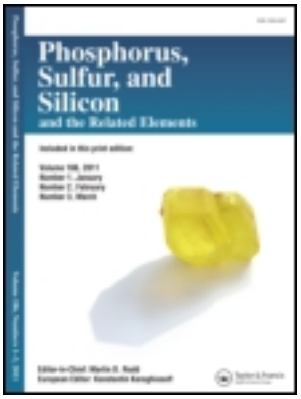

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis and Antimicrobial Properties of New Thiosemicarbazide, 1,2,4-Triazole, and 1,3,4-Thiadiazole Derivatives of Sulfanylacetic Acid

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SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF NEW THIOSEMICARBAZIDE, 1,2,4-TRIAZOLE, AND 1,3,4-THIADIAZOLE DERIVATIVES OF SULFANYLACETIC ACID

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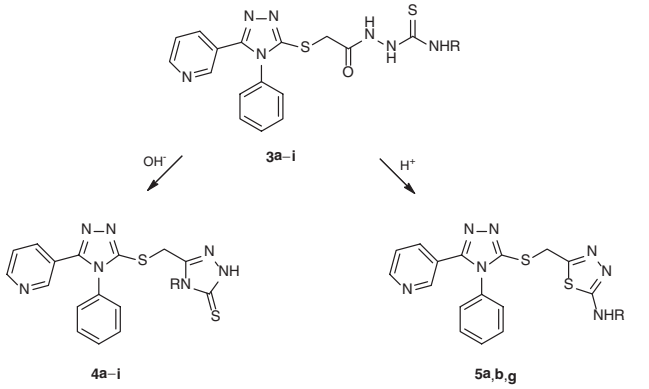
GRAPHICAL ABSTRACT



R = Ph (**a**), 4-MeOC$_6$H$_4$ (**b**), CH$_2$Ph (**c**), *c*-Hex (**d**), 4-BrC$_6$H$_4$ (**e**), Et (**f**), 4-ClC$_6$H$_4$ (**g**), 4-MeOC$_6$H$_4$CH$_2$ (**h**), CH$_2$CH=CH$_2$ (**i**)

Abstract *1,2,4-triazole and 1,3,4-thiadiazole derivatives are still considered a viable lead structure for the synthesis of more efficient antimicrobial agents having a broad spectrum of activity. This study presents the synthesis and antimicrobial evaluation of a new series of substituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives. Reaction of 4-phenyl-5-(pyridin-3-yl)-4H-1,2,4-triazole-3-thione with ethyl bromoacetate yields the corresponding ethyl acetate (**1**). In the subsequent reaction with 100% hydrazine hydrate, the hydrazide (**2**) was obtained, which was converted with isothiocyanates to new acyl derivatives of thiosemicarbazide (**3a–l**). The cyclization of these compounds in alkaline media resulted in the formation of new derivatives of 1,2,4-triazole (**4a–i**), whereas in acidic media new derivatives of 1,3,4-thiadiazole (**5a**,**b**,**g**) were obtained. All synthesized compounds were screened for their in vitro antimicrobial activities.*

Keywords Thiosemicarbazide; 1,2,4-triazole; 1,3,4-thiadiazole; antimicrobial activity; cyclization reaction

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INTRODUCTION

Five-membered aromatic systems having three heteroatoms are still being studied because of their interesting biological properties. Among them, the 1,2,4-triazole and 1,3,4-thiadiazole derivatives possess a wide range of various pharmacological activities, which mainly depend on the nature of the substituents. Some of them obtained in the course of previous investigations and tested on animals showed potential action on the central nervous system.[1] Others exhibited analgesic,[2] antifungal,[3,4] antibacterial,[5–7] antiviral,[8,9] antiphlogistic,[10,11] and antituberculous[12] activity.

Research and development of potent and effective antimicrobial agents represents one of the most important advances in therapeutics, not only in the control of serious infections but also in the prevention and treatment of some infectious complications. Therefore, 1,2,4-triazole and 1,3,4-thiadiazole derivatives are still considered a viable lead structure for the synthesis of more efficient antimicrobial agents with a broad spectrum of activities.

The common method for the preparation of 1,2,4-triazoles and 1,3,4-thiadiazoles is the cyclization reaction of thiosemicarbazide derivatives. In earlier papers,[13–15] it was stated that the cyclization was affected not only by pH of the medium but also by the nature of substituents in the thiosemicarbazide derivatives. The course of the cyclization in alkaline and acidic media for thiosemicarbazide derivatives of formic, benzoic, and nicotinic acids was investigated.[16] Cyclization of thiosemicarbazide derivatives of formic and nicotinic acids in alkaline and in acidic media leads to 1,2,4-triazole derivatives. In alkaline media, the cyclization of benzoic acid thiosemicarbazide derivatives results in the formation of 1,2,4-triazoles, while in acidic media, 1,3,4-thiadiazole derivatives were obtained.[16]

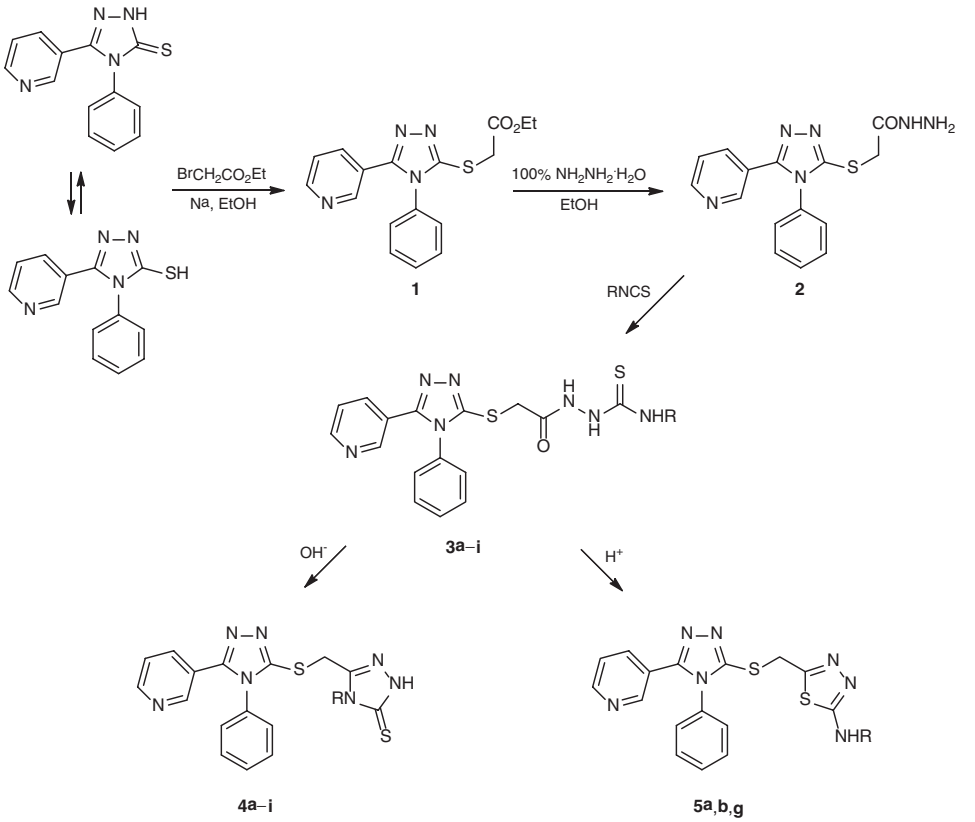
In view of the facts mentioned above and in continuation of our search for various biologically active molecules as well as the encouraging antibacterial activity of some 1,2,4-triazole derivatives has prompted us to synthesize some novel compounds and to carry out preliminary investigations on their antimicrobial activity.[17] In this paper, we report the synthesis and spectral studies of novel series of substituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives and the evaluation of their *in vitro* antibacterial activity.

