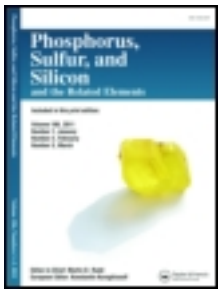

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis and antimicrobial evaluation of new Schiff base hydrazones bearing 1,2,4-triazole moiety

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Synthesis and antimicrobial evaluation of new Schiff base hydrazones bearing 1,2,4-triazole moiety

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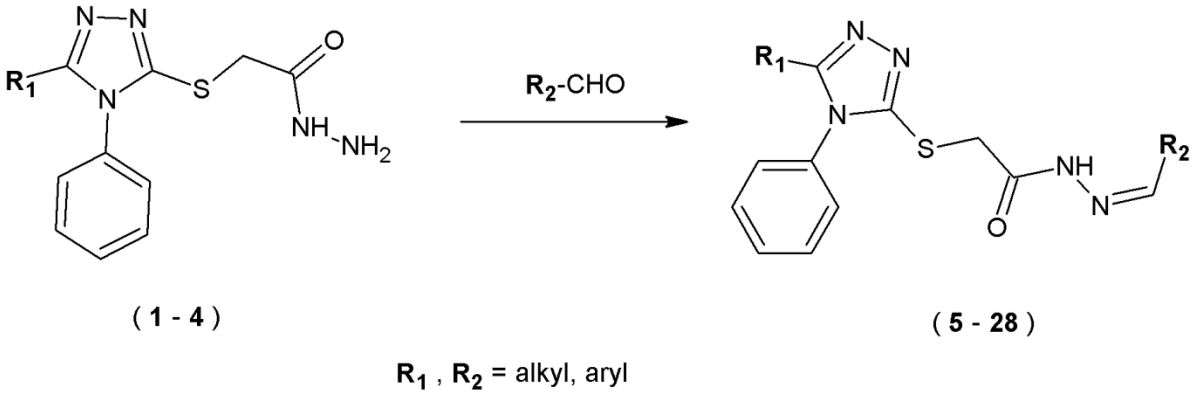
Abstract

This study presents the synthesis and spectral analysis of new Schiff base hydrazone derivatives. New compounds were prepared by the reaction of [(4-phenyl-5-substituted-4*H*-1,2,4-triazol-3-

yl)sulfanyl]acetohydrazide with various aldehydes. The structures of the prepared compounds was confirmed by means of ^{1}H NMR, ^{13}C NMR and elemental analyses. All synthesized compounds were screened for their *in vitro* antimicrobial activity by using the agar dilution technique against 4 reference strains of Gram-positive and Gram-negative bacteria and 12 yeasts belonging to *Candida spp*.



(1 - 4) (5 - 28)

R_1 , R_2 = alkyl, aryl

Keywords: antimicrobial activity, Schiff base hydrazone derivatives, 1,2,4-triazole derivatives

Introduction

The Schiff base hydrazone nucleus has attracted great and growing interest in chemistry and biology for many years due to its facile synthesis and wide applications [1-4].

We report here the synthesis and spectral, as well as antimicrobial characterization, of new Schiff base hydrazones obtained by the condensation reaction of substituted acetohydrazides containing 1,2,4-triazole system with various aldehydes.

Schiff bases have gained importance because of their pharmacological properties such as antibacterial [5-7], antifungal [8-10], anticancer [11, 12] and antiviral agents [13, 14]. Similarly, the occurrence of 1,2,4-triazole system in numerous biologically active molecules has been recognized to possess activities such as analgesic [15], antibacterial [16-18], antifungal [19, 20], anti-inflammatory [21], antitumor [22, 23] and antitubercular [24].

Additionally, some of the present day drugs such as Ribavirin (antiviral agent) [25], Rizatriptan (anitmigraine agent) [26], Alprazolam (anxiolytic agent) [27], Fluconazole and Intraconazole (antifungal agents) [28] are the best examples of potent molecules possessing triazole nucleus.

Since the 1,2,4-triazole moiety seems to be a possible pharmacophore in various pharmacologically active agents, we decided to synthesize compounds with this function coupled with Schiff bases. The choice of these ligands was motivated by two objectives. First, to provide

new 1,2,4-triazole derivatives and to enrich the biological study of Schiff base hydrazones containing 1,2,4-triazole moieties.

In the view of these facts, the aim of this study is to obtain new Schiff base hydrazone derivatives incorporating the 1,2,4-triazole moiety as antimicrobial agents with a view to obtain better therapeutic results.

Results and discussion

Chemistry

The synthetic pathway leading to the new Schiff base hydrazone derivatives **5-28** was carried out according to the steps shown in Scheme 1. The substituents of compounds **5-28** are presented in Table 1. In the initial step, ethyl [(4-phenyl-5-substituted-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetates were synthesized via reaction of 4-phenyl-5-substituted-4*H*-1,2,4-triazol-3-thiones with ethyl bromoacetate in the presence of sodium ethanolate. The treatment of esters with hydrazine hydrate afforded the corresponding acetohydrazides **1-4**. The condensation reaction of [(4-phenyl-5-substituted-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetohydrazides with various alkyl and aryl aldehydes, in the presence of glacial acetic acid in ethanol, gave good yield of new products **5-28**. Compounds **11**, **12**, **13**, **16**, **17**, **18**, **20**, **22**, **23**, **24**, **25**, **27**, **28** are registered in CAS (Chemical Abstracts Service) database but without references and methods of synthesis.

[Insert Scheme 1]

[Insert Table 1]

All the newly obtained compounds are air stable solids and soluble in DMSO at ambient temperature. The purity of the synthesized compounds was checked by elemental analyses and thin layer chromatography. The structures of prepared derivatives were determined on the basis of ^{1}H NMR and ^{13}C NMR spectra and all of the synthesized compounds have satisfactory analyses for their proposed structures. ^{1}H NMR spectral results for all compounds together with hydrogen assignments and ^{13}C NMR spectra results are presented in Experimental Section.

