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Synthesis and Antimicrobial Evaluation of New 1-{[4-(4-Halogenophenyl)-4*H*-1,2,4-triazol-3-yl]sulfanyl}acetyl-4-substituted Thiosemicarbazides and Products of Their Cyclization

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ABSTRACT: *By the reaction of hydrazides of 4-(4-halogenophenyl)-4H-1,2,4-triazol-3-yl-sulfanyl acetic acid with isothiocyanate, 1-acyl-4-substituted thiosemicarbazide derivatives (***7–19***) were obtained. The cyclization of compounds (***7–19***) in the presence of 2% NaOH led to the formation of compounds (***20–26***) containing two 1,2,4-triazole rings connected by a methylenesulfanyl group. The new compounds were tested for their in vitro antimicrobial activity. Some of the tested compounds (***9, 12, 18, 21, 22***) showed activity against the reference strains of Gram-positive bacteria with the MIC (minimal inhibitory concentration) = 125 to >1000 μg/mL.* © 2011 Wiley Periodicals, Inc. Heteroatom Chem 23:117–121, 2012; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20758

INTRODUCTION

1,2,4-Triazoles and their derivatives represent an interesting group of compounds, possessing a wide spectrum of biological activities, such as antibacterial [1,2], antifungal [3,4], antitubercular [5,6], anti-inflammatory [7,8], anticancer [9,10], analgesic [11], antiviral [12], anticonvulsant [13,14], and central nervous system [15] activity. Among them, a large number of 1,2,4-triazole-3-thiones have been reported as antibacterial substances [16–22]. In view of these facts, in the present paper, we have described several novel thiosemicarbazide derivatives with the 1,2,4-triazole-3-thione moiety at the position 1 for which their products were obtained after intramolecular cyclization. The newly synthesized compounds were evaluated for their antimicrobial activity.

RESULTS AND DISCUSSION

Chemistry

In the present work, the ethyl esters of 4-(4-halogenophenyl)-4*H*-1,2,4-triazol-3-yl-sulfanyl acetic acid (**1–3**) were used as starting materials, which were obtained by the reaction of 4-(4-halogenophenyl)-4*H*-1,2,4-triazole-3-thione with ethyl bromoacetate in the presence of sodium

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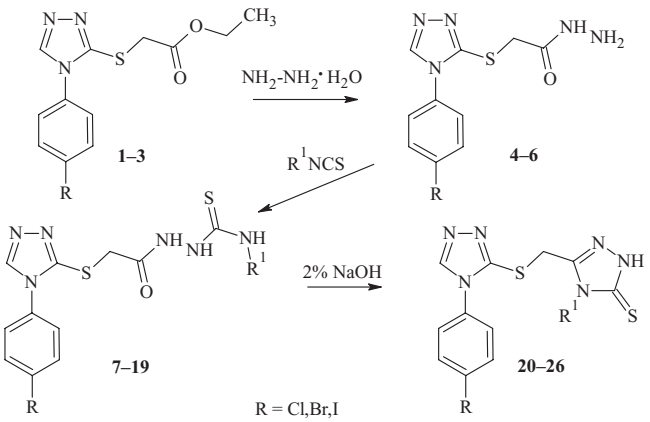

FIGURE 1 Synthesis of 1-acyl-4-substituted thiosemicarbazide derivatives (**7–19**) and products of their cyclization (**20–26**).

ethanolate. The reaction of 1,2,4-triazole-3-thione derivatives with ethyl bromoacetate proceeds according to the classical mechanism of the second-order nucleophilic substitution (S_N2). The pathway of this reaction was confirmed by quantum mechanical calculations [23]. The structures of these compounds were confirmed by X-ray analyses [24]. The next stage of investigation was the synthesis of 4-(4-halogenophenyl)-4*H*-1,2,4-triazol-3-yl-sulfanyl acetic acid hydrazides. By the reaction of the ethyl esters of 4-(4-halogenophenyl)-4*H*-1,2,4-triazol-3-yl-sulfanyl acetic acid (**1–3**) with hydrazine hydrate in anhydrous ethanol, the corresponding hydrazides (**4–6**) were obtained. The hydrazides (**4–6**) were used as substrates in the reaction with appropriate isothiocyanates. In this way, 1-acyl-4-substituted thiosemicarbazide derivatives (**7–19**) were obtained. The cyclization of thiosemicarbazide derivatives in an alkaline medium led to compounds **20–26**. The described reactions are shown in Fig. 1.

