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RESEARCH ARTICLE

Synthesis and *in vitro* antimicrobial activity of new 4-phenyl-5-methyl-4*H*-1,2,4-triazole-3-thione derivatives

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Abstract

This study presents the synthesis, spectral analysis and antimicrobial evaluation of a new series of substituted 1,2,4-triazole (**5a–i**) and 1,3,4-thiadiazole derivatives (**9a, c, g, h**). New compounds were obtained by cyclization reaction of acyl thiosemicarbazide derivatives in the presence of alkaline and acidic media. All synthesized compounds were screened for their *in vitro* antimicrobial activities. Nine of the compounds had potential activity against Gram-positive bacteria (MIC = 3.91–500 μg/mL). Some compounds showed good activity especially against: *Micrococcus luteus* ATCC 10240 (MIC = 3.91–31.25 μg/mL), *Bacillus subtilis* ATCC 6633 (MIC = 15.63– 62.5 μg/mL), and *Staphylococcus aureus* ATCC 25923 (MIC = 15.63–125 μg/mL).

Keywords: Antimicrobial activity, 1,2,4-triazole, 1,3,4-thiadiazole derivatives, MIC, MBC

Introduction

A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules. Among them the derivatives of 1,2,4-triazole and 1,3,4-thiadiazole have played an important role in the medicinal chemistry. These heterocycles have been found to possess a wide spectrum of biological activities such as analgesic[1], antifungal[2,3], antibacterial[4–8], antiviral[9,10], antiphlogistic[11–14] and antituberculous[15] action.

The treatment of infectious diseases still remains an important and challenging problem because of a combination factors including emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens witch particular relevance. In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotics resistant bacterial strains in the last decades constitutes a substantial need for the new class of antimicrobial agents.

Triazole and thiadiazole in particular substituted 1,2,4-trazoles and 1,3,4-thiadiazole are among the various heterocycles that have received the most attention during last decades as potential antimicrobial agents.

In the view of these facts and as a continuation of our research on the biological properties of triazole and thiadiazole we have designed and synthesized series of new potentially active 1,2,4-triazole and 1,3,4-thiadiazole and evaluation of their *in vitro* antibacterial activity.

Experimental

Chemistry

Melting points were determined in Fisher-Johns blocks (Pittsburgh, US) and presented without any corrections. The IR spectra (ν, cm^{-1}) were recorded in KBr tablets using a Specord IR-75 spectrophotometer (Germany). The ^1H 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. Chemical shifts are given in ppm (δ-scale). The MS spectra were recorded on a Thermo-Finnigan Trace DSQ GC MS apparatus (Waltham, Massachusetts, US). Chemicals were purchased from Merck Co. or Lancaster and used without further purification.

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The purity of obtained compounds was checked by TLC on aluminium oxide 60 F_{254} plates (Merck Co. Whitehouse Station, New Jersey US), in a $CHCl_3$/C_2H_5OH (10:1, v/v) solvent system with UV visualization (λ = 254 nm).

Elemental analysis of the obtained compounds was performed for C, H, N, S. The maximum percentage differences between calculated and with found values for each element were within the error and amounted to ±0.4%.

Ethyl [(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] acetate (2)

0.23 g (10 mmol) of sodium was added to 5 mL of anhydrous ethanol. The solution was placed in a three-necked flask equipped with reflux condenser and closed with a tube of $CaCl_2$ and mercury stirred. The content was mixed till sodium dissolved completely and then 1.91 g (10 mmol) of 4-phenyl-5-methyl-4*H*-1,2,4-triazole-3-thione (**1**) was added. Then 1.22 mL ethyl bromoacetate was added drop by drop. The content of the flask was mixed for 4 hrs and left at room temperature for 12 hrs. Then 10 mL of anhydrous ethanol was added and heated for 1 hr. The mixture was filtered off inorganic compounds. After cooling the precipitate was filtered off and crystallized from ethanol.

Yield: 55.6%; M. p. 72–74°C. IR (KBr), ν (cm^{-1}): 3109 (CH aromatic), 2935, 1444 (CH aliphatic), 1698 (C=O), 1597 (C=N), 682 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.16 (t, *J* = 7 Hz, 3H, CH_3), 2.19 (s, 3H, CH_3), 3.99 (s, 2H, CH_2), 4.03–4.12 (q, *J* = 7,5 Hz, 2H, CH_2,), 7.42–7.64 (m, 5H, 5CH aromatic). Analysis for $C_{13}H_{15}N_3O_2S$ (277.34); Calculated: C, 56.30; H, 5.45; N, 15.15; S, 11.56; Found: C, 56.11; H, 5.43; N, 15.21; S, 11.60.

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

0.5 mL of 100% hydrazine hydrate was added to 2.77g (10 mmol) compound (**2**) in 10 mL of anhydrous ethanol. The mixture was put to refrigerator for 24 hrs. After that, the precipitation of hydrazide (**3**) was filtered off, dried and crystallized form ethanol.

Yield: 72.8%; M. p. 86–88°C. 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, 5CH aromatic), 9.30 (s, 1H, NH). Analysis for $C_{11}H_{13}N_5OS$ (263.32); Calculated: C, 50.17; H, 4.98; N, 26.60; S, 12,18; Found: C, 50.04; H, 4.96; N, 26.51; S, 12,16.

Derivatives of 4-substituted-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide

Method A (for compounds 4a–4l)

A mixture of hydrazide (**3**) 2.63g (10 mmol) and 10 mmol appropriate isothiocyanate was heated in an oil bath at 45-95°C for 8-16 hrs. The product was washed with diethyl ether to remove unreacted isothiocyanate. Then it was filtered off, dried and crystallized from: ethanol (**4a, 4b, 4c, 4f, 4g, 4h, 4j, 4k, 4l**), butanol (**4i**) or methanol (**4d, 4e**).

Method B (for compounds 4a, 4f, 4h, 4j)

10 mmol of appropriate isothiocyanate was added to 2.63 g (10 mmol) of hydrazide (**3**) in 10 mL of anhydrous diethyl ether. The mixture placed in a conical bulb was mixed for 5 min and left in room temperature for 24 hrs. The precipitation of thiosemicarbazide (**4a, 4f, 4h, 4j**) was filtered off, dried and crystallized form ethanol. Obtained compounds had the same melting points as the compounds obtained by the Method A.