In the ^{1}H NMR spectra of the compounds **5-28** the singlet peak due to the hydrazone proton (NH) appeared in the region of δ 8.11-11.98 ppm, which confirmed the successful formation of the desired products, whereas the singlet signal for -S-CH_2- protons was observed in the region of δ 3.43-4.55 ppm. All other aliphatic and aromatic protons were observed at the expected regions.

In the ^{13}C NMR spectra of **5-28** derivatives the carbon of -S-CH_2- group had a typical signal at about 30 ppm. Similarly, the presence of the =CH group was also confirmed by a signal at about 150 ppm and for C=O group at 166 ppm. All other aliphatic and aromatic protons were observed at expected regions.

Microbiology

According to our screening results obtained by agar dilution method with 1000 mg/L of the newly synthesized compounds, no activity against the reference strains of Gram-positive or

Gram-negative bacteria was found. Among the tested compounds, only compound **5** exhibited moderate inhibitory effect against the reference strains of yeasts, as monitored by an appearance of visible growth reduction. On the basis of the MIC values obtained by microdilution method it was shown that compound **5** had some, incubation-time dependent activity against both reference strains and clinical isolates of *Candida albicans* (Table 2). After 24 h incubation, MIC ranged from 62.5 to 250 mg/L, while after 48 h incubation - from 250 to ≥1000 mg/L. *C. parapsilosis* was included as a recommended reference strain for testing antifungals with activity against *Candida* spp. MIC values in the case of this strain were 125 or 250 mg/L after 24 or 48 h incubation, respectively.

The opportunistic yeasts belonging to *Candida* spp., mainly *Candida albicans*, are the source of invasive or non-invasive yeast infections in patients, especially those with compromised immune system function and those hospitalized with serious underlying diseases and their high mortality [29-35]. In general, the use of the available antifungals is limited because of their toxicity, side effects, drug interactions, limited routes, and the emergence of drug-resistant and drug-low-susceptible strains.

MIC is mainly used to establish the activity of potentially antimicrobial agents against *e.g.* the tested fungi and is important for clinical or epidemiological studies. According to CLSI and EUCAST definitions and literature data [25-38], MIC obtained after only 24 h of incubation should be used to determine the antifungal effect of widely used antifungal agents (e.g. fluconazole, voriconazole). According to Pfaller *et al*. [36, 39, 40] and Ostrosky-Zeichner *et al*. [41], the earlier reading of e.g. fluconazole predicted therapeutic outcomes and success as accurately as the 48 h MICs. Based on the experimental data, the Subcommittees of CLSI and

EUCAST have included the option to read MICs for *Candida* species after 24 h incubation. According to Pfaller *et al*. [39], the 24h MIC test reliably identifies fluconazole resistance among *Candida* spp.

Data presented in this paper indicate that compound **5** possessed some activity against the tested *C. albicans* strains. However, after the prolonged to 48 h incubation, a significant decrease of the antifungal activity of derivative **5** was found, as monitored by an increase of MIC values from 4 to >16 times in the second reading, depending on the strain. The effect of the prolonged incubation in the presence of antifungal agents increasing the MIC value has already been described in the literature [42, 43]. As found by other authors [42, 43], the influence of the incubation time as a test variable may depend on the nature of the preparation, *e.g.* the various liposomal formulations of amphotericin B. The specific formulation of this antifungal may influence the mechanism of action and/or the intracellular concentration at the site of antifungal action. It is probable that our observation may be due to a decrease of the compound concentration within yeast cells belonging to *C. albicans*, leading to an increase of MIC values. This problem requires further studies, essential for searching new derivatives showing better and stable antifungal activity.

Conclusions

In the present paper, we synthesized and characterized by ^{1}H NMR, ^{13}C NMR and elemental analyses a new series of acetohydrazide derivatives. All the newly prepared compounds were screened for their antimicrobial activity by agar dilution technique against 4 reference strains of

Gram-positive and Gram-negative bacteria and 12 yeasts belonging to *Candida spp*. Antimicrobial study revealed that compounds had no activity against the reference strains of Gram-positive or Gram-negative bacteria, but derivative - 2-[(4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(2-hydroxyphenyl)methylidene]acetohydrazide (**5**) exhibited moderate inhibitory effect against the reference strains of *Candida* spp. yeasts.

Experimental

General

All reagents were purchased from Sigma-Aldrich (Munich, Germany) and Merck Co. (Darmstadt, Germany) and used without further purification. Melting points were determined in Fisher-Johns blocks (Fisher Scientific, Schwerte, Germany) and presented without any corrections. The IR spectra (ν, cm^{-1}) were recorded in KBr tablets using a Specord IR-75 spectrophotometer (Carl Zeiss, Jena, Germany). The ^{1}H NMR spectra were recorded on a Bruker Avance 300 apparatus (Bruker BioSpin GmbH, Rheinstetten/Karlsruhe, Germany) in DMSO-d_6 with TMS as internal standard. The ^{13}C NMR spectra were recorded on a Bruker Avance 300 apparatus. Chemical shifts are given in ppm (δ-scale). The purity of obtained compounds was checked by TLC on aluminium oxide 60 F254 plates (Merck Co. Whitehouse Station, New Jersey, USA), in a $CHCl_3/C_2H_5OH$ (10:1, v/v) solvent system. The spots were detected by exposure to a UV lamp at 254 nm. Elemental analyses of the obtained compounds was performed for C, H, N on AMZ 851 CHX analyser (PG, Gdańsk, Poland). The maximum

percentage differences between calculated and found values for each element were within the error and amounted to ± 0.4%.