The structures of newly obtained compounds (**7–26**) were confirmed by using elemental analysis and the ^{1}H NMR method.

In the ^{1}H NMR spectra, all the compounds showed proton signals that are typical for the CH group of the 1,2,4-triazole-3-thione ring in the range of 8.84–8.98 ppm. All the thiosemicarbazide derivatives (**7–19**) showed three proton signals of the NHNHC(S)NH group in the 8.85–10.54 ppm range. The cyclic compounds (**20–26**) showed proton signals in the 13.57–13.91 ppm range that are typical for the NH group of the 1,2,4-triazole ring. All derivatives with a 4-substituted phenyl ring showed typical signals for two doublets in the 7.12–7.96 ppm range (J = 7.5–10.0 Hz).

Antimicrobial Evaluation

The in vitro antimicrobial activity of the synthesized compounds (**7–10, 12–14, 16–23, 25, 26**) was evaluated by using the broth microdilution method against the reference strains of Gram-negative and Gram-positive bacterial species. According to our screening results, compounds **9, 12, 18, 21**, and **22** showed potential antibacterial activity against Gram-positive bacteria—the *Staphylococcus* species or *Micrococcus luteus* ATCC (American Type Culture Collection) 10240; no activity against Gram-negative bacteria was found. Based on data obtained by using the broth microdilution method, the most effective compounds against Gram-positive species were thiosemicarbazide derivatives **9** and **12** with the 4-methylphenyl substituent at position 4 for *M. luteus* ATCC 10240 (minimal inhibitory concentration [MIC] = 125–250 μg/mL) or for *S. aureus* ATCC 25923 (MIC = 250 μg/mL) and *S. epidermidis* ATCC 12228 (MIC = 250 μg/mL) (Table 1). The compound **18** with a benzyl substitutent at position 4 of thiosemicarbazide derivatives showed somewhat better activity against *S. epidermidis* ATCC 12228 (MIC = 125 μg/mL), but it was not active against *S. aureus* ATCC 25923 (MIC >1000 μg/mL). Cyclic derivatives **21** and **22** showed an inhibitory effect against *S. epidermidis* ATCC 12228 (MIC = 500 μg/mL), but to a lesser extent, and were not active against *S. aureus* ATCC 25923

TABLE 1 Influence of Some Newly Synthesized Compounds on the Growth of Gram-Positive Bacteria on the Basis of MIC (μg/mL) Values Determined by Using the Broth Dilution Method

Compound	*Staphylococcus aureus ATCC 25923*	*Staphylococcus epidermidis ATCC 12228*	*Micrococcus Luteus ATCC 10240*
9	250	250	250
12	250	250	125
18	>1000	125	125
21	>1000	500	>1000
22	>1000	500	250

(MIC >1000 μg/mL). Among cyclic compounds, only compound **22** showed activity against *M. luteus* ATCC 10240 (MIC = 250 μg/mL). According to our results, the MICs of cefuroxime, which have been extensively used to treat bacterial infections, were 0.24–1.95 μg/mL for the *Staphylococcus* species and 0.49–31.25 μg/mL for the other Gram-positive bacteria. Other tested compounds had no effect on Gram-positive and Gram-negative bacterial species.

EXPERIMENTAL

Chemistry

Melting points were determined in a Fischer–Johns block (Sanyo, Japan) and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance DPX 250 in DMSO-d_6 using tetramethylsilane as an internal standard. The purity of all compounds was checked by TLC on aluminum oxide 60 F_{254} plates (Merck) in a $CHCl_3/C_2H_5OH$ (10:1, v/v) solvent system with UV visualization (λ = 254 nm). The elemental microanalysis for C, H, and N was performed on an AMZ 851 CHX analyzer, and the results were within ±0.4% of the theoretical value.

Synthesis of 1-({[4-(4-Halogenophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)-4-substituted Thiosemicarbazides (**7–19**) *General Procedure*. To 0.005 mol of substances **4, 5**, or **6**, 0.005 mol of corresponding isothiocyanate was added. Then the mixture was heated in an oil bath at 50–60°C for 12 h. The obtained product was washed with diethyl ether, dried, and recrystallized from ethanol. The compounds **13**, **16–19** were recrystallized from the mixture DMF:H_2O (1:1).