RESULTS AND DISCUSSION

Chemistry

4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thione, which can exist in two tautomeric forms, was the starting material for the synthesis of new derivatives, which contain two 1,2,4-triazole rings or one 1,2,4-triazole, and one 1,3,4-thiadiazole ring connected by an $-S-CH_2-$ bridge. The thione was obtained using a method described earlier by cyclization of 1-(pyridin-3-yl)-4-phenyl thiosemicarbazide in alkaline medium. Depending on the conditions used, further reaction of 4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thione can lead either to *S*- or to *N*-derivatives. We have investigated the reaction with ethyl bromoacetate. On the basis of the results described in previous papers,[18,19] elemental and spectral analyses as well as X-ray crystallography data of similar compounds, it was found that the reaction leads to the formation of an *S*-derivative.

Reaction of 4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thione with ethyl bromoacetate in the presence of sodium ethanolate gave ethyl [(4-phenyl-(5-pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetate (**1**), which was converted to the hydrazide **2** by reaction with 100% hydrazine hydrate (Scheme 1).



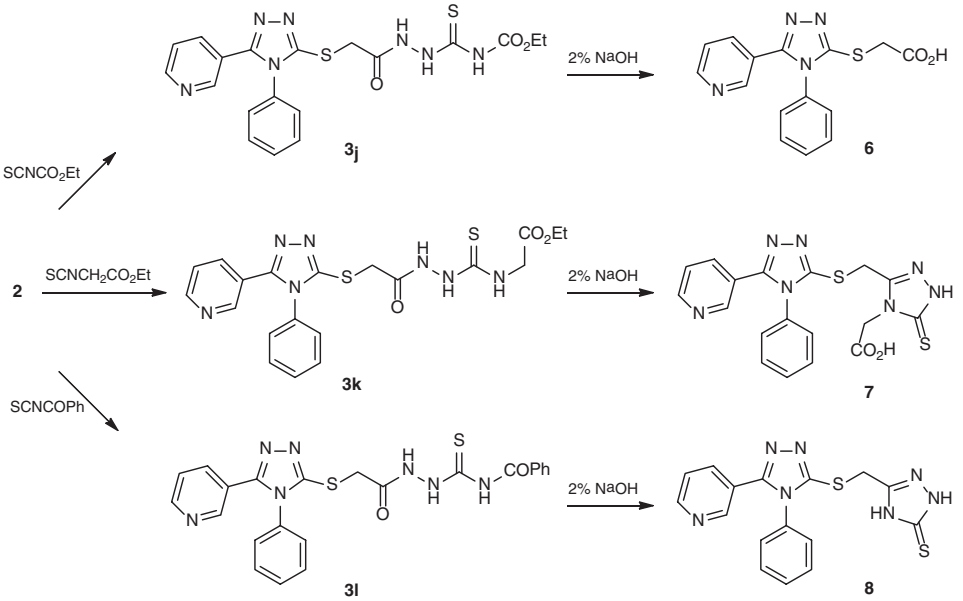
R = Ph (**a**), 4-MeOC$_6$H$_4$ (**b**), CH$_2$Ph (**c**), *c*-Hex (**d**), 4-BrC$_6$H$_4$ (**e**), Et (**f**), 4-ClC$_6$H$_4$ (**g**), 4-MeOC$_6$H$_4$CH$_2$ (**h**), CH$_2$CH=CH$_2$ (**i**)

Scheme 1

Reactions of the hydrazide **2** with aliphatic, aromatic, ethoxycarbonyl, ethoxycarbonylmethyl, and benzoyl isothiocyanates were carried out following two different experimental procedures. The new thiosemibarbazide derivatives (**3a**–**l**) were obtained by heating the reactants in an oil bath without a solvent at temperatures between 50 °C and 110 °C. The thiosemicarbazide derivatives **3a**–**d** and **3f** were prepared by reaction of the hydrazide **2** with the respective isothiocyanate in diethyl ether as solvent.

Cyclization reaction of the thiosemicarbazide derivatives **3a–i** with 2% aqueous solution of sodium hydroxide leads to the new 4*H*-1,2,4-triazol-3(2*H*)-thione derivatives **4a–i**.

Hydrolysis was observed in the case of the cyclization of thiosemicarbazide **3j** in alkaline media, which resulted in the formation of [(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetic acid **6**. This compound was described earlier,[20] but it was obtained in a different way. The cyclization in alkaline media of the thiosemicarbazide **3k**, which contains an ethoxycarbonylmethyl group, was also accompanied by hydrolysis of the ester group and yielded the 4*H*-1,2,4-triazole-3(2*H*)-thione **7**. In the case of 4-benzoyl thiosemicarbazide derivative **3l**, the cyclization in alkaline media gave the 2,5-dihydro-4*H*-1,2,4-triazole-3(2*H*)-thione **8** (Scheme 2).



Scheme 2

The cyclization reaction in acidic media of the new thiosemicarbazide derivatives enabled us to obtain new the new 1,3,4-thiadiazoles **5a**,**b**,**g**.

The reactions were performed according to Schemes 1 and 2; the substituents are listed in Table 1. The structure of the compounds obtained was confirmed by elementary analysis, IR, and ^{1}H NMR spectra and for some of the compounds also by the ^{13}C NMR and MS spectra.