4-Phenyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetyl} thiosemicarbazide (4a)

IR (KBr), ν (cm^{-1}): 3195 (NH), 3098 (CH aromatic), 2985, 1428 (CH aliphatic), 1701 (C=O), 1608 (C=N), 1518 (C-N), 1338 (C=S), 675 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 3.86 (s, 2H, CH_2), 7.11-7.59 (m, 10H, 10CH aromatic), 9.77, 9.82, 10.35 (3s, 3H, 3NH). ^{13}C NMR δ (ppm): 10.97 (CH_3), 34.78 (-S-CH_2-), 125.04, 125.62, 127.15, 127.97, 128.99, 130.08 (10$CH_{aromatic}$), 133.15, 139.02 (2$C_{aromatic}$), 149.36 (C-S), 152.58 (C-3 triazole), 166.99 (C=O), 180.64 (C=S). MS m/z (%): 398 (M^+, 0.05), 383 (1), 307 (0.05), 263 (7), 232 (16), 190 (100), 158 (2), 149 (9), 135 (48), 118 (8), 93 (11), 91 (17), 77 (52). Analysis for $C_{18}H_{18}N_6OS_2$ (398.50); Calculated: C, 54.25; H, 4.55; N, 21.09; S, 16.09; Found: C, 54.30; H, 4.54; N, 21.03; S, 16.11.

4-(4-Bromophenyl)-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (4b)

IR (KBr), ν (cm^{-1}): 3243 (NH), 3098 (CH aromatic), 2952 (CH aliphatic),1710 (C=O), 1611 (C=N), 1351 (C=S), 687 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 7.21-7.65 (m, 9H, 9CH aromatic), 9.87, 9.96, 10.38 (3s, 3H, 3NH). Analysis for $C_{18}H_{17}BrN_6OS_2$ (477.40); Calculated: C, 45.28; H, 3.59; N, 17.60; S, 13.43; Found: C, 45.44; H, 3.60; N, 17.59; S, 13.40.

4-(4-Chlorophenyl)-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (4c)

IR (KBr), ν (cm^{-1}): 3211 (NH), 3113 (CH aromatic), 2973 (CH aliphatic), 1695 (C=O), 1603 (C=N), 1334 (C=S), 676 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 7.20-7.73 (m, 9H, 9CH aromatic), 9.86, 9.97, 10.39 (3s, 3H, 3 NH). Analysis for $C_{18}H_{17}ClN_6OS_2$ (432.95); Calculated: C, 49.93; H, 3.96; N, 19.41; S, 14.81; Found: C, 50.01; H, 3.95; N, 19.42; S, 14.85.

4-(4-Methoxyphenyl)-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (4d)

IR (KBr), ν (cm^{-1}): 3197 (NH), 3110 (CH aromatic), 2965, 1439 (CH aliphatic), 1705 (C=O), 1607 (C=N), 1512 (C-N), 1346 (C=S), 679 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.18 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 6.79-7.65

(m, 9H, 9CH aromatic), 9.61, 9.68, 10.32 (3s, 3H, 3NH). Analysis for $C_{19}H_{20}N_6O_2S_2$ (428.53); Calculated: C, 53.25; H, 4.70; N, 19.61; S, 14.96; Found: C, 53.08; H, 4.71; N, 19.55; S, 14.92.

4-Benzyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetyl} thiosemicarbazide (4e)

IR (KBr), ν (cm^{-1}): 3199 (NH), 3109 (CH aromatic), 2978, 1429 (CH aliphatic), 1708 (C=O), 1608 (C=N), 1517 (C-N), 1336 (C=S), 684 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.14 (s, 3H, CH_3), 3.91 (s, 2H, CH_2), 4.83 (s, 2H, CH_2), 7.22-7.69 (m, 10H, 10CH aromatic), 8.84, 9.55, 10.40 (3s, 3H, 3NH). Analysis for $C_{19}H_{20}N_6OS_2$ (412.53); Calculated: C, 55.32; H, 4.89; N, 20.37; S, 15.54; Found: C, 55.49; H, 4.90; N, 20.44; S, 15.59.

4-(4-Methoxybenzyl)-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (4f)

IR (KBr), ν (cm^{-1}): 3212 (NH), 3092 (CH aromatic), 2983, 1442, 752 (CH aliphatic), 1708 (C=O), 1598 (C=N), 1508 (C-N), 1346 (C=S), 683 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.18 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 4.75 (s, 2H, CH_2), 6.84–7.67 (m, 9H, 9CH aromatic), 8.78, 9.49, 10.37 (3s, 3H, 3NH). Analysis for $C_{20}H_{22}N_6O_2S_2$ (442.56); Calculated: C, 54.28; H, 5.01; N, 18.99; S, 14.49; Found: C, 54.07; H, 5.03; N, 19.01; S, 14.52.

4-Cyclohexyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetyl} thiosemicarbazide (4g)

IR (KBr), ν (cm^{-1}): 3198 (NH), 3079 (CH aromatic), 2977, 1444, 763 (CH aliphatic), 1701 (C=O), 1591 (C=N), 1520 (C-N), 1341 (C=S), 681 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15-1.71 (m, 10H, $5CH_2$ cyclohexyl), 2.18 (s, 3H, CH_3), 3.68 (s, 2H, CH_2), 4.14 (s, 1H, CH cyclohexyl), 7.45-7.75 (m, 5H, 5CH aromatic), 9.23, 9.30, 10.10 (3s, 3H, 3NH). Analysis for $C_{18}H_{24}N_6OS_2$ (404.55); Calculated: C, 53.44; H, 5.98; N, 20.77; S, 15.85; Found: C, 53.63; H, 5.96; N, 20.73; S, 15.81.

4-Ethyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] acetyl} thiosemicarbazide (4h)

IR (KBr), ν (cm^{-1}): 3211 (NH), 3099 (CH aromatic), 2984, 1445, 751 (CH aliphatic), 1695 (C=O), 1604 (C=N), 1513 (C-N), 1345 (C=S), 671 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (t, J = 7.5 Hz, 3H, CH_3), 2.23 (s, 3H, CH_3), 3.51-3.55 (q, J = 5 Hz, 2H, CH_2), 3.72 (s, 2H, CH_2), 7.45-7.62 (m, 5H, 5CH aromatic), 8.35, 9.30, 10.18 (3s, 3H, 3NH). Analysis for $C_{14}H_{18}N_6OS_2$ (350.46); Calculated: C, 47.98; H, 5.18; N, 23.98; S, 18.30; Found: C, 48.13; H, 5.16; N, 24.06; S, 18.35.