1-({[4-(4-Chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)-4-phenylthiosemicarbazide (**7**). Yield 80%, mp 194–196°C. ^{1}H NMR δ (ppm): 4.04 (s, 2H, CH_2); 7.23–7.60 (m, 5H, C_6H_5); 7.67 (d, 2H, 4-Cl-C_6H_4, J = 7.5 Hz); 7.76 (d, 2H, 4-Cl-C_6H_4, J = 7.5 Hz); 8.97 (s, 1H, CH); 9.80, 9.83, 10.47 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{17}H_{15}ClN_6OS_2$: C 48.74, H 3.61, N 20.06; Found: C 48.67, H 3.60, N 20.08.

4-Benzyl-1-({[4-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)thiosemicarbazide (**8**). Yield 78%, mp 176–178°C. ^{1}H NMR δ (ppm): 4.02 (s, 2H, CH_2); 4.85 (d, 2H, $C_6H_5C\underline{H}_2$, J = 7.5 Hz); 7.25–7.43 (m, 5H, C_6H_5); 7.63 (d, 2H, 4-Cl-C_6H_4, J = 7.5 Hz); 7.76 (d, 2H, 4-Cl-C_6H_4, J = 7.5 Hz); 8.93 (s, 1H, CH); 8.85, 9.58, 10.42 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}ClN_6OS_2$: C 49.94, H 3.96, N 19.41; Found: C 49.90, H 3.94, N 19.39.

1-({[4-(4-Chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)-4-(4-methylphenyl)thiosemicarbazide (**9**). Yield 73%, mp 166–168°C. ^{1}H NMR δ (ppm): 2.35 (s, 3H, CH_3); 4.04 (s, 2H, CH_2); 7.19 (d, 2H, 4-CH_3-$C_6\underline{H}_4$, J = 7.5 Hz); 7.42 (d, 2H, 4-CH_3-$C_6\underline{H}_4$, J = 7.5 Hz); 7.66 (d, 2H, 4-Cl-C_6H_4, J = 7.5 Hz); 7.76 (d, 2H, 4-Cl-C_6H_4, J = 7.5 Hz); 8.97 (s, 1H, CH); 9.72, 9.76, 10.45 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}ClN_6OS_2$: C 49.94, H 3.96, N 19.41; Found: C 49.89, H 3.97, N 19.42.

1-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)-4-phenylthiosemicarbazide (**10**). Yield 81%, mp 190–192°C. ^{1}H NMR δ (ppm): 3.96 (s, 2H, CH_2); 7.12–7.53 (m, 9H, C_6H_5 & 4-Br-C_6H_4); 8.89 (s, 1H, CH); 9.71, 9.74, 10.39 (3s, 3H, 3× NH). Elemental anal. (%) Calcd for $C_{17}H_{15}BrN_6OS_2$: C 44.07, H 3.26, N 18.14; Found: C 44.09, H 3.25, N 18.07.

4-Benzyl-1-({[4-(4-bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)thiosemicarbazide (**11**). Yield 77%, mp 184–186°C. ^{1}H NMR δ (ppm): 3.93 (s, 2H, CH_2); 4.77 (d, 2H, $C_6H_5C\underline{H}_2$, J = 7.5 Hz); 7.25–7.47 (m, 7H, C_6H_5 and 4-Br-C_6H_4); 7.80 (d, 2H, 4-Br-C_6H_4, J = 7.5 Hz); 8.85 (s, 1H, CH); 8.78, 9.49, 10.36 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}BrN_6OS_2$: C 45.29, H 3.59, N 17.60; Found: C 45.22, H 3.60, N 17.54.