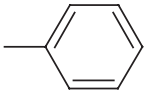
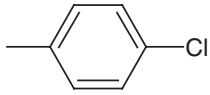
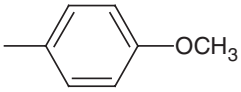
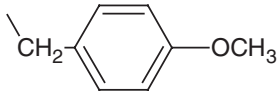
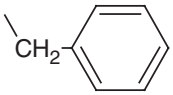
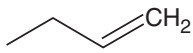
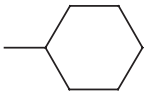
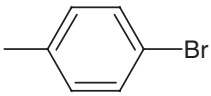
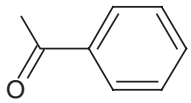
In the IR spectra of the 1,2,4-triazole compounds **4a**–**i**, **7**, and **8**, characteristic absorption bands at about 1500 cm^{-1} corresponding to C—N group and in the range of 1600 cm^{-1} corresponding to C=N group were observed. The IR spectra of the new 1,3,4-thiadiazole derivatives **5a**,**b**,**g** displayed characteristic absorption bands in the range of 1500 cm^{-1} corresponding to the C—N group and in the range of 1600 cm^{-1} corresponding to the C=N group.

The ^{1}H NMR spectra of the thiosemicarbazide derivatives **3a**–**l** show three proton signals typical for the NH protons in the range of 8.4–12.9 ppm. For the new 1,2,4-triazole compounds **4a**–**g**, **7**, and **8**, the signal of the NH proton was observed at 13.6–14.3 ppm. The 1,3,4-thiadiazole derivatives **5a**,**b**,**g** showed one typical proton signal at 10.1–10.3 ppm. ^{13}C NMR spectroscopic data were obtained for compounds **3b**,**c**, **4a**–**c**, and **5a**, while compounds **3a**,**c** and **4b**–**d** were also characterized by MS spectrometry.

Microbiology

All new compounds were screened for in vitro antibacterial activity by the agar well diffusion method. For compounds **3b**,**e**,**g**–**k**, and **4b**,**g**–**i**, which showed potential inhibitory effect on the growth of bacteria, minimal inhibitory concentration (MIC) values were estimated by microdilution technique.

Table 1 Substituents of compounds **3a–l**, **4a–i**, and **5a**,**b**,**g**

Compounds	R	Compounds	R
3a, 4a, 5a		**3g, 4g, 5g**	
3b, 4b, 5b		**3h, 4h**	
3c, 4c		**3i, 4i**	
3d, 4d		**3j**	$COOC_2H_5$
3e, 4e		**3k**	$CH_2COOC_2H_5$
3f, 4f	C_2H_5	**3l**	

According to the screening results obtained with 1000 μg/mL of the new compounds by the method mentioned above, some of them possess activity only against the Gram-positive bacteria tested (Table 2); no activity against Gram-negative bacteria was found. In our experiments, MICs of available antibiotics such as gentamicin and cefuroxime, which are extensively used to treat bacterial infections, were also estimated for comparison.

Table 2 The inhibitory activities of the synthesized compounds against Gram-positive bacteria on the basis of MIC (μg/mL) values determined by broth microdilution method

Compound	*Sa*25923	*Sa*6538	*Se*12228	*Bs*6633	*Bc*10876	*Ml*10240
3b	1000	1000	1000	1000	1000	1000
3e	**125**	**125**	**500**	**500**	**500**	**62.5**
3g	1000	1000	1000	1000	>1000	**125**
3h	1000	1000	>1000	>1000	>1000	1000
3i	>1000	1000	>1000	1000	1000	1000
3j	**500**	>1000	1000	1000	>1000	**500**
3k	>1000	>1000	1000	1000	1000	1000
3l	**125**	**250**	**250**	**125**	**250**	**125**
4b	1000	**250**	1000	1000	1000	**125**
4g	1000	1000	1000	1000	1000	**250**
4h	>1000	>1000	>1000	1000	1000	1000
4i	>1000	1000	>1000	1000	1000	1000
Cefuroxime	0.49	1.95	0.49	0.49	31.25	0.49
Gentamicin	0.015	0.06	0.06	0.015	0.49	0.12

Sa25923, *Staphylococcus aureus* ATCC 25923; Sa6538, *Staphylococcus aureus* ATCC 6538; Se12228, *Staphylococcus epidermidis* ATCC 12228; Bs6633, *Bacillus subtilis* ATCC 6633; Bc10876, *Bacillus cereus* ATCC 10876; Ml10240, *Micrococcus luteus* ATCC 10240.

On the basis of the MIC values (Table 2) obtained by broth microdilution method, it becomes evident that the highest activity against Gram-positive bacteria is shown by compounds **3e** (MIC = 62.5–500 μg/mL) and **3l** (MIC = 125–250 μg/mL). This activity may be due to the introduction of a 4-bromophenyl (**3e**) and a benzoyl (**3l**) substituent in the 4 position of the thiosemicarbazide derivative. Our investigations also show that the acyclic thiosemicarbazide derivatives display a better antimicrobial activity than the cyclic compounds for all reference strains of Gram-positive bacteria. For comparison, MIC values for reference strains of the tested bacteria were 0.015–0.49 μg/mL for gentamicin and 0.49–31.25 μg/mL for cefuroxime.

In summary, among the tested new compounds, only **3e** and **3l** exhibited moderate antibacterial activity against both pathogenic and opportunistic Gram-positive bacteria and may be of use for searching new derivatives showing better antimicrobial activity.

EXPERIMENTAL

Chemistry

Melting points were determined in Fisher–Johns blocks and are uncorrected. The IR spectra (ν, cm^{-1}) were recorded in KBr tablets using a Specord IR-75 spectrophotometer. The ^{1}H NMR spectra were recorded with a Bruker Avance 300 apparatus in DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. The ^{13}C NMR spectra were recorded with a Bruker Avance 300 instrument. Chemical shifts are given in ppm (δ-scale). The MS spectra were recorded with a Thermo-Finnigan Trace DSQ GC-MS apparatus. Chemicals were purchased from Merck Co. or Lancaster and used without further purification.

The purity of the compounds obtained was checked by TLC on aluminum oxide 60 F_{254} plates (Merck), in the $CHCl_3/C_2H_5OH$ (10:1, *v/v*) solvent system with UV visualization (λ = 254 nm).

Ethyl [(4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] Acetate (1). Approximately 0.23g (10 mmol) of sodium was added to 5 mL of anhydrous ethanol (EtOH). The solution was placed in a three-necked flask equipped with reflux condenser and closed with a tube of $CaCl_2$ and stirred. Stirring was continued until the sodium dissolved completely and then 2.54 g (10 mmol) of 4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thione was added. Then, 1.22 mL of ethyl bromoacetate was added dropwise. The content of the flask was stirred for 4 h and left at r.t. for 12 h. Then, 10 mL of anhydrous EtOH was added and the reaction solution was heated for 1 h. From the mixture, the inorganic compounds were removed by filtration. After cooling of the reaction mixture, the precipitate formed was filtered off and crystallized from EtOH. Yield: 2.43 g (71.4%); mp 72 °C–74 °C (dec.). IR (KBr), ν (cm^{-1}): 3110 (CH aromatic), 2982, 1445 (CH aliphatic), 1722 (C=O), 1602 (C=N), 669 (C−S). ^{1}H NMR (DMSO-d_6): δ = 1.20 (t, J = 6 Hz, 3H, CH_3), 4.12 (s, 2H, CH_2), 4.13 (q, J = 5 Hz, 2H, CH_2), 7.37–8.58 (m, 9H, arom-H). Anal. calcd. for $C_{17}H_{16}N_4O_2S$ (340.4): C, 59.98; H, 4.74; N, 16.46; S, 9.42; Found: C, 59.85; H, 4.76; N, 16.41; S, 9.37%.