4-Allyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] acetyl} thiosemicarbazide (4i)

IR (KBr), ν (cm^{-1}): 3208 (NH), 3101 (CH aromatic), 2984, 1426, 759 (CH aliphatic), 1699 (C=O), 1600 (C=N), 1501 (C-N), 1341 (C=S), 679 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.26 (s, 3H, CH_3), 3.84 (s, 2H, CH_2), 4.16 (d, J = 5 Hz, 2H, CH_2), 5.02-5.13 (dd, 2H, = CH_2), 5.78-5.89 (m, 1H, CH), 7.43-7.62 (m, 5H, 5CH aromatic), 8.45, 9.40, 10.25 (3s, 3H, 3NH). Analysis for $C_{15}H_{18}N_6OS_2$ (362.47); Calculated: C, 49.70; H, 5.00; N, 23.18; S, 17.69; Found: C, 49.67; H, 4.98; N, 23.13; S, 17.73.

4-Ethoxycarbonyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (4j)

IR (KBr), ν (cm^{-1}): 3194 (NH), 3095 (CH aromatic), 2983, 1448, 762 (CH aliphatic), 1722 (C=O acidic), 1697 (C=O), 1603 (C=N), 1511 (C-N), 1338 (C=S), 691 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.21 (t, J = 7.5 Hz, 3H, CH_3), 2.18 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 4.11-4.20 (q, J = 7.5 Hz, 2H, CH_2), 7.32-7.71 (m, 5H, 5CH aromatic), 10.41, 10.88, 11.38 (3s, 3H, 3NH). Analysis for $C_{15}H_{18}N_6O_3S_2$ (394.47); Calculated: C, 45.67; H, 4.60; N, 21.30; S, 16.26; Found: C, 45.53; H, 4.61; N, 21.33; S, 16.31.

4-Ethoxycarbonylmethyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (4k)

IR (KBr), ν (cm^{-1}): 3191 (NH), 3089 (CH aromatic), 2965, 1455, 761 (CH aliphatic), 1740 (C=O acidic), 1708 (C=O), 1610 (C=N), 1499 (C-N), 1349 (C=S), 687 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.16 (t, J = 5 Hz, 3H, CH_3), 2.18 (s, 3H, CH_3), 3.71 (d, J = 5 Hz, 2H, CH_2), 3.87 (s, 2H, CH_2), 4.05-4.08 (q, J = 2,5 Hz, 2H, CH_2), 7.43-7.59 (m, 5H, 5CH aromatic), 8.82, 9.66, 10.45 (3s, 3H, 3NH). Analysis for $C_{16}H_{20}N_6O_3S_2$ (408.50); Calculated: C, 47.04; H, 4.93; N, 20.57; S, 15.70; Found: C, 47.09; H, 4.92; N, 20.59; S, 15.68.

4-Benzoyl-1-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetyl} thiosemicarbazide (4l)

IR (KBr), ν (cm^{-1}): 3155 (NH), 3098 (CH aromatic), 2980, 1451 (CH aliphatic), 1748 (C=O acidic), 1705 (C=O), 1611 (C=N), 1515 (C-N), 1324 (C=S), 671 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.20 (s, 3H, CH_3), 3.95 (s, 2H, CH_2), 7.38-7.99 (m, 10H, 10CH aromatic), 11.12, 11.31, 11.75 (3s, 3H, 3NH). Analysis for $C_{19}H_{18}N_6O_2S_2$ (426.51); Calculated: C, 53.50; H, 4.25; N, 19.70; S, 15.03; Found: C, 53.58; H, 4.26; N, 19.63; S, 15.08.

Derivatives of 4-substituted-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5a–5i)

General procedure

A mixture of thiosemicarbazide (**4a–4i**) (10 mmol) and 20–40 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2h. Then, the solution was neutralized with diluted hydrochloric acid and the formed precipitate was filtered off and crystallized from: ethanol (**5a, 5b, 5e, 5f, 5h**), butanol (**5c, 5i**) or methanol (**5d, 5g**).

4-Phenyl-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5a)

IR (KBr), ν (cm^{-1}): 3156 (NH), 3103 (CH aromatic), 2955, 1449 (CH aliphatic), 1599 (C=N), 1511 (C-N), 1335 (C=S), 679 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3),

4.09 (s, 2H, CH_2), 7.30-7.64 (m, 10H, 10CH aromatic), 13.90 (s, 1H, NH). ^{13}C NMR δ (ppm): 10.91 (-CH_3), 27.28 (-S-CH_2-), 128.21, 128.68, 128.95, 129.36, 129.61, 129.94 (10$CH_{aromatic}$), 132.94, 133.16 (2$C_{aromatic}$), 147.46 (C-3 triazole), 148.14 (C-3` triazole), 153.06 (C-S), 168.24 (C=S). MS m/z (%): 380 (M^+, 0.03), 348 (0.04), 326 (0.05), 313 (1), 268 (9), 204 (1), 190 (100), 158 (1.5), 149 (19), 135 (2), 118 (5), 91 (7), 77 (19). Analysis for $C_{18}H_{16}N_6S_2$ (380.49); Calculated: C, 56.82; H, 4.24; N, 22.09; S, 16.85; Found: C, 56.69; H, 4.23; N, 22.02; S, 16.91.

4-(4-Bromophenyl)-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5b)

IR (KBr), ν (cm^{-1}): 3174 (NH), 3111 (CH aromatic), 2979, 1449 (CH aliphatic), 1610 (C=N), 1500 (C-N), 1322 (C=S), 688 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 2.17 (s, 3H, CH_3), 4.06 (s, 2H, CH_2), 7.27-7.62 (m, 9H, 9CH aromatic), 13.87 (s, 1H, NH). Analysis for $C_{18}H_{15}BrN_6S_2$ (459.38); Calculated: C, 47.06; H, 3.29; N, 18.29; S, 13.96; Found: C, 46.88; H, 3.29; N, 18.34; S, 14.01.

4-(4-Chlorophenyl)-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5c)

IR (KBr), ν (cm^{-1}): 3165 (NH), 3100 (CH aromatic), 2956, 1443 (CH aliphatic), 1611 (C=N), 1504 (C-N), 1312 (C=S), 683 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 2.21 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 7.14-7.73 (m, 9H, 9CH aromatic), 13.90 (s, 1H, NH). Analysis for $C_{18}H_{15}ClN_6S_2$ (414.93); Calculated: C, 52.19; H, 3.64; N, 20.25; S, 15.45; Found: C, 52.29; H, 3.63; N, 20.28; S, 15.42.