1-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)-4-(4-methylphenyl)thiosemicarbazide (**12**). Yield 71%, mp 176–178°C. ^{1}H NMR δ (ppm): 2.27 (s, 3H, CH_3); 3.96 (s, 2H, CH_2); 7.12 (d, 2H, 4-CH_3-$C_6\underline{H}_4$, J = 7.5 Hz); 7.35 (d, 2H, 4-CH_3-$C_6\underline{H}_4$, J = 7.5 Hz); 7.52 (d, 2H, 4-Br-C_6H_4, J = 7.5 Hz); 7.81 (d, 2H, 4-Br-C_6H_4, J = 7.5 Hz); 8.89 (s, 1H, CH); 9.64, 9.68, 10.37 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}BrN_6OS_2$: C 45.29, H 3.59, N 17.60; Found: C 45.20, H 3.57, N 17.58.

1-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)-4-(2-methylphenyl)thiosemicarbazide (**13**). Yield 72%, mp 184–186°C. ^{1}H NMR δ (ppm): 2.12 (s, 3H, CH_3); 4.00 (s, 2H, CH_2); 7.09–7.22 (m, 4H, 2-CH_3-$C_6\underline{H}_4$,); 7.50 (d, 2H, 4-Br-C_6H_4, J = 7.5 Hz); 7.74 (d, 2H, 4-Br-C_6H_4, J = 7.5 Hz); 8.84 (s, 1H, CH); 9.51, 9.66, 10.41 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}BrN_6OS_2$: C 45.29, H 3.59, N 17.60; Found: C 45.31, H 3.60, N 17.57.

4-Allyl-1-({[4-(4-bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)thiosemicarbazide (**14**). Yield 68%, mp 168–170°C. ^{1}H NMR δ (ppm): 4.01 (s, 2H, CH_2); 4.19–4.24 (m, 2H, $C\underline{H}_2CH{=}CH_2$); 5.05–5.21 (m, 2H, $CH_2CH{=}C\underline{H}_2$); 5.80–5.98 (m, 1H, $CH_2C\underline{H}{=}CH_2$); 7.58 (d, 2H, 4-Br-$C_6H_4$, J = 10.0 Hz); 7.89 (d, 2H, 4-Br-C_6H_4, J = 10.0 Hz); 8.98 (s, 1H, CH); 8.46 (t, 1H, NH, J = 5.0 Hz); 9.50, 10.35 (2s, 2H, 2× NH).

Elemental anal. (%), Calcd for $C_{14}H_{15}BrN_6OS_2$: C 39.35, H 3.54, N 19.67; Found: C 39.32, H 3.56, N 19.61.

*1-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}acetyl)-4-cyclohexylthiosemicarbazide (***15**). Yield 73%, mp 187–190°C. ^{1}H NMR δ (ppm): 1.02–1.72 (m, 10H, C_6H_{11}); 3.85 (s, 2H, CH_2); 4.17 (s, 1H, C_6H_{11}); 7.52 (d, 2H, 4-Br-C_6H_4, $J = 7.5$ Hz); 7.82 (d, 2H, 4-Br-C_6H_4, $J = 7.5$ Hz); 8.94 (s, 1H, CH); 7.67, 9.25, 10.13 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{17}H_{21}BrN_6OS_2$: C 43.50, H 4.51, N 17.90; Found: C 43.37, H 4.50, N 17.86.

*1-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}acetyl)-4-ethylthiosemicarbazide (***16**). Yield 79%, mp 186–188°C. ^{1}H NMR δ (ppm): 1.07 (m, 3H, $CH_2C\underline{H}_3$); 3.52 (m, 2H, $C\underline{H}_2CH_3$); 3.89 (s, 2H, CH_2); 7.52 (d, 2H, 4-Br-C_6H_4, $J = 10.0$ Hz); 7.82 (d, 2H, 4-Br-C_6H_4, $J = 10.0$ Hz); 8.93 (s, 1H, CH); 8,26 (t, 1H, NH, $J = 5.0$ Hz); 9.31, 10.22 (2s, 2H, 2× NH). Elemental anal. (%), Calcd for $C_{13}H_{15}BrN_6OS_2$: C 37.59, H 3.64, N 20.23; Found: C 37.60, H 3.64, N 20.18.

*1-({[4-(4-Iodophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}acetyl)-4-phenylthiosemicarbazide (***17**). Yield 70%, mp 176–178°C. ^{1}H NMR δ (ppm): 3.96 (s, 2H, CH_2); 7.12–7.52 (m, 7H, C_6H_5 & 4-I-C_6H_4); 7.97 (d, 2H, 4-I-C_6H_4, $J = 7.5$ Hz); 8.88 (s, 1H, CH); 9.71, 9.75, 10.39 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{17}H_{15}IN_6OS_2$: C 40.01, H 2.96, N 16.47; Found: C 39.97, H 2.94, N 16.48.