[(4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] Acetohydrazide (2). Approximately 0.5 mL of 100% hydrazine hydrate was added to 3.40 g (10 mmol) of compound **1** in 10 mL of anhydrous EtOH. The mixture was left at r.t. for 24 h. The precipitate of the hydrazide **2** was filtered off, dried, and crystallized from EtOH. Yield: 2.94 g (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): $\delta = 3.93$ (s, 2H, CH_2), 4.31 (s, 2H, NH_2), 7.37–8.58 (m, 9H, arom-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, S, 9.82; Found: C, 55.16; H, 4.35; N, 25.80; S, 9.77%.

Thiosemicarbazides 3a–3l

Method A (for compounds 3a–l). A mixture of hydrazide **2** 3.26 g (10 mmol) and 10 mmol of the respective isothiocyanate was heated in an oil bath at 50 °C–110 °C for 8–20 h. The product was washed with diethyl ether to remove unreacted isothiocyanate. Then, it was separated by filtration, and dried and crystallized from EtOH (**3a**,**c**,**f**,**h**,**i–l**), butanol (**3b**,**d**,**e**), or methanol (**3g**).

Method B (for compounds 3a–d,f). Approximately 10 mmol of the appropriate isothiocyanate was added to 3.26 g (10 mmol) of hydrazide **2** in 10 mL of anhydrous diethyl ether. The mixture was placed in a conical bulb, stirred for 5 min, and left at r.t. for 24 h. The precipitate of the thiosemicarbazide (**3a–d**,**f**) was separated by filtration, and dried and crystallized from EtOH. The compounds thus obtained showed the same melting points as the compounds obtained by the Method A.

1-{[(4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl}-4-phenyl Thiosemicarbazide (3a). Yield: 3.29 g (71.4%). Temperature of reaction: 50 °C for 14 h, mp 172 °C–174 °C (dec.). IR (KBr), ν (cm^{-1}): 3201 (NH), 3103 (CH aromatic), 2983 (CH aliphatic), 1701 (C=O), 1611 (C=N), 1501 (C−N), 1322 (C=S), 679 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.93$ (s, 2H, CH_2), 7.30–8.58 (m, 14H, arom-H), 9.36 (s, 1H, NH), 9.68 (s, 1H, NH), 10.44 (s, 1H, NH). MS m/z (%): 461 (M^+, 2), 383 (1), 326 (5), 295 (12), 254 (38), 253 (46), 221 (4), 195 (7), 181 (8), 149 (9), 119 (15), 105 (38), 91 (34), 78 (33), 77 (100). Anal. calcd. for $C_{22}H_{19}N_7OS_2$ (461.6): C, 57.25; H, 4.15; N, 21.24; S, 13.89; Found C, 57.32; H, 4.13; N, 21.28; S, 13.94%.

4-(4-Methoxyphenyl)-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3b). Yield: 4.06 g (82.6%). Temperature of reaction: 50 °C for 16 h, mp 172 °C–174 °C (dec.). IR (KBr), ν (cm^{-1}): 3208 (NH), 3092 (CH aromatic), 2973, 1442, 756 (CH aliphatic), 1702 (C=O), 1601 (C=N), 1520 (C−N), 1339 (C=S), 683 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.67$ (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 6.85–8.57 (m, 13H, arom-H), 9.59 (s, 1H, NH), 9.66 (s, 1H, NH), 10.41 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta = 34.4$ (−S−CH_2−), 55.2 (−OCH_3), 113.2 (CH), 122.8 (CH), 122.9 (CH), 123.5 (CH), 127.3 (CH), 127.6 (CH), 130.1 (CH), 130.4 (CH), 131.7 (CH), 133.2 (C), 135.3 (C), 148.2 (C), 150.6 (C), 156.8 (C−S), 165.9 (C_3 triazole), 166.7 (C=O), 181.1 (C=S). Anal. calcd. for $C_{23}H_{21}N_7O_2S_2$ (491.6): C, 56.19; H, 4.30; N, 19.94; S, 13.04; Found C, 55.97; H, 4.29; N, 19.92; S, 13.10%.

4-Benzyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3c). Yield: 3.97 g (83.5%). Temperature of reaction: 50 °C for 10 h, mp 180 °C–182 °C (dec.). IR (KBr), ν (cm^{-1}): 3211 (NH), 3097 (CH aromatic), 2981, 1447 (CH aliphatic), 1705 (C=O), 1601 (C=N), 1518 (C−N), 1347 (C=S), 685 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.92$ (s, 2H, CH_2), 4.75 (s, 2H, CH_2), 7.14–8.53 (m, 14H, arom-H), 9.36 (s, 1H, NH), 9.54 (s, 1H, NH), 10.41 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta = 33.7$ (−S−CH_2−), 46.7 (−CH_2−), 123.5 (CH), 123.7 (CH), 126.6 (CH), 127.0 (CH), 127.7 (CH), 127.9 (CH), 130.1 (CH), 130.3 (CH), 130.4 (CH), 130.6 (CH), 133.3 (C), 135.4 (C), 139.4 (C), 152.6 (C−S), 166.0 (C_3 triazole), 166.8 (C=O), 182.1 (C=S). MS m/z (%): 475 (M^+, 0.8), 384 (1.5), 326 (6), 295 (12), 254 (35), 253 (37), 221 (3), 195 (7), 149 (16), 119 (7), 91 (100), 78 (11), 77 (31). Anal. calcd. for

$C_{23}H_{21}N_7OS_2$ (475.6): C, 58.08; H, 4.45; N, 20.61; S, 13.48; Found C, 57.88; H, 4.44; N, 20.59; S, 13.44%.

4-Cyclohexyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetyl} Thiosemicarbazide (3d). Yield: 3.83 g (81.9%). Temperature of reaction: 80 °C for 12 h, mp 185–187 °C (dec.). IR (KBr), ν (cm^{-1}): 3220 (NH), 3099 (CH aromatic), 2978, 1434, 765 (CH aliphatic), 1703 (C=O), 1610 (C=N), 1516 (C−N), 1343 (C=S), 695 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 1.03$–1.79 (m, 10H, cyclohexyl-CH_2), 3.93 (s, 2H, CH_2), 4.12 (m, 1H, cyclohexyl-CH), 7.37–8.59 (m, 9H, arom-H), 9.29 (s, 1H, NH), 9.36 (s, 1H, CH), 10.19 (s, 1H, NH). Anal. calcd. for $C_{22}H_{25}N_7OS_2$ (467.6): C, 56.51; H, 5.39; N, 20.97; S, 13.71; Found C, 56.47; H, 5.34; N, 21.01; S, 13.80%.