4-(4-Methoxyphenyl)-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5d)

IR (KBr), ν (cm^{-1}): 3144 (NH), 3093 (CH aromatic), 2945, 1466 (CH aliphatic), 1602 (C=N), 1504 (C-N), 1328 (C=S), 683 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 2.16 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 6.82-7.59 (m, 9H, 9CH aromatic), 13.79 (s, 1H, NH). Analysis for $C_{19}H_{18}N_6S_2$ (410.51); Calculated: C, 55.59; H, 4.42 N, 20.47; S, 15.62; Found: C, 55.80; H, 4.43; N, 20.51; S, 15.68.

4-Benzyl-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5e)

IR (KBr), ν (cm^{-1}): 3150 (NH), 3097 (CH aromatic), 2981, 1439, 765 (CH aliphatic), 1602 (C=N), 1501 (C-N), 1333 (C=S), 679 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3), 4.26 (s, 2H, CH_2), 5.28 (s, 2H, CH_2), 7.18-7.66 (m, 10H, 10CH aromatic), 13.89 (s, 1H, NH). Analysis for $C_{19}H_{18}N_6S_2$ (394.52); Calculated: C, 57.84; H, 4.60; N, 21.30; S, 16.25; Found: C, 57.75; H, 4.61; N, 21.24; S, 16.21.

4-(4-Methoxybenzyl)-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5f)

IR (KBr), ν (cm^{-1}): 3179 (NH), 3079 (CH aromatic), 2974, 1446, 759 (CH aliphatic), 1614 (C=N), 1512 (C-N), 1329 (C=S), 669 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 6.80-7.68 (m, 9H, 9CH aromatic), 13.93 (s, 1H, NH). Analysis for $C_{20}H_{20}N_6OS_2$ (424.54); Calculated: C, 56.58; H, 4.75; N, 19.79; S, 15.10; Found: C, 56.55; H, 4.73; N, 19.82; S, 15.03.

4-Cyclohexyl-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5g)

IR (KBr), ν (cm^{-1}): 3164 (NH), 3068 (CH aromatic), 2989, 1455, 764 (CH aliphatic), 1610 (C=N), 1509 (C-N), 1335 (C=S), 670 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 1.04-1.34 (m, 10H, 5CH_2 cyclohexyl), 2.16 (s, 3H, CH_3), 4.35 (s, 2H, CH_2), 4.80 (s, 1H, CH cyclohexyl), 7.31-7.68 (m, 5H, 5CH aromatic), 13.63 (s, 1H, NH). Analysis for $C_{18}H_{22}N_6S_2$ (386.54); Calculated: C, 55.93; H, 5.74; N, 21.74; S, 16.59; Found: C, 55.71; H, 5.72; N, 21.78; S, 16.55.

4-Ethyl-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5h)

IR (KBr), ν (cm^{-1}): 3155 (NH), 3064 (CH aromatic), 2966, 1449, 762 (CH aliphatic), 1608 (C=N), 1505 (C-N), 1346 (C=S), 682 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 1.12 (t, J = 7.5 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.39-3.48 (q, J = 7.5 Hz, 2H, CH_2), 4.38 (s, 2H, CH_2), 7.27-7.66 (m, 5H, 5CH aromatic), 13.66 (s, 1H, NH). Analysis for $C_{14}H_{16}N_6S_2$ (332.45); Calculated: C, 50.58; H, 4.85; N, 25.28; S, 19.29; Found: C, 50.62; H, 4.83; N, 25.33; S, 19.33.

4-Allyl-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (5i)

IR (KBr), ν (cm^{-1}): 3181 (NH), 3099 (CH aromatic), 2984, 1456, 753 (CH aliphatic), 1603 (C=N), 1501 (C-N), 1325 C=S, 679 C-S. ^1H NMR (DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 4.26 (s, 2H, CH_2), 4.58-4.60 (d, J = 5 Hz, 2H, CH_2), 4.95-5.14 (dd, 2H, =CH_2), 5.66-5.83 (m, 1H, CH), 7.32-7.63 (m, 5H, 5CH aromatic), 13.71 (s, 1H, NH). Analysis for $C_{15}H_{16}N_6S_2$ (344.46); Calculated: C, 52.30; H, 4.68; N, 24.40; S, 18.62; Found: C, 52.44; H, 4.69; N, 24.49; S, 18.66.

[(4-Phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] acetic acid (6)

Compound (**6**) was obtained using the same method described earlier for derivatives (**5a–5i**). That is, a mixture of thiosemicarbazide (**4j**) (10 mmol) and 20 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 hrs. Then, the solution was neutralized with diluted hydrochloric acid and the formed precipitate was filtered off and crystallized from ethanol.

Yield: 72.8%; M. p. 232-234°C. IR (KBr), ν (cm^{-1}): 3111 (CH aromatic), 3023 (OH), 2967, 1423 (CH aliphatic), 1720 (C=O), 1597 (C=N), 698 (C-S). ^1H NMR (DMSO-d_6) δ (ppm): 2.56 (s, 3H, CH_3), 4.21 (s, 2H, CH_2), 7.34-7.74 (m, 5H, 5CH aromatic), 12.15 (s, 1H, OH). Analysis for $C_{11}H_{11}N_3O_2S$ (249.29); Calculated: C, 53.00; H, 4.45; N, 16.86; S, 12.86; Found: C, 53.13; H, 4.46; N, 16.89; S, 12.82.

4-Carboxymethyl-5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (7)

Compound (**7**) was obtained using the same method described earlier for derivatives (**5a-5i**). That is, a mixture

of thiosemicarbazide (**4k**) (10 mmol) and 20 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 hrs. Then, the solution was neutralized with diluted hydrochloric acid and the formed precipitate was filtered off and crystallized from ethanol.

IR (KBr), ν (cm^{-1}): 3280 (NH), 3066 (OH), 3044 (CH aromatic), 2980, 1444 (CH aliphatic), 1711 (C=O), 1602 (C=N), 1501 (C-N), 1355 (C=S), 677 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 7.44-7.78 (m, 5H, 5CH aromatic), 9.33 (s, 1H, OH), 13.93 (s, 1H, NH). Analysis for $C_{14}H_{14}N_6O_2S_2$ (362.43); Calculated: C, 46.39; H, 3.89; N, 23.19; S, 17.69; Found: C, 46.44; H, 3.90; N, 23.28; S, 17.72.