*4-Benzyl-1-({[4-(4-iodophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetyl)thiosemicarbazide (***18**). Yield 85%, mp 204–206°C. ^{1}H NMR δ (ppm): 3.95 (s, 2H, CH_2); 4.77 (d, 2H, $C_6H_5C\underline{H}_2$, $J = 5.0$ Hz); 7.15–7.33 (m, 7H, C_6H_5 & 4-I-C_6H_4); 7.97 (d, 2H, 4-I-C_6H_4, $J = 7.5$ Hz); 8.85 (s, 1H, CH); 8,78 (t, 1H, NH, $J = 2.5$ Hz); 9.51, 10.34 (2s, 2H, 2× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}IN_6OS_2$: C 41.23, H 3.27, N 16.03; Found: C 41.20, H 3.30, N 15.97.

*1-({[4-(4-Iodophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}acetyl)-4-(4-methylphenyl)thiosemicarbazide (***19**). Yield 70%, mp 168–170°C. ^{1}H NMR δ (ppm): 2.27 (s, 3H, CH_3); 3.95 (s, 2H, CH_2); 7.12 (d, 2H, 4-CH_3-$C_6\underline{H}_4$, $J = 7.5$ Hz); 7.32–7.36 (m, 4H, 4-I-$C_6\underline{H}_4$ (2H) & 4-CH_3-$C_6\underline{H}_4$ (2H)); 7.96 (d, 2H, 4-I-$C_6\underline{H}_4$ $J = 10.0$ Hz); 8.88 (s, 1H, CH); 9.64, 9.69, 10.37 (3s, 3H, 3× NH). Elemental anal. (%), Calcd for $C_{18}H_{17}IN_6OS_2$: C 41.23, H 3.27, N 16.03; Found: C 43.19, H 3.28, N 15.98.

*5-({[4-(4-Halogenephenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}methyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones (***20–26**). *General Procedure.* To 0.005 mol of compounds **7–11, 14**, or **15**, 10 mL of a 2% solution of NaOH was added. The mixture was refluxed for 2 h. The solution was filtered off. After cooling, the filtrate was acidified by diluted HCl to a pH of 3–4. The resulting precipitate was recrystallized from ethanol.

*5-({[4-(4-Chlorophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}methyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***20**). Yield 78%, mp 177–180°C. ^{1}H NMR δ (ppm): 4.11 (s, 2H, CH_2); 7.15–7.70 (m, 9H, C_6H_5 & 4-Cl-C_6H_4); 8.89 (s, 1H, CH); 13.83 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{17}H_{13}ClN_6S_2$: C 50.93, H 3.27, N 20.96; Found: C 50.84, H 3.27, N 20.79.

*4-Benzyl-5-({[4-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***21**). Yield 81%, mp 143–145°C. ^{1}H NMR δ (ppm): 4.24 (s, 2H, CH_2); 5.24 (s, 2H, $C_6H_5C\underline{H}_2$); 7.17–7.69 (m, 9H, C_6H_5 and 4-Cl-C_6H_4); 8.89 (s, 1H, CH); 13.57 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{18}H_{15}ClN_6S_2$: C 52.10, H 3.64, N 20.25; Found: C 52.03, H 3.59, N 20.26.

*5-({[4-(4-Chlorophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}methyl)-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***22**). Yield 77%, mp 156–158°C. ^{1}H NMR δ (ppm): 2.37 (s, 3H, CH_3); 4.09 (s, 2H, CH_2); 7.12–7.70 (m, 8H, 4-Cl-C_6H_4 and 4-CH_3-$C_6\underline{H}_4$); 8.88 (s, 1H, CH); 13.76 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{18}H_{15}ClN_6S_2$: C 52.10, H 3.64, N 20.25; Found: C 52.01, H 3.61, N 20.20.