4-(4-Bromophenyl)-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3e). Yield: 5.16 g (95.5%). Temperature of reaction: 110 °C for 14 h, mp 163 °C–165 °C (dec.). IR (KBr), ν (cm^{-1}): 3211 (NH), 3093 (CH aromatic), 2977 (CH aliphatic), 1702 (C=O), 1609 (C=N), 1331 (C=S), 689 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.01$ (s, 2H, CH_2), 7.36–8.58 (m, 13H, arom-H), 9.76 (s, 1H, NH), 9.88 (s, 1H, NH), 10.47 (s, 1H, NH). Anal. calcd. for $C_{22}H_{18}BrN_7OS_2$ (540.5): C, 48.89; H, 3.36; N, 18.14; S, 11.86; Br, 14.78; Found C, 48.85; H, 3.37; N, 18.20; S, 11.90%.

4-Ethyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetyl} Thiosemicarbazide (3f). Yield: 3.34 g (80.9%). Temperature of reaction 60 °C for 8 h, mp 194–196 °C (dec.). IR (KBr), ν (cm^{-1}): 3117 (NH), 3092 (CH aromatic), 2972, 1447, 763 (CH aliphatic), 1701 (C=O), 1602 (C=N), 1511 (C−N), 1334 (C=S), 683 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 1.14$ (t, $J = 9$ Hz, 3H, CH_3), 3.60 (q, $J = 7.5$ Hz, 2H, CH_2), 3.83 (s, 2H, CH_2), 7.43–8.56 (m, 9H, arom-H), 8.35 (s, 1H, NH), 9.42 (s, 1H, NH), 10.21 (s, 1H, NH). Anal. calcd. for $C_{18}H_{19}N_7OS_2$ (413.5): C, 52.28; H, 4.63; N, 23.71; S, 15.51; Found C, 52.15; H, 4.62; N, 23.79; S, 15.44%.

4-(4-Chlorophenyl)-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3g). Yield: 4.59 g (92.7%). Temperature of reaction: 100 °C for 16 h, mp 162 °C–164 °C (dec.). IR (KBr), ν (cm^{-1}): 3217 (NH), 3108 (CH aromatic), 2974 (CH aliphatic), 1699 (C=O), 1611 (C=N), 1348 (C=S), 685 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.09$ (s, 2H, CH_2), 7.43–8.64 (m, 13H, arom-H), 9.85 (s, 1H, NH), 9.96 (s, 1H, NH), 10.55 (s, 1H, NH). Anal. calcd. for $C_{22}H_{18}ClN_7OS_2$ (496.0): C, 53.27; H, 3.66; N, 19.77; S, 12.93; Cl, 7.15; Found C, 53.32; H, 3.67; N, 19.70; S, 12.99%.

4-(4-Methoxybenzyl)-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3h). Yield: 4.94 g (97.7%). Temperature of reaction: 55 °C for 12 h, mp 196 °C–198 °C (dec.). IR (KBr), ν (cm^{-1}): 3218 (NH), 3095 (CH aromatic), 2977, 1449, 757 (CH aliphatic), 1701 (C=O), 1607 (C=N), 1519 (C−N), 1336 (C=S), 689 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.75$ (s, 3H, CH_3), 4.05 (s, 2H, CH_2), 4.74 (s, 2H, CH_2), 6.85–8.63 (m, 13H, arom-H), 8.67 (s, 1H, NH), 9.57 (s, 1H, NH), 10.46 (s, 1H, NH). Anal. calcd. for $C_{24}H_{23}N_7O_2S_2$ (505.6): C, 57.01; H, 4.58; N, 19.39; S, 12.68; Found C, 56.92; H, 4.57; N, 19.44; S, 12.72%.

4-Allyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetyl} Thiosemicarbazide (3i). Yield: 3.32 g (78.2%). Temperature of reaction: 50 °C for 12 h, mp 170 °C–172 °C (dec.). IR (KBr), ν (cm^{-1}): 3209 (NH), 3092 (CH aromatic), 2970, 1429, 759 (CH aliphatic), 1701 (C=O), 1611 (C=N), 1520 (C−N), 1336 (C=S), 689 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.99$ (s, 2H, CH_2), 4.18 (d, $J = 5$ Hz, 2H, CH_2,), 5.02–5.13 (m, 2H, $= CH_2$), 5.79–5.88 (m, 1H, CH), 7.40–8.56 (m, 9H, arom-H), 9.37 (s, 1H, NH), 9.47 (s, 1H, NH), 10.35 (s, 1H, NH). Anal. calcd. for $C_{19}H_{19}N_7OS_2$

(425.5): C, 53.63; H, 4.50; N, 23.04; S, 15.07; Found C, 53.76; H, 4.49; N, 22.96; S, 15.00%.

4-Ethoxycarbonyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3j). Yield: 4.45 g (97.4%). Temperature of reaction: 50 °C for 16 h, mp 176 °C–178 °C (dec.). IR (KBr), ν (cm^{-1}): 3199 (NH), 3110 (CH aromatic), 2961, 1448, 755 (CH aliphatic), 1732 (C=O acidic), 1700 (C=O), 1603 (C−N), 1518 (C−N), 1348 (C=S), 690 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 1.26$ (t, $J = 7.5$ Hz, 3H, CH_3), 4.04 (s, 2H, CH_2), 4.10 (q, $J = 7.5$ Hz, 2H, CH_2), 7.48–8.64 (m, 9H, arom-H), 11.13 (s, 1H, NH), 11.45 (s, 1H, NH), 11.61 (s, 1H, NH). Anal. calcd. for $C_{19}H_{19}N_7O_3S_2$ (457.5): C, 49.88; H, 4.18; N, 21.43; S, 14.02; Found C, 49.81; H, 4.19; N, 21.44; S, 14.07%.

4-Ethoxycarbonylmetyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl} Thiosemicarbazide (3k). Yield: 3.82 g (81.2%). Temperature of reaction: 50 °C for 18 h, mp 184 °C–186 °C (dec.). IR (KBr), ν (cm^{-1}): 3201 (NH), 3082 (CH aromatic), 2986, 1484, 751 (CH aliphatic), 1729 (C=O acidic), 1701 (C=O), 1599 (C=N), 1499 (C−N), 1349 (C=S), 684 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 1.20$ (t, $J =$ 7.5 Hz, 3H, CH_3), 4,04 (s, 2H, CH_2), 4.10 (q, $J = 7.5$ Hz, 2H, CH_2), 4.34 (s, 2H, CH_2), 7.50–8.61 (m, 9H, arom-H), 8.79 (s, 1H, NH), 9.74 (s, 1H, NH), 10.53 (s, 1H, NH). Anal. calcd. for $C_{20}H_{21}N_7O_3S_2$ (471.6): C, 50.94; H, 4.49; N, 20.79; S, 13.60; Found C, 51.08; H, 4.50; N, 20.81; S, 13.54%.