Chemistry

Preparation of the compounds

Preparation of [(4-phenyl-5-substituted-4H-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**1-4**)

The 100% hydrazine hydrate (10 mmol) was added to ethyl [(4-phenyl-5-substituted-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetate (10 mmol) in anhydrous ethanol (10 mL). The mixture was put to a refrigerator for 24 h. After that, the precipitation of hydrazide (**1-4**) was filtered off, dried and crystallized from ethanol [44-47].

[(4-Phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**1**)

Yield: 78%; mp 132°C–134°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.89 (s, 2H, CH_2); 4.29 (s, 2H, NH_2); 7.51–7.61 (m, 5H, Ar-H); 8.86 (s, 1H, CH); 9.33 (s, 1H, NH). Anal. calcd. for $C_{10}H_{11}N_5OS$ (249.29): C, 48.14; H, 4.44; N, 28.08; Found: C, 48.16; H; 4.41; N, 28.05%.

[(4-Phenyl-5-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**2**)

Yield: 72.8%; mp. 86°C–88°C (dec.). IR (KBr), ν (cm^{-1}): 3089 (CH aromatic), 2955, 1444 (CH aliphatic), 1711 (C=O), 1601 (C=N), 1503 (C-N), 688 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm) = 2.19 (s, 3H, CH_3), 3.79 (s, 2H, CH_2), 4.21 (s, 2H, NH_2), 7.35-7.64 (m, 5H, Ar-H), 9.30 (s, 1H,

NH). Anal. calcd. for $C_{11}H_{13}N_5OS$ (263.32: C, 50.17; H, 4.98; N, 26.60; Found: C, 50.04; H, 4.96; N, 26.51%.

[(4,5-Diphenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**3**)

Yield: 91.4%, mp. 196°C–198°C (dec.). IR (KBr), m (cm^{-1}): 3105 (CH aromatic), 2980, 1423 (CH aliphatic), 1698 (C=O), 1611 (C=N), 1522 (C–N), 699 (C–S). ^{1}H NMR (DMSO-d_6) δ (ppm) = 3.91 (s, 2H,CH_2), 4.31 (s, 2H, NH_2), 7.31-7.57 (m, 10H, Ar-H), 9.40 (brs, 1H, NH). Anal. calcd. for $C_{16}H_{15}N_5OS$ (325.39); C, 59.06; H, 4.65; N, 21.52; found: C, 59.10; H, 4.63; N, 21.49%.

[(4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**4**)

Yield: 90.2%; mp. 202°C–206°C (dec.). IR (KBr), ν (cm^{-1}): 3079 (CH aromtic), 2977, 1455 (CH aliphatic), 1672 (C=O), 1608 (C=N), 1502 (C-N), 673 (C-S). ^{1}H NMR (DMSO-d_6): δ = 3.93 (s, 2H, CH_2), 4.31 (s, 2H, NH_2), 7.37–8.58 (m, 9H, Ar-H), 9.38 (s, 1H, NH). Anal. calcd. for $C_{15}H_{14}N_6OS$ (326.4): C, 55.20; H, 4.32; N, 25.75; Found: C, 55.16; H, 4.35; N, 25.80%.

Preparation of acetohydrazide derivatives

To a suspension of hydrazide (**1-4**) (10 mmol) in ethanol (20 mL), an equimolar of various aldehydes (10 mmol) was added. The suspension was heated until clear solution was obtained. Then few drops of glacial acetic acid were added as a catalyst. The solution was refluxed for 4

hrs. After the completion of the reaction, the solution was cooled to room temperature. The obtained precipitate was filtered off and crystallized from ethanol.

2-[(4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(2-hydroxyphenyl) methylidene]acetohydrazide (**5**)

Yield: 66%; mp. 124°C–126°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.15 (s, 2H, CH_2); 4.54 (s, 1H, OH); 6.90-7.73 (m, 9H, Ar-H); 8.40 (s, 1H, CH); 8.46 (s, 1H, CH); 8.94 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.1 (-S-CH_2-), 117.4, 120.4, 121.2, 125.6, 128.5, 128.70, 129.2, 129.7, 137.5 (11C_{ar}), 147.5 (C-3 triazole), 150.2 (=CH), 154.5 (C-5 triazole), 158.3 (C_{ar}), 166.5 (C=O). Anal. calcd. for $C_{17}H_{15}N_5O_2S$ (353.4): C, 57.78; H, 4.28; N, 19.82; Found: C, 57.82; H, 4.22; N, 19.85%.

2-[(4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methylphenyl) methylidene]acetohydrazide (**6**)

Yield: 63%; mp. 130°C–132°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.39 (s, 3H, CH_3); 4.13 (s, 2H, CH_2); 7.22-8.20 (m, 9H, Ar-H); 8.20 (s, 1H, CH); 8.93 (s, 1H, NH); 8.98 (s, 1H, CH). ^{13}C NMR: δ (ppm) = 21.2 (-CH_3), 30.2 (-S-CH_2), 125.6, 127.3, 127.8, 129.1, 131.9, 137.5, 138.5 (12C_{ar}), 147.5 (C-3 triazole), 148.6 (=CH), 154.4 (C-5 triazole), 166.0 (C=O). Anal. calcd. for $C_{18}H_{17}N_5OS$ (351.42): C, 61,52; H, 4.88; N, 19.93; Found: C, 61.55; H, 4.89; N, 19.90%.

2-[(4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methoxyphenyl) methylidene]acetohydrazide (**7**)

Yield: 68%; mp. 166°C–168°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.88 (s, 3H, CH_3); 4.13 (s, 2H, CH_2); 6.98-8.07 (m, 9H, Ar-H); 8.18 (s, 1H, CH); 8.69 (s, 1H, NH); 8.94 (s, 1H, CH). ^{13}C NMR: δ (ppm) = 30.1 (-S-CH_2-), 56.1 (-CH_3), 114.6 (2C_{ar}), 125.6, 127.1, 128.7, 129.1, 129.2, 137.5 (9C_{ar}), 147.5 (C-3 triazole), 148.6 (=CH), 154.5 (C-5 triazole), 160.1 (C_{ar}), 166.1 (C=O). Anal. calcd. For $C_{18}H_{17}N_5O_2S$ (367.42): C, 58.84; H, 4.66; N, 19.06; Found: C, 58.87; H, 4.63; N, 19.09%.