*5-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}methyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***23**). Yield 54%, mp 200–202°C. ^{1}H NMR δ (ppm): 4.17 (s, 2H, CH_2); 7.26–7.77 (m, 9H, C_6H_5 and 4-Br-C_6H_4); 8.85 (s, 1H, CH); 13.91 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{17}H_{13}BrN_6S_2$: C 45.85, H 2.94, N 18.87; Found: C 45.79, H 2.95, N 18.79.

*4-Benzyl-5-({[4-(4-bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***24**). Yield 74%, mp 161–164°C. ^{1}H NMR δ (ppm): 4.20 (s, 2H, CH_2); 5.21 (s, 2H, $C_6H_5C\underline{H}_2$); 7.19-7.78 (m, 9H, C_6H_5 and 4-Br-C_6H_4); 8.91 (s, 1H, CH); 13.62 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{18}H_{15}BrN_6S_2$: C 47.06, H 3.29, N 18.29; Found: C 47.10, H 3.27, N 18.26.

*4-Allyl-5-({[4-(4-bromophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***25**). Yield 81%, mp 152–154°C. ^{1}H NMR δ (ppm): 4.31 (s, 2H, CH_2); 4.58 (d, 2H, $C\underline{H}_2CH{=}CH_2$, $J = 5.0$ Hz); 5.00 (d, 1H, $CH_2CH{=}C\underline{H}_2$, $J = 12.5$ Hz); 5.13 (d, 1H, $CH_2CH{=}C\underline{H}_2$, $J = 12.5$ Hz); 5.73–5.84 (m, 1H, $CH_2C\underline{H}{=}CH_2$); 7.40 (d, 2H, 4-Br-$C_6H_4$, $J = 10.0$ Hz); 7.77 (d, 2H, 4-Br-C_6H_4, $J = 10.0$ Hz); 8.92 (s, 1H, CH); 13.68 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{14}H_{13}BrN_6S_2$: C 41.08, H 3.20, N 20.53; Found: C 40.98, H 3.19, N 20.50.

*5-({[4-(4-Bromophenyl)-4H-1,2,4-triazol-3-yl] sulfanyl}methyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (***26**). Yield 70%, mp 144–146°C. ^{1}H NMR δ (ppm): 1.09–1.83 (m, 10H, C_6H_{11}); 4.27 (s, 1H, C_6H_{11}); 4.53 (s, 2H, CH_2); 7.36 (d, 2H, 4-Br-C_6H_4, $J = 7.5$ Hz); 7.78 (d, 2H, 4-Br-C_6H_4, $J = 7.5$ Hz); 8.78 (s, 1H, CH); 13.66 (s, 1H, NH). Elemental anal. (%), Calcd for $C_{17}H_{19}BrN_6S_2$: C 45.23, H 4.24, N 18.62; Found: C 45.19, H 4.21, N 18.58.

Microbiology

Antimicrobial activity tests were carried out against the reference strains of aerobic bacteria, including Gram-positive (*S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *M. luteus* ATCC 10240) or Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027) microorganisms. All of these strains came from the American Type Culture Collection, which is routinely used for the evaluation of antimicrobials. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density conforming to the McFarland standard 0.5 [150×10^6 CFU (colony-forming units/milliliter)].

All stock solutions of the tested compounds were dissolved in dimethyl sulfoxide (DMSO). It was found that DMSO at the final concentration had no influence on the growth of the tested microorganisms.

The in vitro antibacterial activity of the potentially active compounds tested was determined on the basis of the MIC, usually defined as the lowest concentration of the compound at which there is no visible growth of tested microorganisms. The determination of the MIC value was achieved by using the broth microdilution method. The Mueller–Hinton broth was used with a series of twofold dilutions of the tested substances in the final concentration ranging from 3.91 to 1000 μg/mL. Cefuroxime, belonging to the second generation of cephalosporins, was used as a control antimicrobial agent at the final concentration from 0.063 to 500 μg/mL.

In the broth microdilution method, 96-well microplates were used; 198 μL of the Mueller–Hinton broth without or with the tested compound was inoculated with 2 μL of microbial suspension (total volume per each well 200 μL). After incubation (at 35°C for 18 h), spectrophotometric measurements of the optical density (OD_{600}) of the bacterial cultures with or without the tested compounds were performed to determine the MIC. The blank control wells with a twofold dilution of each tested compound were added to the Mueller–Hinton broth without bacteria and were incubated under the same conditions.

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