5-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-2,5-dihydro-4H-1,2,4-triazole-3(2H)-thione (8)

Compound (**8**) was obtained using the same method described earlier for derivatives (**5a–5i**). That is, a mixture of thiosemicarbazide (**4l**) (10 mmol) and 20 mL of 2% aqueous solution of sodium hydroxide was refluxed for 2 hrs. Then, the solution was neutralized with diluted hydrochloric acid and the formed precipitate was filtered off and crystallized from ethanol.

IR (KBr), ν (cm^{-1}): 3198 (NH), 3082 (CH aromatic), 2989, 1432 (CH aliphatic), 1612 (C=N), 1505 (C-N), 1343 (C=S), 688 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.15 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 7.16-7.57 (m, 5H, 5CH aromatic), 13.65 (s, 1H, NH), 13.84 (s, 1H, NH). Analysis for $C_{12}H_{12}N_6S_2$ (304.39); Calculated: C, 47.35; H, 3.97; N, 27.61; S, 21.07; Found: C, 47.51; H, 3.98; N, 27.72; S, 21.02.

Derivatives of (5-aminosubstituted)-2-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (9a, 9c, 9g, 9h)

General method (for compounds 9a, 9c, 9g, 9h)

10 mmol of 4-substituted-1-{[(4-phenyl-5-methyl-4*H*-1-,2,4-triazol-3-yl)sulfanyl]acetyl} thiosemicarbazide (**4a**, **4c**, **4g**, **4h**) was dissolved in 10–20 mL diluted sulphuric acid and stirred in a closed bulb for 1 hr. After that the solution was poured out on crushed ice (50 g) and stirred until the ice was completely dissolved. Later the solution was neutralized with ammonium hydroxide. Formed precipitate was filtered off, dried, and crystallized from: ethanol (**9a**, **9h**), butanol (**9c**) or methanol (**9g**).

(5-Aminophenyl)-2-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (9a)

IR (KBr), ν (cm^{-1}): 3237 (NH), 2999 (CH aromatic), 2980, 1448 (CH aliphatic), 1620 (C=N), 1499 (C-N), 695 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.20 (s, 3H, CH_3), 4.56 (s, 2H, CH_2), 6.89–7.59 (m, 10H, 10CH aromatic), 10.31 (s, 1H, NH). Analysis for $C_{18}H_{16}N_6S_2$ (380.49); Calculated: C, 56.82; H, 4.24; N, 22.09; S, 16.85; Found: C, 56.76; H, 4.23; N, 22.16; S, 16.89.

[5-Amino-(4-chlorophenyl)]-2-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (9c)

IR (KBr), ν (cm^{-1}): 3198 (NH), 3076 (CH aromatic), 2934, 1455 (CH aliphatic), 1598 (C=N), 1503 (C-N), 687 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.27 (s, 3H, CH_3), 4.63 (s, 2H, CH_2), 7.41-7.67 (m, 9H, 9CH aromatic), 10.13 (s, 1H, NH). Analysis for $C_{18}H_{15}ClN_6S_2$ (414.93); Calculated: C, 52.10; H, 3.64; N, 20.25; S, 15.45; Found: C, 52.08; H, 3.65; N, 20.20; S, 15.49.

5-Aminocyclohexyl-2-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (9g)

IR (KBr), ν (cm^{-1}): 3276 (NH), 3099 (CH aromatic), 2975, 1460, 766 (CH aliphatic), 1608 (C=N), 1509 (C-N), 677 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.12-1.85 (m, 10H, $5CH_2$ cyclohexyl), 2.22 (s, 3H, CH_3), 3.74 (s, 2H, CH_2), 4.17 (s, 1H, CH cyclohexyl), 7.35-7.69 (m, 5H, 5CH aromatic), 10.10 (s, 1H, NH). Analysis for $C_{18}H_{22}N_6S_2$ (386.54); Calculated: C, 55.93; H, 5.74; N, 21.74; S, 16.59; Found: C, 56.06; H, 5.76; N, 21.66; S, 16.63.

5-Aminoethyl-2-{[(4-phenyl-5-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (9h)

IR (KBr), ν (cm^{-1}): 3289 (NH), 3077 (CH aromatic), 2944, 1433, 779 (CH aliphatic), 1605 (C=N), 1502 (C-N), 671 (C-S). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.20 (t, J = 7.5 Hz, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.24-3.30 (q, J = 5 Hz, 2H, CH_2), 3.86 (s, 2H, CH_2), 7.35–7.75 (m, 5H, 5CH aromatic), 10.17 (s, 1H, NH). Analysis for $C_{14}H_{16}N_6S_2$ (332.45); Calculated: C, 50.57; H, 4.85; N, 25.28; S, 19.29; Found: C, 50.38; H, 4.84; N, 25.25; S, 19.33.

Microbiology

Materials and methods

A panel reference strains of aerobic bacteria from American Type Culture Collection, including 4 Gram-positive bacteria - *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240 and 4 Gram-negative bacteria – *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027, were used. Microbial suspensions with an optical density of 0.5 McFarland standard – 150×10^6 CFU/mL (CFU – colony forming units) were prepared in sterile 0.85% NaCl. All stock solutions of the tested compounds were prepared in DMSO (dimethyl sulfoxide). The medium with DMSO at the final concentration and without the tested compounds served as control - no microbial growth inhibition was observed.