_N_-[(4-bromophenyl)methylidene]-2-[(4-phenyl-4_H_-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (8)

Yield: 75%; mp. 140°C–142°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.07 (s, 2H, CH_2); 7.50-8.03 (m, 9H, Ar-H); 8.21 (s, 1H, CH); 8.85 (s, 1H, CH); 11.71 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.1 (-S-CH_2-), 123.9, 125.6, 128.8, 129.1, 129.3, 132.4, 132.5, 137.5 (12C_{ar}), 147.5 (C-3 triazole), 148.7 (=CH), 154.6 (C-5 triazole), 166.1 (C=O). Anal. calcd. for $C_{17}H_{14}BrN_5OS$ (416.29): C, 49.05; H, 3.39; N, 16.82; Found: C, 49.01; H, 3.40; N, 16.85%.

2-[(5-methyl-4-phenyl-4_H_-1,2,4-triazol-3-yl)sulfanyl]-_N_-(propylidene)acetohydrazide (9)

Yield: 25%; mp. 100°C–102°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.02 (t, J = 7.5 Hz, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.18-2.26 (m, 2H, CH_2), 4.23 (s, 2H, CH_2), 7.28-7.62 (m, 5H, Ar-H), 7.89 (t, J = 5.0 Hz, 1H, =CH), 11.20 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 9.5 (-CH_3), 12.4 (-CH_3), 12.3 (-CH_2-), 30.2 (-S-CH_2), 128.3, 129.9, 130.2, 136.2 (6C_{ar}), 145.6 (=CH), 156.0 (C-5 triazole); 158.4 (C-3 triazole), 166.1 (C=O). Anal. calcd. for $C_{14}H_{17}N_5OS$ (303.38): C, 55.42; H, 5.65; N, 23.08; Found: C, 55.38; H, 5.69; N, 23.15%.

2-[(5-methyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(2-methylpropylidene) acetohydrazide (**10**)

Yield: 70%; mp. 132°C–134°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.06 (d, J = 2.5 Hz, 3H, CH_3), 1.10 (d, J = 2,5 Hz, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.42-2.48 (m, 1H, CH), 4.29 (s, 2H, CH_2), 7.30-7.69 (m, 5H, Ar-H), 7.99 (d, J = 5.0 Hz, 1H, =CH), 11.21 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.4 (-CH_3), 18.2 (2xCH_3), 29.7 (-CH), 30.1 (-S-CH_2-), 128.4, 129.9, 130.3, 136.3 (6C_{ar}), 150.8 (=CH), 156.1 (C-5 triazole), 158.5 (C-3 triazole), 166.0 (C=O). Anal. calcd. for $C_{15}H_{19}N_5OS$ (317.41): C, 56.76; H, 6.03; N, 22.06; Found: C, 56.71; H, 6.01; N, 22.09%.

2-[(5-methyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(2-hydroxyphenyl)methylidene] acetohydrazide (**11**)

CAS registry number: 62372-96-7. Yield: 68%; mp. 200°C–202°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.26 (s, 3H, CH_3), 4.04 (s, 2H, CH_2), 4.41 (s, 1H, OH), 6.89-7.76 (m, 9H, Ar-H), 8.52 (s, 1H, =CH), 9.65 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.5 (-CH_3), 30.6 (-S-CH_2-), 117.4, 120.4, 128.3, 128.5, 129.9, 130.2, 136.2 (11C_{ar}), 156.0 (C-5 triazole), 158.3 (C_{ar}), 158.4 (C-3 triazole), 166.1 (C=O). Anal. calcd. for $C_{18}H_{17}N_5O2S$ (367.42): C, 58.84; H, 4.66; N, 19.06; Found: C, 58.89; H, 4.70; N, 19.04%.

2-[(5-methyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(3-nitrophenyl)methylidene] acetohydrazide (**12**)

CAS registry number: 62373-03-9.Yield: 93%; mp. 184°C–186°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.24 (s, 3H, CH_3), 4.47 (s, 2H, CH_2), 7.51-8.58 (m, 9H, Ar-H), 8.96 (s, 1H, =CH),

10.20 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.6 (-CH_3), 30.1 (-S-CH_2-), 122.3, 124.1, 128.3, 129.9, 130.2, 132.6, 136.2, 137.4, 147.4 (12C_{ar}), 147.9 (=CH), 156.2 (C-5 triazole), 158.4 (C-3 triazole), 166.2 (C=O). Anal. calcd. for $C_{18}H_{16}N_6O_3S$ (396.42): C, 54.54; H, 4.07; N, 21.20; Found: C, 54.69; H, 4.02; N, 21.27%.

2-[(5-methyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methylphenyl)methylidene] acetohydrazide (**13**)

CAS registry number: 62373-03-9. Yield: 73%; mp. 186°C–188°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.24 (s, 3H, CH_3); 2.38 (s, 3H, CH_3); 4.04 (s, 2H, CH_2); 7.21-8.19 (m, 9H, Ar-H); 8.71 (s, 1H, CH); 11.60 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.5 (-CH_3), 21.1 (-CH_3), 30.1 (-S-CH_2-), 127.3, 128.4, 129.1, 129.9, 130.2, 131.9, 136.2, 138.5 (12C_{ar}), 148.6 (=CH), 156.1 (C-5 triazole), 158.4 (C-3 triazole), 166.1 (C=O). Anal. calcd. for $C_{19}H_{19}N_5OS$ (365,45): C, 62.44; H, 5.24; N, 19.16; Found: C, 62.49; H, 5.22; N, 19.11%.