4-Benzoyl-1-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetyl} Thiosemicarbazide (3l). Yield: 4.82 g (98.4%). Temperature of reaction: 50 °C for 16 h, mp 162 °C–164 °C (dec.). IR (KBr), ν (cm^{-1}): 3186 (NH), 3079 (CH aromatic), 2976, 1456 (CH aliphatic), 1748 (C=O acidic), 1708 (C=O), 1609 (C=N), 1511 (C−N), 1359 (C=S), 689 (C–S). ^{1}H NMR (DMSO-d_6): $\delta = 4.16$ (s, 2H, CH_2), 7.51–8.63 (m, 14H, arom-H), 11.39 (s, 1H, NH), 11.87 (s, 1H, NH), 12.92 (s, 1H, NH). Anal. calcd. for $C_{23}H_{19}N_7O_2S_2$ (489.6): C, 56.43; H, 3.91; N, 20.03; S, 13.10; Found C, 56.37; H, 3.92; N, 19.95; S, 13.03%.

4*H*-1,2,4-Triazole-3(2*H*)-thiones (4a–4i): General Procedure

A mixture of the thiosemicarbazide **3a–i** (10 mmol) and 20–40 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 h. Then, the solution was neutralized with diluted hydrochloric acid and the precipitate formed was separated by filtration and crystallized from EtOH (**4a**,**b**,**f**,**h**), butanol (**4e**,**g**,**i**), or methanol (**4c**,**d**).

5-{[(4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4-phenyl-4*H*-1,2,4-triazole-3(2*H*)-thione (4a). Yield: 3.36 g (75.8%); mp 210 °C–212 °C (dec.). IR (KBr), ν (cm^{-1}): 3169 (NH), 3079 (CH aromatic), 2955 (CH aliphatic), 1602 (C=N), 1506 (C−N), 1368 (C=S), 689 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.19$ (s, 2H, CH_2), 7.15–8.57 (m, 14H, arom-H), 13.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta =$ 27.3 (−S−CH_2−), 122.8 (CH), 123.4 (CH), 123.6 (CH), 127.5 (CH), 127.6 (CH), 128.2 (CH), 128.7 (CH), 129.3 (CH), 129.6 (CH), 130.3 (CH), 133.2 (C), 135.4 (C), 135.8 (C), 150.6 (C_3· triazole), 150.9 (C–S), 152.5 (C_3 triazole), 168.2 (C = S). Anal. calcd. for $C_{22}H_{17}N_7S_2$ (443.5): C, 59.57; H, 3.86; N, 22.10; S, 14.46; Found C, 59.43; H, 3.85; N, 22.06; S, 14.50%.

4-(4-Methoxyphenyl)-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4b). Yield: 4.30 g (90.9%); mp 105 °C–107 °C (dec.). IR (KBr), ν (cm^{-1}): 3189 (NH), 3091 (CH aromatic), 2974,

1456 (CH aliphatic), 1610 (C=N), 1500 (C−N), 1355 (C=S), 695 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.85$ (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 7.06–8.57 (m, 13H, arom-H), 13.82 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta = 33.1$ (−S−CH_2−), 55.6 (−OCH_3), 114.5 (CH), 123.6 (CH), 125.6 (CH), 127.6 (CH), 128.4 (CH), 128.9 (CH), 129.6 (CH), 129.8 (CH), 130.4 (CH), 133.2 (C), 135.8 (C), 136.1 (C), 159.8 (C), 150.5 (C_3· triazole), 159.7 (C−S), 168.9 (C_3 triazole), 169.2 (C=S). MS m/z (%): 473 (M^+, 0.02), 368 (0.22), 354 (0.05), 328 (0.52), 312 (0.62), 295 (0.14), 268 (5), 254 (100), 253 (91), 221 (4), 195 (21), 168 (7), 149 (23), 119 (21), 104 (20), 91 (27), 78 (25), 77 (67). Anal. calcd. for $C_{23}H_{19}N_7OS_2$ (473.6): C, 58.33; H, 4.04; N, 20.70; S, 13.54; Found C, 58.52; H, 4.03; N, 20.69 S, 13.59%.

4-Benzyl-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4c). Yield: 3.46 g (75.7%); mp 103 °C–105 °C (dec.). IR (KBr), ν (cm^{-1}): 3149 (NH), 3076 (CH aromatic), 2958, 1421, 757 (CH aliphatic), 1611 (C=N), 1506 (C−N), 1351 (C=S), 693 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.36$ (s, 2H, CH_2), 5.30 (s, 2H, CH_2), 7.29–8.58 (m, 14H, arom-H), 13.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta = 26.4$ (−S−CH_2), 45.9 (CH_2), 122.9 (CH), 123.6 (CH), 123.9 (CH), 127.0 (CH), 127.7 (CH), 128.6 (CH), 128.7 (CH), 129.5 (CH), 130.1 (CH), 130.4 (CH), 133.0 (C), 135.3 (C), 135.8 (C), 150.3 (C_3· triazole), 150.6 (C−S), 152.4 (C_3 triazole), 168.8 (C=S). MS m/z (%): 457 (M^+, 0.04), 368 (0.02), 344 (0.03), 312 (0.03), 295 (0.5), 268 (3), 221 (5), 195 (22), 181 (7), 168 (9), 149 (17), 119 (12), 104 (17), 91 (34), 78 (19), 77 (51). Anal. calcd. for $C_{23}H_{19}N_7S_2$ (457.6): C, 60.37; H, 4.18; N, 21.43; S, 14.01; Found C, 60.60; H, 4.18; N, 21.38; S, 14.08%.

4-Cyclohexyl-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4d). Yield: 3.56 g (79.3%); mp 162 °C–164 °C (dec.). IR (KBr), ν (cm^{-1}): 3164 (NH), 3072 (CH aromatic), 2983, 1459, 767 (CH aliphatic), 1606 (C=N), 1503 (C−N), 1344 (C=S), 680 (C−S). ^{1}H NMR (DMSO-d_6): $\delta =$ 1.18–1.72 (m, 10H, cyclohexyl-CH_2), 4,05 (s, 2H, CH_2), 4.47 (m, 1H, cyclohexyl-CH), 7.34–8.58 (m, 9H, arom-H), 13,56 (s, 1H, NH). MS m/z (%): 449 (M^+, 0.2), 367 (0.03), 339 (0.08), 292 (0.06), 254 (61), 253 (60), 221 (14), 195 (15), 181 (5), 149 (26), 127 (8), 119 (23), 104 (17), 91 (32), 83 (12), 78 (41), 77 (100). Anal. calcd. for $C_{22}H_{23}N_7S_2$ (449.6): C, 58.77; H, 5.16; N, 21.81; S, 14.26; Found C, 58.87; H, 5.18; N, 21.76; S, 14.30%.