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

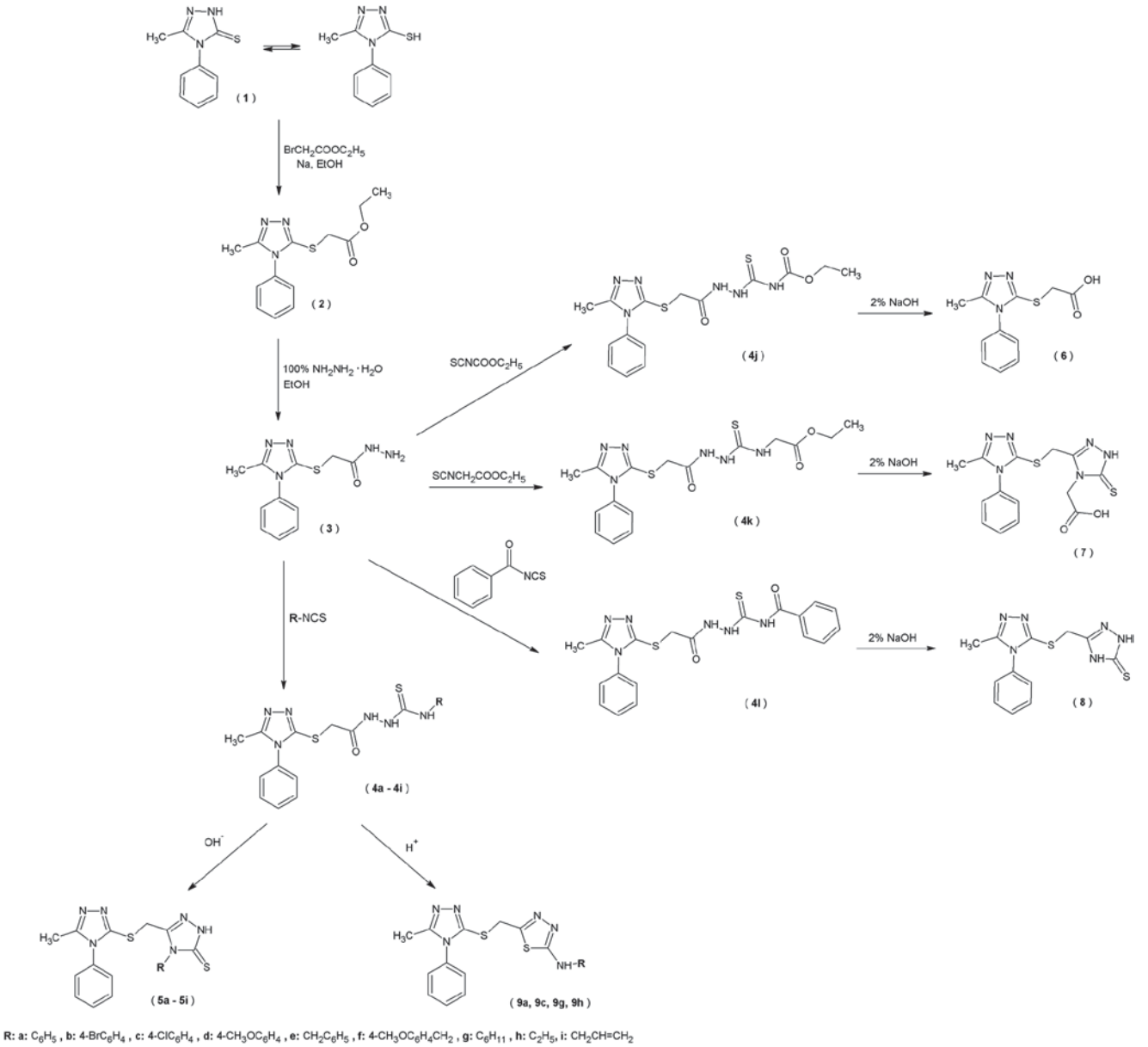
The antibacterial activity *in vitro* of the potentially active compounds were determined by broth microdilution method on the basis of minimal inhibitory concentration (MIC), usually defined as the lowest concentration of the compound at which there was no visible growth of

microorganisms. The 96-well microplates were used; 198 μL of Mueller-Hinton broth without or with a series of two-fold dilution of the tested compound in the range of final concentrations from 3.91 to 1000 μg/mL (0.24 to 1000 μg/mL for **9a, c, g, h**) 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 without bacterial suspension were incubated under the same conditions. For some substances with promising antibacterial activity the values of minimal bactericidal concentration (MBC), defined as the lowest concentration of each compound that resulted in >99.9% reduction in CFU (colony forming units) of the initial inoculum, was also determined. The MBC was calculated using broth microdilution technique by plating out onto Mueller Hinton agar plates the contents of wells (20 μL) that showed no visible bacterial growth after previous incubation under the conditions mentioned above; incubation of plates was conducted at 35°C for 18 h. Both MIC and MBC values were given in μg/mL. On the basis of MBC/MIC ratio, bactericidal (MBC/MIC ≤ 4) or bacteriostatic (MBC/MIC > 4) effect was assessed[16,17]. Cefuroxime (belonging to 2nd generation of cephalosporins) and gentamicin was used as control antimicrobial agent at final concentration from 0.063 to 500 μg/mL.

Results and discussion

Chemistry

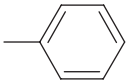
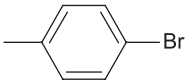
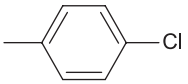
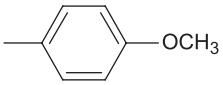
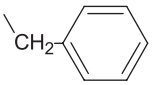
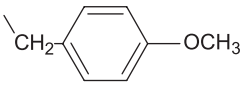
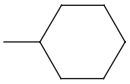
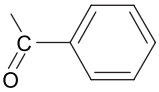
The common method for the preparation of 1,2,4-triazole and 1,3,4-thiadiazole is the cyclization reaction of thiosemicarbazide derivatives. In earlier papers[18,19] it was



R: a: C_6H_5, b: 4-BrC_6H_4, c: 4-ClC_6H_4, d: 4-$CH_3OC_6H_4$, e: $CH_2C_6H_5$, f: 4-$CH_3OC_6H_4CH_2$, g: C_6H_{11}, h: C_2H_5, i: $CH_2CH{=}CH_2$

Scheme 1. Synthesis of new thiosemicarbazide, 1,2,4-triazole and 1,3,4-thiadiazole derivatives.

Table 1. Physicochemical data and synthesis parameters for thiosemicarbazide derivatives (**4a–4l**).