2-[(5-methyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methoxyphenyl) methylidene]acetohydrazide (**14**)

Yield: 74%; mp. 118°C–120°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.24 (s, 3H, CH_3); 3.84 (s, 3H, CH_3); 4.04 (s, 2H, CH_2); 6.96-7.92 (m, 9H, Ar-H); 8.17 (s, 1H, CH); 8.68 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.4 (-CH_3), 30.1 (-S-CH_2), 50.0 (-CH_3), 114.3 (2C_{ar}), 127.1, 128.3, 129.1, 129.9, 130.2, 136.2 (9C_{ar}), 148.6 (=CH), 156.0 (C-5 triazole), 158.4 (C-3 triazole), 160.1 (C_{ar}), 166.0 (C=O). Anal. calcd. for $C_{19}H_{19}N_5O_2S$ (381.45): C, 59.82; H, 5.02; N, 18.36; Found: C, 59.85; H, 5.05; N, 18.41%.

_N_-[(4-bromophenyl)methylidene]-2-[(5-methyl-4-phenyl-4_H_-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (15)

Yield: 65%; mp. 126°C–128°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.26 (s, 3H, CH_3); 4.03 (s, 2H, CH_2); 7.49-8.22 (m, 9H, Ar-H); 8.75 (s, 1H, CH); 10.04 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.4 (-CH_3), 30.0 (-S-CH_2-), 123.9, 128.3, 129.3, 129.9, 130.2, 132.3, 132.5, 136.2 (12C_{ar}), 148.7 (=CH), 156.1 (C-5 triazole), 158,4 (C-3 triazole), 166.0 (C=O). Anal. calcd. for $C_{18}H_{16}BrN_5OS$ (430.32): C, 50.24; H, 3.75; N, 16.27; Found: C, 50.29; H, 3.70; N, 16.31%.

2-[(5-methyl-4-phenyl-4_H_-1,2,4-triazol-3-yl)sulfanyl]-_N_-[(pyridin-4-yl)methylidene]acetohydrazide (16)

CAS registry number 351510-77-5. Yield: 42%; mp. 186°C–188°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.29 (s, 3H, CH_3); 4.46 (s, 2H, CH_2); 7.46-8.95 (m, 9H, Ar-H); 8.56 (s, 1H, =CH); 10.15 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 12.5 (-CH_3), 30.0 (-S-CH_2-), 122.3, 128.3, 129.9, 130.2, 136.2, 140.7 (9C_{ar}), 148.6 (=CH), 150, 1 (2C_{ar}), 156.0 (C-5 triazole), 158.4 (C-3 triazole), 166.1 (C=O). Anal. calcd. for $C_{17}H_{16}N_6OS$ (352.41): C, 57.94; H, 4.58; N, 23.85; Found: C, 23.85; H, 4.61; N, 23.90%.

2-[(4,5-diphenyl-4_H_-1,2,4-triazol-3-yl)sulfanyl]-_N_-[(2-hydroxyphenyl) methylidene]acetohydrazide (17)

CAS Registry Number: 62372-96-7. Yield: 85%; mp. 212°C–214°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.15 (s, 2H, CH_2); 4.54 (s, 1H, OH); 6.90-7.74 (m, 14H, Ar-H); 8.37 (s, 1H, CH); 8.48 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.2 (-S-CH_2), 117.6, 120.7, 121.3, 125.8, 128.5, 128.6,

128.8, 130.1, 137.4 (17C_{ar}), 150.7 (=CH), 158.5 (C_{ar}) 159.5 (C-3 triazole), 166.5 (C=O), 167.4 (C-5 triazole). Anal. calcd. for $C_{23}H_{19}N_5O_2S$ (429.49): C, 64.32; H, 4.46; N, 16.31; Found: C, 64.35; H, 4.48; N, 16.35%.

2-[(4,5-diphenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methylphenyl) methylidene]acetohydrazide (18)

CAS Registry Number: 93300-42-6. Yield: 84%; mp. 214°C–216°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.39 (s, 3H, CH_3); 4.14 (s, 2H, CH_2); 7.30-7.67 (m, 14H, Ar-H); 8.04 (s, 1H, CH); 8.22 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 21.1 (-CH_3), 30.0 (-S-CH_2), 125.6, 127.3, 128.6, 128.8, 129.1, 129.5, 130.1, 130.6, 131.9, 137.4, 138.5 (18C_{ar}), 148.5 (=CH), 159.5 (C-3 triazole), 166.1 (C=O), 167.4 (C-5 triazole). Anal. calcd. for $C_{24}H_{21}N_5OS$ (427.52): C, 67.43; H, 4.95; N, 16.38; Found: C, 67.48; H, 4.98; N, 16.42%.

2-[(4,5-diphenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methoxyphenyl) methylidene]acetohydrazide (19)

Yield: 93%; mp. 210°C–212°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.76 (s, 3H, CH_3); 4.04 (s, 2H, CH_2); 6.96-7.60 (m, 14H, Ar-H); 7.93 (s, 1H, CH); 8.11 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.2 (-S-CH_2-), 56.8 (-CH_3), 114.8 (2C_{ar}), 125.6, 128.4, 128.6, 128.8, 129.1, 129.5, 130.1, 137.4 (15C_{ar}), 148.2 (=CH), 159.5 (C-3 triazole), 160.3 (C_{ar}), 166.1 (C=O), 167.4 (C-5 triazole). Anal. calcd. for $C_{24}H_{21}N_5O_2S$ (443.52): C, 64.99; H, 4.77; N, 15.79; Found: C, 64.96; H, 4.72; N, 15.75%.