4-(4-Bromophenyl)-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4e). Yield: 4.68 g (89.6%); mp 150 °C–152 °C (dec.). IR (KBr), ν (cm^{-1}): 3177 (NH), 3096 (CH aromatic), 2975, 1463 (CH aliphatic), 1605 (C=N), 1508 (C−N), 1324 (C=S), 679 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.22$ (s, 2H, CH_2), 7.27–8.58 (m, 13H, arom-H), 13.90 (s, 1H, NH). Anal. calcd. for $C_{22}H_{16}BrN_7S_2$ (522.4): C, 50.58; H, 3.09; N, 18.77; S, 12.27; Br, 15.29; Found C, 50.64; H, 3.10; N, 18.80; S, 12.21%.

4-Ethyl-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4f). Yield: 3.17 g (80.1%); mp 218 °C–220 °C (dec.). IR (KBr), ν (cm^{-1}): 3145 (NH), 3081 (CH aromatic), 2962, 1429, 763 (CH aliphatic), 1612 (C=N), 1507 (C−N), 1333 (C=S), 695 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 1.04$ (t, $J = 7.5$ Hz, 3H, CH_3), 3.70 (q, $J = 7.5$ Hz, 2H, CH_2), 4.06 (s, 2H, CH_2), 7.36–8.57 (m, 9H, arom-H), 13.98 (s, 1H, NH). Anal. calcd. for $C_{18}H_{17}N_7S_2$ (395.5): C, 54.66; H, 4.33; N, 24.79; S, 16.21; Found C, 54.51; H, 4.34; N, 24.76; S, 16.18%.

4-(4-Chlorophenyl)-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4g). Yield: 4.69 g (98.1%); mp 118 °C–120 °C (dec.). IR (KBr), ν (cm^{-1}): 3153 (NH), 3068 (CH aromatic), 2975,

1449 (CH aliphatic), 1598 (C=N), 1507 (C−N), 1314 (C=S), 678 (C−S). ^{1}H NMR (DMSO-d_6): δ = 4.23 (s, 2H, CH_2), 7.30–8.58 (m, 13H, arom-H), 13.92 (s, 1H, NH). Anal. calcd. for $C_{22}H_{16}ClN_7S_2$ (478.0): C, 55.28; H, 3.37; N, 20.51; S, 13.42; Cl, 7.42; Found C, 55.08; H, 3.36; N, 20.52; S, 13.37%.

4-(4-Methoxybenzyl)-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4h). Yield: 3.11 g (63.8%); mp 112 °C–114 °C (dec.). IR (KBr), ν (cm^{-1}): 3152 (NH), 3061 (CH aromatic), 2972, 1463, 751 (CH aliphatic), 1610 (C=N), 1507 (C−N), 1338 (C=S), 679 (C−S). ^{1}H NMR (DMSO-d_6): δ = 3.72 (s, 3H, CH_3), 4.31 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 6.83–8.60 (m, 13H, arom-H), 13.84 (s, 1H, NH). Anal. calcd. for $C_{24}H_{21}N_7OS_2$ (487.6): C, 59.12; H, 4.34; N, 20.11; S, 13.15; Found C, 59.07; H, 4.33; N, 20.08; S, 13.20%.

4-Allyl-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (4i). Yield: 3.19 g (78.2%); mp 170 °C–172 °C (dec.). IR (KBr), ν (cm^{-1}): 3187 (NH), 3086 (CH aromatic), 2976, 1443, 749 (CH aliphatic), 1602 (C=N), 1501 (C−N), 1357 (C=S), 678 (C−S). ^{1}H NMR (DMSO-d_6): δ = 4.38 (s, 2H, CH_2), 4.64 (d, J = 5 Hz, 2H, CH_2,), 5.18–5.25 (m, 2H, = CH_2), 5.83–5.95 (m, 1H, CH), 7.38–8.58 (m, 9H, arom-H), 13.74 (s, 1H, NH). Anal. calcd. for $C_{19}H_{17}N_7S_2$ (407.5): C, 56.00; H, 4.20; N, 24.06; S, 15.74; Found C, 55.92; H, 4.24; N, 24.10; S, 15.79%.

Synthesis of 1,3,4-thiadiazoles 5a,b,g

Method A (for compounds 5a,b,g). Approximately 10 mmol of the respective thiosemicarbazide **3a**,**b**,**g** was dissolved in 10–20 mL of diluted sulfuric acid and stirred in a closed bulb for 1 h. Subsequently, the solution was poured on crushed ice (50 g) and stirred until the ice was completely dissolved. The solution was neutralized with ammonium hydroxide. The precipitate formed was separated by filtration, dried, and crystallized from EtOH (**5a**,**b**) or butanol (**5g**).

Method B (for compound 5a). Around 20 mL of 10% ethanolic solution of hydrochloric acid was added to thiosemicarbazide **3a** and the reaction mixture was heated under reflux for 1 h. Subsequently, the solution was left at r.t. for 24 h. The precipitate formed was separated by filtration, dried, and crystallized from EtOH. The compound obtained had the same melting point as the compound obtained by Method A.

Method C (for compound 5g). A mixture of 10 mmol of thiosemicarbazide **3g** in 10 mL of anhydrous acetic acid was refluxed for 1 h. Subsequently, the solution was left at r.t. for 12 h. The precipitate formed was separated by filtration, dried, and crystallized from butanol. The compound thus obtained had the same melting point as the compound obtained by Method A.

(2-Aminophenyl)-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (5a). Yield: 3.23 g (72.8%); mp 182 °C–184 °C (dec.). IR (KBr), ν (cm^{-1}): 3249 (NH), 3098 (CH aromatic), 2948 (CH aliphatic), 1609 (C=N), 1507 (C−N), 691 (C−S). ^{1}H NMR (DMSO-d_6): δ = 4.63 (s, 2H, CH_2), 7.39–8.55 (m, 14H, arom-H), 10.34 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ = 29.4 (−S−CH_2−), 116.1 (CH), 120.6 (CH), 121.5 (CH), 122.2 (CH), 126.3 (CH), 128.7 (CH), 128.8 (CH), 129.0(CH), 146.9 (CH), 149.3 (CH), 131.9 (C), 134.0 (C), 139.2 (C), 149.9 (C−S), 151.3 (C_3 triazole), 154.3 (C_2 thiadiazole), 164.1 (C_5 thiadiazole). Anal. calcd. for

$C_{22}H_{17}N_7S_2$ (443.5): C, 59.57; H, 3.86; N, 22.10; S, 14.46; Found C, 59.81; H, 3.87; N, 22.03; S, 14.51%.