Compound	R	Yield (%)	M. p. (°C)	Temp. of reaction (°C)	Time of reaction (hrs)	Molecular weight	Molecular formula
4a		92.7	176–178	50	10	398.50	$C_{18}H_{18}N_6OS_2$
4b		89.6	116–118	95	14	477.40	$C_{18}H_{17}BrN_6OS_2$
4c		86.8	128–130	80	14	432.95	$C_{18}H_{17}ClN_6OS_2$
4d		98.3	130–132	50	12	428.53	$C_{19}H_{20}N_6O_2S_2$
4e		97.8	168–170	45	12	412.53	$C_{19}H_{20}N_6OS_2$
4f		77.8	152–154	50	12	442.56	$C_{20}H_{22}N_6O_2S_2$
4g		83.9	151–153	50	10	404.55	$C_{18}H_{24}N_6OS_2$
4h	$-C_2H_5$	82.5	162–164	60	8	350.46	$C_{14}H_{18}N_6OS_2$
4i	$-CH_2-CH=CH_2$	83.4	163–165	50	12	362.47	$C_{15}H_{18}N_6OS_2$
4j	$-COOC_2H_5$	75.5	75–77	50	14	394.47	$C_{15}H_{18}N_6O_3S_2$
4k	$-CH_2COOC_2H_5$	75.1	88–90	50	16	408.50	$C_{16}H_{20}N_6O_3S_2$
4l		98.1	68–70	50	16	426.51	$C_{19}H_{18}N_6O_2S_2$

stated that the reaction of cyclization was affected not only by pH of the medium but also by the nature of substituents in thiosemicarbazide derivatives.

Cyclization of thiosemicarbazide in the presence of alkaline media usually promotes reaction to obtain 1,2,4-triazole system, while in acidic media 1,3,4-thiadiazole derivatives were obtained[20].

By the cyclization in alkaline media of 1-methyl-4-phenyl thiosemicarbazide, 4-phenyl-5-methyl-4*H*-1-,2,4-triazole-3-thione (**1**) was obtained. This compound, can exist in two tautomeric forms, was a starting material for synthesis of new derivatives, which consist of two 1,2,4-triazole systems or 1,2,4-triazole and 1,3,4-thiadiazole systems connected with S-methylene bridge. Depending on the conditions used, 4-phenyl-5-methyl-4*H*-1,2,4-triazole-3-thione (**1**) can lead either to the S- or N-derivatives. In this paper the reaction of this compound with ethyl bromoacetate was investigated (**2**). Basing on the results of the previous papers, the elemental and spectral analysis as well as on the X-ray crystallography of similar compound, it was revealed and confirmed that the reaction leads to the formation of S-derivative[21].

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

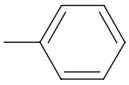
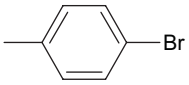
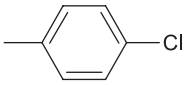
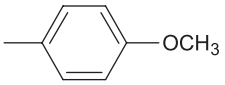
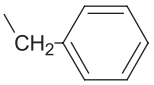
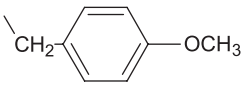
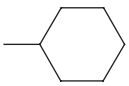
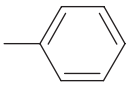
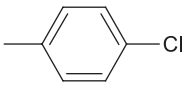
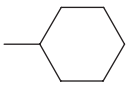
Reactions of hydrazide (**3**) with various isothiocyanates were carried out in two ways. All new thiosemicarbazide derivatives (**4a–4l**) were obtained by heating substracts in an oil bath, temperatures were selected experimentally (t = 45–95°C). Thiosemicarbazide derivatives (**4a, 4f**, **4h**, **4j**) were products of the reaction hydrazide (**3**) with appropriate isothiocyanates in the presence of diethyl ether.

New thiosemicarbazide derivatives (**4a–4i**) in cyclization reaction with 2% aqueous solution of sodium hydroxide lead to a new group of 1,2,4-triazole derivatives (**5a–5i**).

The hydrolysis was observed in the case of cyclization of 4-ethoxycarbonyl-1-substituted thiosemicarbazide (**4j**) in alkaline media, which led to obtain [(4-phenyl-5-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetic acid (**6**). This compound was described earlier[22], but it was obtained in a different way. The cyclization in alkaline media of thiosemicarbazide which contains ethoxycarbonylmethyl group (**4k**) and benzoyl (**4l**) in the 4 position led to obtain 1,2,4-triazole derivatives (**7**,**8**).

The cyclization reaction in acidic media of new derivatives of thiosemicarbazide enabled us to

Table 2. Physicochemical data of 1,2,4-triazole and 1,3,4-thiadiazole derivatives (**5a–5i, 7, 8, 9a, c, g, h**).

Compound	R	Yield (%)	M. p. (°C)	Molecular weight	Molecular formula
5a		81.2	122–124	380.49	$C_{18}H_{16}N_6S_2$
5b		81.6	106–108	459.38	$C_{18}H_{15}BrN_6S_2$
5c		65.2	124–126	414.93	$C_{18}H_{15}ClN_6S_2$
5d		62.6	162–164	410.51	$C_{19}H_{18}N_6OS_2$
5e		83.6	122–124	394.52	$C_{19}H_{18}N_6S_2$
5f		83.4	79–81	424.54	$C_{20}H_{20}N_6OS_2$
5g		80.2	114–116	386.54	$C_{18}H_{22}N_6S_2$
5h	$-C_2H_5$	85.9	145–147	332.45	$C_{14}H_{16}N_6S_2$
5i	$-CH_2-CH=CH_2$	39.5	158–160	344.46	$C_{15}H_{16}N_6S_2$
7	$-CH_2COOH$	87.8	226–228	362.43	$C_{14}H_{14}N_6O_2S_2$
8	–H	28.1	105–107	304.39	$C_{12}H_{12}N_6S_2$
9a		78.9	112–114	380.49	$C_{18}H_{16}N_6S_2$
9c		52.6	106–108	414.93	$C_{18}H_{15}ClN_6S_2$
9g		62.2	122–124	386.54	$C_{18}H_{22}N_6S_2$
9h	$-C_2H_5$	67.8	138–140	332.45	$C_{14}H_{16}N_6S_2$

obtain new derivatives of 1,3,4-thiadiazole, that is: (5-aminosubstituted)-2-{[(4-phenyl-5-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**9a**, **9c**, **9g**, **9h**).

The structure of the obtained compounds was confirmed by elementary analysis, IR and ^{1}H NMR spectra. Some of the compounds were also submitted to ^{13}C NMR and MS spectra analysis.

In the IR spectra of the compounds which consist of two 1,2,4-triazole system (**5a–5i**, **7**, **8**), the following characteristic absorption bands were observed: about 1500 cm^{-1} corresponding to C-N group and in the range of 1600 cm^{-1} – corresponding to C=N group. Then in the IR spectra of the new derivatives of 1,3,4-thiadiazole (**9a**, **9c**, **9g**, **9h**) the following characteristic absorption bands were observed: in the range of 1500 cm^{-1} - corresponding to C-N group and in the range of 1600 cm^{-1} – corresponding to C=N group.