_N_-[(4-bromophenyl)methylidene]-2-[(4,5-diphenyl-4_H_-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**20**)

CAS Registry Number: 306756-39-8. Yield: 87%; mp. 202°C–204°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.08 (s, 2H, CH_2); 7.31-7.97 (m, 14H, Ar-H); 8.16 (s, 1H, CH); 9.00 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.0 (-S-CH_2-), 124.0, 125.6, 128.4, 128.6, 128.8, 129.3, 129.5, 130.1, 130.6, 132.4, 132.5, 137.4 (18C_{ar}), 148.7 (=CH), 159.5 (C-3 triazole), 166.1 (C=O), 167.5 (C-5 triazole). Anal. calcd. for $C_{23}H_{18}BrN_5OS$ (492.39): C, 56.10; H, 3.68; N, 14.22; Found: C, 56.13; H, 3.72; N, 14.26%.

2-{[4-phenyl-5-(pyridin-3-yl)-4_H_-1,2,4-triazol-3-yl]sulfanyl}-_N_-(propylidene) acetohydrazide (**21**)

Yield: 44%; mp. 134°C–136°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1,01 (t, J = 5,0 Hz, 3H, CH_3), 2,18-2,23 (m, 2H, CH_2), 4,37 (s, 2H, CH_2), 7,30-8,57 (m, 9H, Ar-H), 8,08 (t, J = 5,0 Hz, 1H, =CH), 11,24 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 9.5 (-CH_3), 23.3 (-CH_2-), 30.1 (-S-CH_2-), 126.1, 128.4, 128.8, 130.1, 132.1, 134.6, 137.4 (9C_{ar}), 145.7 (=CH), 146.4, 146.9 (2C_{ar}), 159.5 (C-3 triazole), 162.4 (C-5 triazole), 166.2 (C=O). Anal. calcd. for $C_{18}H_{18}N_6OS$ (366,44): C, 59,00; H, 4,95; N, 22,93; Found: C, 59.03; H, 4,93; N, 22,98%.

2-{[4-phenyl-5-(pyridin-3-yl)-4_H_-1,2,4-triazol-3-yl]sulfanyl}-_N_-(2-methylpropylidene) acetohydrazide (**22**)

CAS registry number 901628-30-6. Yield: 60%; mp. 200°C–202°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.95 (d, J = 2,5 Hz, 3H, CH_3), 1.01 (d, J = 2,5 Hz, 3H, CH_3), 2.46-2.52 (m, 1H, CH),

4,33 (s, 2H, CH_2), 7.24-8.55 (m, 9H, Ar-H), 8.43 (d, *J* = 5,0 Hz, 1H, =CH), 11.17 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 18.2 (2xCH_3), 29.7 (-CH), 30.0 (-S-CH_2-), 126.2, 128.4, 128.8, 130.1, 132.1, 134.6, 137.4, 146.4, 146.7 (11C_{ar}), 150.8 (=CH), 159.6 (C-3 triazole), 162.5 (C-5 triazole), 166.0 (C=O). Anal. calcd. for $C_{19}H_{20}N_6OS$ (380.47): C, 59.98; H, 5.30; N, 22.09; Found: C, 59.91; H, 5.34; N, 22.13%.

2-{[4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl]sulfanyl}-*N*-[(2-hydroxyphenyl)methylidene]acetohydrazide (**23**)

CAS registry number 903116-56-3. Yield: 90%; mp. 210°C–212°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.43 (s, 2H, CH_2), 4.11 (s, 1H, OH), 6.82-8.56 (m, 13H, Ar-H), 8.41 (s, 1H, =CH), 10.23 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.1 (-S-CH_2), 117.3, 120.4, 121.2, 126.3, 128.5, 128.6, 128.8, 129.8, 130.2, 132.3, 134.7, 137.9, 146.4, 146.9 (16C_{ar}), 150.9 (=CH), 158.4 (C_{ar}), 159.5 (C-3 triazole), 162.4 (C-5 triazole), 166.2 (C=O). Anal. calcd. for $C_{22}H_{18}N_6O_2S$ (430.48): C, 61.38; H, 4.21; N, 19.52; Found: C, 61.43; H, 4.28; N, 19.58%.

2-{[4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl]sulfanyl}-*N*-[(3-nitrophenyl)methylidene]acetohydrazide (**24**)

CAS registry number 903107-17-5. Yield: 98%; mp. 202°C–204°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.55 (s, 2H, CH_2), 7.38-8.71 (m, 13H, Ar-H), 8.91 (s, 1H, =CH), 11.98 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.1 (-S-CH_2-), 122.3, 124.1, 128.4, 128.7, 129.9, 130.0, 132.6, 134.6, 137.4, 137.8, 146.3, 146.9, 147.4 (17C_{ar}), 147.9 (=CH), 159.4 (C-3 triazole), 162.4 (C-5 triazole), 166.1 (C=O). Anal. calcd. for $C_{22}H_{17}N_7O_3S$ (459.48): C, 57.51; H, 3.73; N, 21.34; Found: C, 57.54; H, 3.76; N, 21.36%.

2-[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methylphenyl) methylidene]acetohydrazide (**25**)

CAS Registry Number: 903408-50-4. Yield: 65%; mp. 184°C–186°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.31 (s, 3H, CH_3); 4.10 (s, 2H, CH_2); 7.22-8.57 (m, 13H, Ar-H); 8.15 (s, 1H, CH); 9.93 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 21.0 (-CH_3), 30.1 (-S-CH_2-), 126.1, 127.4, 128.4, 128.7, 129.2, 130.1, 131.9, 132.2, 134.6, 137.4, 138.5, 146.3, 146.9 (17C_{ar}), 148.8 (=CH), 159.6 (C-3 triazole), 162.3 (C-5 triazole), 166.0 (C=O). Anal. calcd. for $C_{23}H_{20}N_6OS$ (428.50): C, 64.47; H, 4.70; N, 19.61; Found: C, 64.50; H, 4.72; N, 19.63%.

