pyridine-H), 7.59 (m, 3H, Ar), 7.39 (t, 1H, -CH=N-), 6.96 (m, 3H, pyridine-H), 6.42 (s, 1H, =N-NH-), 1.61 (d, 2H, -CH_2); GS/MS: 338 (m/z); Anal. Calcd. for $C_{16}H_{14}N_6OS$: C, 56.79; H, 4.17; N, 24.84; S, 9.49. Found: C, 56.65; H, 4.13; N, 24.79; S, 9.54.

N'-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethylidene)isonicotinohydrazide **3l*:*** Grey solid with 84% yield, m.p. 232-234 ^oC; IR (KBr) cm^{-1}: 3048 (pyridine); 1663 (C=O); 1598 (-C=N-); 1557 (N-H+C-N); 699 (-C-S-); ^1H NMR (400 Mz, $DMSOd_6$) δ ppm: 8.54 (d, 2H, pyridine-H), 8.25 (s, 1H, 1,2,4-triazole-H), 7.53 (t, 1H, -CH=N-), 7.22 (d, 2H, pyridine-H), 6.95 (s, 1H, =N-NH-), 3.15 (d, 2H, -CH_2), 2.78 (s, 3H, -CH_3); GS/MS: 276 (m/z); Anal. Calcd. for $C_{11}H_{12}N_6OS$: C, 47.81; H, 4.38; N, 30.41; S, 11.60. Found: C, 47.65; H, 4.41; N, 30.30; S, 11.54.

N'-(2-((5-phenyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)isonicotinohydrazide **3m***:* Yellow solid with 93% yield, m.p. 170-172 ^oC;IR (KBr) cm^{-1}: 3042 (pyridine); 1681 (C=O); 1639 (-C=N-); 1572 (N-H+C-N); 690 (-C-S-); ^1H NMR (400 Mz, $DMSOd_6$ δ ppm: 13.51 (s, 1H, NH-triazole), 9.06 (d, 2H, pyridine-H), 8.71 (d, 2H, Ar), 8.41 (d, 2H, pyridine-H), 7.69 (m, 3H, Ar), 7.42 (t, 1H, -CH=N-), 6.94 (s, 1H, =N-NH-), 3.09 (d, 2H, -CH_2); GS/MS: 338 (m/z); Anal. Calcd. for $C_{16}H_{14}N_6OS$: C, 56.79; H, 4.17; N, 24.84; S, 9.48. Found: C, 56.91; H, 4.19; N, 24.71; S, 9.41.

As a result of this work, the new series of ylidenhydrazides of 2-((4-R-5-R_1-4H-1,2,4-triazol-3-yl)thio)acetaldehydes were synthesized. The antimicrobial activity was studied for 13 new compounds on 10 strains of microorganisms in 3 different concentrations (0.1; 0.2; 0.5%). According to the results

of the study, some regularities the dependence "structure-activity" have been established. The obtained results can serve as a basis for the targeted search for compounds with pronounced antibacterial action. There is no doubt that the nature of microorganisms, chemical structure and antimicrobial variations are interrelated. Increasing the concentration of test substances is predicted increasing the antimicrobial activity in almost all cases. The most active compound in our study is N'-(2-((5-methyl-1H-1,2,4-triazol-3-yl)thio)ethylidene)-4-nitrobenzohydrazide **3i** (ZOI **Sta.s.** and **S.p** in 0.5% at the level of 25 and 26 mm, respectively).

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AUTHOR CONTRIBUTIONS

Concept: *R.S., O. PAN*; Design: *O. POL., N.N., M.D.*; Control: *R.S., O. PAN.*; Sources: *O. POL., N.N.*; Materials: *M.D.*; Data Collection and / or Processing: *R.S., O. PAN, O. POL., N.N., M.D.*; Analysis and / or Interpretation: *O. PAN., O. POL.*; Literature Review: *M.D.*; Manuscript Writing: *R.S.*; Critical Review: *R.S., O. PAN, O. POL., N.N., M.D.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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