2-[Amino-(4-methoxyphenyl)]-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl) sulfanyl]methyl}-1,3,4-thiadiazole (5b). Yield: 4.16 g (87.8%); mp 192 °C–194 °C (dec.). IR (KBr), ν (cm^{-1}): 3199 (NH), 3056 (CH aromatic), 2981, 1468 (CH aliphatic), 1603 (C=N), 1500 (C−N), 679 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.65$ (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 7.32–8.60 (m, 13H, arom-H), 10.16 (s, 1H, NH). Anal. calcd. for $C_{23}H_{19}N_7OS_2$ (473.6): C, 58.33; H, 4.04; N, 20.70; S, 13.54; Found C, 58.25; H, 4.05; N, 20.62; S, 13.49%.

2-[Amino-(4-chlorophenyl)]-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl) sulfanyl]methyl}-1,3,4-thiadiazole (5g). Yield: 4.27 g (89.3%); mp 208 °C–210 °C (dec.). IR (KBr), ν (cm^{-1}): 3281 (NH), 3066 (CH aromatic), 2951 (CH aliphatic), 1600 (C=N), 1510 (C−N), 692 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.71$ (s, 2H, CH_2), 7.35–8.58 (m, 13H, arom-H), 10.26 (s, 1H, NH). Anal. calcd. for $C_{22}H_{16}ClN_7S_2$ (478.0): C, 55.28; H, 3.37; N, 20.51; S, 13.42; Cl, 7.42; Found C, 55.11; H, 3.38; N, 20.56; S, 13.48%.

[(4-Phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetic acid (6). Compound **6** was obtained using the same method described for derivatives **4a–i**. A mixture of thiosemicarbazide **3j** (10 mmol) and 20 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 h. Subsequently, the solution was neutralized with diluted hydrochloric acid and the precipitate formed was separated by filtration and crystallized from EtOH. Yield: 2.47 g (79.2%); mp 222 °C–224 °C (dec.). IR (KBr), ν (cm^{-1}): 3106 (CH aromatic), 3099 (OH), 2983 (CH aliphatic), 1710 (C=O), 1604 (C=N), 669 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.02$ (s, 2H, CH_2), 7.41–8.57 (m, 9H, arom-H), 12.68 (s, 1H, OH). Anal. calcd. for $C_{15}H_{12}N_4O_2S$ (312.3): C, 57.68; H, 3.87; N, 17.94; S, 10.26; Found C, 57.90; H, 3.88; N, 17.87; S, 10.21%.

4-Carboxymethyl-5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl) sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione (7). Compound **7** was obtained using the same method described for derivatives **4a–i**. A mixture of thiosemicarbazide **3k** (10 mmol) and 20 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 h. Subsequently, the solution was neutralized with diluted hydrochloric acid and the precipitate formed was separated by filtration and crystallized from EtOH. Yield: 1.18 g (27.8%); mp 222 °C–224 °C (dec.). IR (KBr), ν (cm^{-1}): 3311 (NH), 3067 (OH), 3041 (CH aromatic), 2946, 1461, (CH aliphatic), 1699 (C=O), 1600 (C=N), 1502 (C−N), 1345 (C=S), 699 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 4.51$ (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 7.39–8.63 (m, 9H, arom-H), 13.85 (s, 1H, OH), 14.30 (s, 1H, NH). Anal. calcd. for $C_{18}H_{15}N_7O_2S_2$ (425.5): C, 50.81; H, 3.55; N, 23.04; S, 15.07; Found C, 50.64; H, 3.54; N, 23.10; S, 15.12%.

5-{[(4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-2,5-dihydro-4*H*-1,2,4-triazole-3(2*H*)-thione (8). Compound **8** was obtained using the same method described for derivatives **4a–i**. A mixture of thiosemicarbazide **3l** (10 mmol) and 20 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 h. Subsequently, the solution was neutralized with diluted hydrochloric acid and the precipitate formed was separated by filtration and crystallized from EtOH. Yield: 2.39 g (65.0%); mp 152 °C–154 °C (dec.). IR (KBr), ν (cm^{-1}): 3187 (NH), 3034 (CH aromatic), 2984 (CH aliphatic), 1597 (C=N), 1505 (C−N), 1322 (C=S), 679 (C−S). ^{1}H NMR (DMSO-d_6): $\delta = 3.87$ (s, 2H, CH_2), 7.43–8.56 (m, 9H, arom-H), 13.67 (s, 1H, NH), 13.99 (s, 1H, NH). Anal. calcd.

for $C_{16}H_{13}N_7S_2$ (367.4): C, 52.30; H, 3.57; N, 26.68; S, 17.45; Found C, 52.42; H, 3.58; N, 26.61; S, 17.39%.

MICROBIOLOGY

Materials and Methods

A panel of 10 reference strains of aerobic bacteria from American Type Culture Collection, including Gram-positive *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240, and Gram-negative *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027, were used. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard – 150×10^6 CFU/mL (CFU – colony forming units). All stock solutions of the tested compounds were dissolved in dimethyl sulfoxide (DMSO). The medium with DMSO at the final concentration and without the tested compounds served as control – no microbial growth inhibition was observed.

Preliminary in vitro antimicrobial potency of the tested compounds was screened using the agar dilution method on the basis of the bacterial growth inhibition on the Mueller–Hinton agar to which the tested compounds at concentration 1000 μg/mL were added. The plates were poured on the day of testing. Approximately 10 μL of each bacterial suspension was put onto the prepared solid media. The plates were incubated at 37 °C for 18 h.

The *in vitro* antibacterial activity of the potentially active tested compounds was determined on the basis of MIC, usually defined as the lowest concentration of a compound at which there was no visible growth of tested microorganisms. Determination of the MIC value was achieved by broth microdilution method according to CLSI (Clinical and Laboratory Standards Institute) recommendation with some modifications.[21] Mueller–Hinton broth was used with a series of two-fold dilutions of the tested substances in the range of final concentrations from 3.91 to 1000 μg/mL. Cefuroxime (belonging to second generation of cephalosporins) and gentamicin were used as control antimicrobial agents at a final concentration from 0.063 to 500 μg/mL.

In broth microdilution method, 96-well microplates were used; 198 μL of Mueller–Hinton broth with or without 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 optical density (OD_{600}) of the bacterial cultures with or without the tested compounds were performed in order to determine MIC. The blank control wells with two-fold dilution of each of the tested compounds added to Mueller–Hinton broth (total volume – 200 μL) without bacteria were incubated under the same conditions.

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