2-[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-[(4-methoxyphenyl) methylidene]acetohydrazide (**26**)

Yield: 44%; mp. 193°C–195°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.76 (s, 3H, CH_3); 4.07 (s, 2H, CH_2); 6.88-8.55 (m, 13H, Ar-H); 8.00 (s, 1H, CH); 8.12 (s, 1H, NH). ^{13}C NMR, δ (ppm): 30.1 (-S-CH_2), 56.2 (-CH_3), 114.4 (2C_{ar}), 126.1, 127.1, 128.4, 128.8, 129.1, 130.1, 132.1, 134.6, 137.4, 146.4, 146.9 (14C_{ar}), 148.6 (=CH), 159.5 (C-3 triazole), 160.1 (C_{ar}), 162.4 (C-5 triazole), 166.7 (C=O). Anal. calcd. for $C_{23}H_{20}N_6O_2S$ (444.5089): C, 62.15; H, 4.54; N, 18.91; Found: C, 62.18; H, 4.57; N, 18.79%.

***N*-[(4-bromophenyl)methylidene]-2-{[4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl]sulfanyl}acetohydrazide** (**27**)

CAS registry number 903407-49-8. Yield: 76%; mp. 228°C–230°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.10 (s, 2H, CH_2), 7.36-8.57 (m, 13H, Ar-H), 7.98 (s, 1H, =CH), 10.16 (s, 1H, NH).

^{13}C NMR: δ (ppm) = 30.2 (-S-CH_2-), 124.0, 126.2, 128.3, 128.7, 129.3, 130.0, 132.1, 132.5, 134.6, 137.4, 146.5, 146.8 (17C_{ar}), 148.6 (=CH), 159.4 (C-3 triazole), 162.3 (C-5 triazole), 166.1 (C=O). Anal. calcd. for $C_{22}H_{17}BrN_6OS$ (493.38): C, 53.56; H, 3.47; N, 17.03; Found: C, 53.62; H, 3.41; N, 17.09%.

2-{[4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl]sulfanyl}-*N*-[(pyridin-4-yl)methylidene]acetohydrazide (**28**)

CAS registry number 901624-83-7. Yield: 92%; mp. 198°C–200°C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.54 (s, 2H, CH_2), 7.35-8.64 (m, 13H, Ar-H), 7.99 (s, 1H, =CH), 11.96 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 30.1. (-S-CH_2-), 122.3, 126.1, 128.4, 130.1, 132.2, 134.6, 137.4, 140.7, 146.4, 146.9 (14C_{ar}), 148.7 (=CH), 150.1 (2C_{ar}), 159.5 (C-3 triazole), 162.5 (C-5 triazole), 166.2 (C=O). Anal. calcd. for: $C_{21}H_{17}N_7OS$ (415.47): C, 60.71; H, 4.12; N, 23.60; Found: C, 60.78; H, 4.07; N, 23.67%.

Microbiology

Materials and methods

The antimicrobial activity of the tested compounds was screened on the panel of American Type Culture Collection (ATCC) reference strains of Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, S*taphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240), and of the Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027). Moreover, we used the yeasts belonging to *Candida* spp. - two reference strains (*C. albicans* ATCC 2091 and *C. parapsilosis* ATCC 22019) and ten clinical

isolates of *C. albicans* from the Department of Pharmaceutical Microbiology of Medical University of Lublin collection.

Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard - 150 x 10^6 CFU/mL (CFU - colony forming units). All stock solutions of the tested compounds were prepared in dimethyl sulfoxide (DMSO). Mueller-Hinton agar (for bacteria) or Mueller-Hinton agar supplemented with 2% glucose and buffered at pH 5.6 (for fungi) were used for examination of antibacterial or antifungal activity. The Mueller-Hinton medium plus 2% glucose was used instead of RPMI 1640. It should be noted that Mueller-Hinton medium is readily available, possess as good batch-to-batch reproducibility and supports the growth of most pathogenic yeasts [48].

In the first step the *in vitro* antimicrobial potency of all tested compounds was screened using the agar dilution method on the basis of the microbial growth inhibition on the agar medium to which the tested compounds at concentrations 1000 μg/mL were added, as described elsewhere [49]. The medium with DMSO at the final concentration and without the tested compounds served as a negative control; no microbial growth inhibition was observed. The plates were poured on the day of testing. 10 μL of each microbial suspension was put onto the prepared solid media. The plates were pre-incubated at room temperature for 1-1.5 h and then were incubated at 35^{o}C for 18 h for bacteria and for 24-48 h for yeasts.

Subsequently, for compound **5** showing inhibitory effect on the growth of tested yeasts, MIC was estimated by broth microdilution technique, according to the Clinical and Laboratory Standards Institute (CLSI) standards M27-A3 for yeasts [36] and European Committee on Antimicrobial Susceptibility Testing (EUCAST), Subcommittee of Antifungal Susceptibility

Testing [37], with some modification. Microdilution technique was developed using sterile 96-well microplates, which were inoculated with a yeast suspension of optical density of 0.5 McFarland standard. A 2 µL of the suspension was put into 198 µL of Mueller Hinton broth buffered at pH 5.6 supplemented with 2% glucose containing two-fold dilution of tested compound at the final concentration from 7.82 to 1000 mg/L. The medium without the tested compound was used as negative control. After incubation (at 35^{o}C for 24 or 48 h), the optical density (OD_{600}) measurements were determined spectrophotometrically for microbial culture in broth medium. The MIC (minimal inhibitory concentration) values, defined as the lowest concentration of a compound that prevents visible growth of the tested microorganisms, were determined after 24 and 48 h by comparison with the fungal growth in control (compound free) medium. The blank control wells with two-fold dilution of each of the tested compounds added to broth medium (total volume - 200 µL) without the yeast suspension were incubated under the same conditions. MIC is usually defined as the lowest concentration of compound that inhibit of the growth of tested microorganisms [37].