^{1}H NMR spectra of the thiosemicarbazide derivatives (**4a–4l**) show three proton signals typical for the NH group in the δ 8.35-11.75 ppm range. Whereas for the new compounds consisting of two 1,2,4-triazole system (**5a–5i**, **7**, **8**) one proton signal of the NH group was observed in the δ 13.63-13.93 ppm range. The 1,3,4-thiadiazole derivatives (**9a**, **9c**, **9g**, **9h**) had one typical proton signal of NH group in the δ 10.10–10.31 ppm range.

The reactions were performed according to Scheme 1. Physicochemical data and synthesis parameters for thiosemicarbazide derivatives (**4a–4l**) are presented in the Table 1. The Table 2 contains physicochemical data for 1,2,4-triazole and 1,3,4-thiadiazole derivatives (**5a–5i, 7, 8, 9a, 9c, 9g, 9h**).

Microbiology

According to the preliminary results obtained by agar dilution method, nine of newly synthesized compounds possessed potential activity against Gram-positive bacteria. None of the compounds had inhibitory effect on the Gram-negative bacteria growth.

Table 3. The influence of newly synthesized compounds on the growth of Gram-positive bacteria of on the basis of MIC (μg/mL) or MBC (μg/mL) values obtained by the broth microdilution method.

Species	Sa25923		Se12228		Bs6633		Ml10240	
Compound	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4a	1000	nd	1000	nd	1000	nd	**250**	nd
4b	**31.25**	1000	**31.25**	>1000	**31.25**	62.5	**7.8**	250
4c	**31.25**	1000	**62.5**	>1000	**31.25**	500	**15.63**	125
4e	1000	nd	1000	nd	1000	nd	1000	nd
4f	1000	nd	>1000	nd	1000	nd	1000	nd
4g	**500**	nd	1000	nd	1000	nd	**125**	nd
4h	1000	nd	1000	nd	1000	nd	1000	nd
4i	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
4l	**125**	>1000	**62.5**	>1000	**15.63**	250	**31.25**	>1000
5a	1000	nd	1000	nd	1000	nd	1000	nd
5b	**15.63**	>1000	**31.25**	>1000	**15.63**	31.25	**15.63**	62.5
5c	1000	>1000	>1000	>1000	**500**	>1000	**7.8**	62.5
5e	1000	nd	1000	nd	1000	nd	1000	nd
5f	>1000	nd	>1000	nd	500	nd	>1000	nd
5g	1000	nd	1000	nd	1000	nd	1000	nd
9a	**125**	>1000	>1000	>1000	**62.5**	>1000	**125**	>1000
9c	**15.63**	1000	**31.25**	1000	**31.25**	31.25	**3.91**	250
Cefuroxime	0.49	nd	0.49	nd	0.49	nd	0.49	nd
Gentamicin	0.015	nd	0.06	nd	0.015	nd	0.12	nd

Abbreviations: nd - not determined, Sa25923 - *Staphylococcus aureus* ATCC 25923, Se12228 - *Staphylococcus epidermidis* ATCC 12228, Bs6633 - *Bacillus subtilis* ATCC 6633, Ml10240 - *Micrococcus luteus* ATCC 10240.

On the basis of MIC and MBC values obtained by broth microdilution method it was shown that antibacterial activity was species-dependent (Table 3). The most sensitive bacteria to newly synthesized derivatives were *Bacillus subtilis* ATCC 6633 (MIC = 15.63–62.5 μg/mL), *Micrococcus luteus* ATCC 10240 (MIC = 3.91–125 μg/mL) and *Staphylococcus aureus* ATCC 25923 (MIC = 15.63–125 μg/mL).

The compounds **4b, 4c, 4l, 5b, 9c** had good activity against nonpathogenic *Bacillus subtilis* ATCC 6633 (MIC = 15.63–31.25 μg/mL, MBC = 31.25–500 μg/mL), while compounds **4b, 4c, 4l, 5b, 5c, 9c**– against opportunistic *Micrococcus luteus* ATCC 10240 (MIC = 7.8–31.25 μg/mL, MBC = 31.25– >1000 μg/mL).

The highest activity against pathogenic *Staphylococcus aureus* ATCC 25923 and opportunistic *Staphylococcus epidermidis* ATCC 12228 had **4b, 4c, 4l, 5b, 9a, 9c** compounds (MIC = 15.63–62.5 μg/mL, MBC ≥ 1000 μg/mL).

According to our results, the potentially active compounds had mainly bacteriostatic effect (MBC/MIC ratio from 8 to ≥32); bactericidal were only compounds **5b** against *B. subtilis* ATCC 6633 or *M. luteus* ATCC 10240 (MBC/MIC = 2) and **4b** against *B. subtilis* ATCC 6633 (MBC/MIC = 2).

Among all compounds the highest activity had **9c** with bacteriostatic effect against *Micrococcus luteus* ATCC 10240 (MIC = 3.91 μg/mL, MBC = 250 μg/mL, MBC/MIC = 64), *Staphylococcus aureus* ATCC 25923 (MIC = 15.63 μg/mL, MBC = 1000 μg/mL, MBC/MIC = 64), *Staphylococcus epidermidis* ATCC 12228 (MIC = 31.25 μg/mL, MBC = 1000 μg/mL, MBC/MIC = 32) and with bactericidal effect against *Bacillus subtilis* ATCC 6633 (MIC = MBC = 31.25 μg/mL, MBC/MIC = 1).

In our experiments MICs of available antibiotics such as cefuroxime and gentamicin, that had been extensively used to treat of bacterial infections, were also estimated. MIC values for reference strains of the tested bacteria were 0.015 to 0.12 μg/mL for gentamicin and 0.49 μg/mL for cefuroxime.

The compounds **4b, 4c, 4l, 5b, 5c, 9a, 9c** with good activity against the reference Gram-positive bacteria may be regarded as precursor compounds for searching new derivatives showing better antimicrobial activity against pathogenic (e.g. *Staphylococcus aureus*) or opportunistic (e.g. *Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis*) bacteria. Today due to several serious problems such as growing drug resistance of bacteria or undesirable side effects of drugs it is very important to find new substances with antimicrobial activity.

Declaration of interest

The authors declare no conflict of interest.

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