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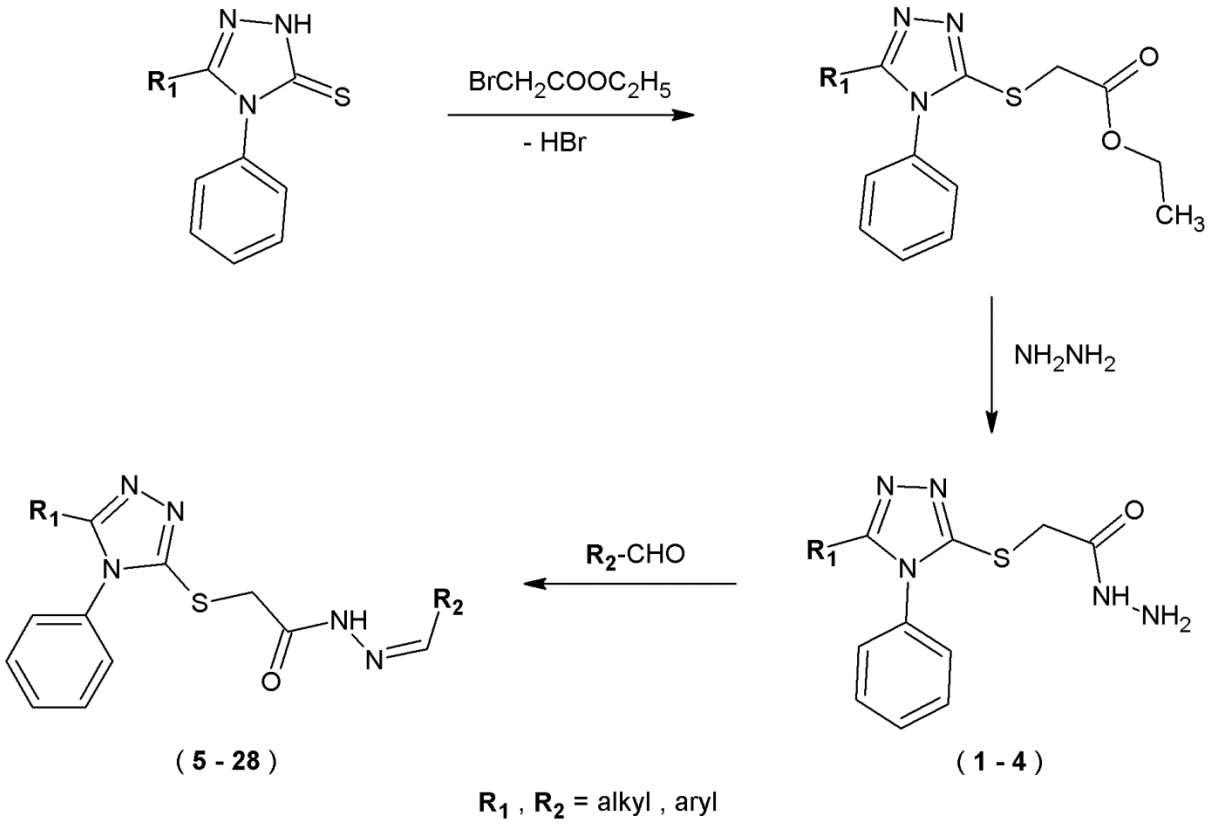

Table 1. Substituents of compounds **1 - 28**.

Compounds	**R_1**	**R_2**
1	H	-
2	CH_3	-
3	C_6H_5	-
4	3-C_5H_4N	-
5	H	2-OHC_6H_4
6	H	4-$CH_3C_6H_4$
7	H	4-$CH_3OC_6H_4$
8	H	4-BrC_6H_4
9	CH_3	C_3H_7
10	CH_3	$(CH_3)_2CHCH_2$

11	CH_3	2-OHC_6H_4
12	CH_3	3-$NO_2C_6H_4$
13	CH_3	4-$CH_3C_6H_4$
14	CH_3	4-$CH_3OC_6H_4$
15	CH_3	4-BrC_6H_4
16	CH_3	4-C_5H_4N
17	C_6H_5	2-OHC_6H_4
18	C_6H_5	4-$CH_3C_6H_4$
19	C_6H_5	4-$CH_3OC_6H_4$
20	C_6H_5	4-BrC_6H_4
21	3-C_5H_4N	C_3H_7
22	3-C_5H_4N	$(CH_3)_2CHCH_2$
23	3-C_5H_4N	2-OHC_6H_4
24	3-C_5H_4N	3-$NO_2C_6H_4$
25	3-C_5H_4N	4-$CH_3C_6H_4$
26	3-C_5H_4N	4-$CH_3OC_6H_4$
27	3-C_5H_4N	4-BrC_6H_4
28	3-C_5H_4N	4-C_5H_4N

Table 2. The influence of compound **5** on the growth of *Candida* spp.

Candida spp.	**MIC (mg/L)**	
	24 h	**48 h**
Candida parapsilosis ATCC 22019	125	250
Candida albicans ATCC 2091	125	1000
Candida albicans 104	62.5	>1000
Candida albicans 105	250	>1000
Candida albicans 106	62.5	1000
Candida albicans 107	62.5	500
Candida albicans 109	250	>1000
Candida albicans 110	250	>1000
Candida albicans 111	125	500
Candida albicans 112	62.5	1000
Candida albicans 114	62.5	500
Candida albicans 115	250	1000

*MIC values were determined using the broth microdilution method in the Mueller-Hinton supplemented with 2% glucose and spectrophotometric (OD_{600}) readings