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Synthesis and biological activity of 1,3,4-oxa(thia)diazole, 1,2,4-triazole-5-(thio)one and *S*-substituted derivatives of 3-((2-carboxyethyl)phenylamino)propanoic acid

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Abstract A series of novel derivatives of 3-((2-carboxyethyl)phenylamino)propanoic acid bearing two identical substituted 1,3,4-oxadiazole, 1,3,4-thiadiazole, or 1,2,4-triazole moieties were synthesized and their molecular structures were confirmed by IR, ^{1}H, ^{13}C NMR spectroscopy, and elemental analysis data. The synthesized compounds were screened for their reducing power, free radical scavenging, plant growth regulating, and antimicrobial properties. 5,5′-((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione) showed excellent antioxidant activity, three times higher than that of the antibiotic control (cefazolin). 2,2′-((((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))diacetate exhibited notable bactericidal activity against *Pseudomonas aeruginosa* with MBC and MIC values 7.3 μg/ml, whereas 12 compounds displayed significant fungicidal activity against *Candida albicans* with MIC value 3.9 μg/ml.

Graphical Abstract Synthesis of compounds **2**–**27**. R: **2**, **5**, **7, 10, 13, 15-27** C_6H_5–; **3**, **8**, **11** 4-Cl-C_6H_4–; **4**, **9**, **12** CH_3–; **6**, **14** 2.6-$(CH_3)_2$-C_6H_3–; R′: **15** CH_3CH_2–; **16** $(CH_3)_2CH$–; **17** $(CH_3)_2CHCH_2$–; **18** $CH_3CH_2CH_2CH_2$–; **19** $(CH_3)_2CHCH_2CH_2$–; **20** $CH_3CH_2CH_2CH_2CH_2$–; **21** NH_2COCH_2–; **22** $C_6H_5CH_2$–; **23** $C_6H_5COCH_2$–; **24** 4-$ClC_6H_4COCH_2$–; **25** 4-$NO_2C_6H_4COCH_2$–; **26** $C_2H_5OCOCH_2$–.

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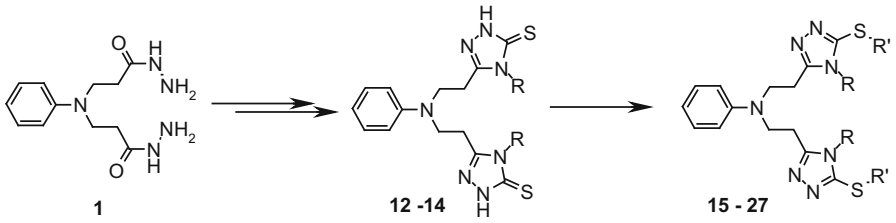


Keywords Propanehydrazide · 1,3,4-Oxadiazole · 1,3,4-Thiadiazole · 1,2,4-Triazole · Antioxidative activity · Antibacterial activity

Introduction

The worsening ecological situation in the world, changing habits of people, and increasing number of population in large cities influence the weakening human immunity, emergence of new diseases, spread of new bacteria strains, and mutation of viruses, etc. Activity of currently used drug preparations decreases or they become completely inactive. Therefore, search for new biologically active substances and development of novel pharmaceuticals is an important ongoing process. Among different five-membered heterocyclic systems, sulfur and nitrogen-containing ones have gained importance as they constitute the structural features of many bioactive naturally occurring or synthetic compounds.

Oxadiazoles and thiadiazoles are widely used in medicinal and agricultural chemistry [1–3]. Substituted 1,3,4-oxadiazole derivatives possess a wide array of biological activities such as antitubercular [4], antitumor [5], antibacterial and antifungal [4, 6, 7], anti-convulsant [8], anti-inflammatory [9], and antioxidative [10].

1,3,4-Thiadiazoles are another important class of heterocyclic compounds due to their numerous biological activities [3] including anticancer [11–13], antimicrobial and antifungal [14, 15], and antioxidative [16] ones.

1,2,4-Triazole scaffold is yet another important pharmacophore since compounds bearing this moiety have been shown to display anti-inflammatory and analgesic [17], antitumor [18], antioxidative [19], antibacterial [13, 14, 20], and antifungal [21] properties. 1,2,4-Triazole derivatives, e.g., flucarbazone and amicarbazone, are used as herbicides [22].

Cyclization of hydrazine derivatives is a well-known method for the synthesis of polyfunctionalized derivatives of 1,3,4-thiadiazole, 1,3,4-oxadiazole, and 1,2,4-triazole [3, 23, 24].

As a continuation of ongoing efforts on the synthesis of azole derivatives with potential biological activities and encouraged by the already published data on the biological activity of 3-arylaminopropanehydrazides and their derivatives [6, 19, 25–27], we report the synthesis of novel derivatives of 3,3′-(phenylimino)bis-propanehydrazide containing two identical heterocyclic moieties and evaluation of their antioxidative, growth regulating and antimicrobial properties.

Experimental

Materials and methods

Chemistry

All the reagents and chemicals were obtained from commercial sources and used without further purification. Melting points were measured on a B-540 Melting Point Analyzer and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Varian Unity Inova (300 MHz) and Bruker Avance III/400 (400 MHz) spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) calibrated from TMS (0 ppm) as an internal standard for ^{1}H NMR, and DMSO-d_6 (39.5 ppm) for ^{13}C NMR. The *J* constants are given in Hz. IR spectra (ν, cm^{-1}) were recorded on a Perkin–Elmer BX FT–IR spectrometer using KBr pellets. Elemental analyses were performed on a CE-440 elemental analyzer. TLC was performed using Silica gel 60 F254 (Kieselgel 60 F254) (Merck, Darmstadt, Germany) plates.

*2,2′-(3,3′-(Phenylazanediyl)bis(propanoyl))bis(N-phenylhydrazine-1-carboxamide) (**2**)* Synthesis and characteristics have been described earlier [27].

*2,2′-(3,3′-(Phenylazanediyl)bis(propanoyl))bis(N-(4-chlorophenyl)hydrazine-1-carboxamide) (**3**)* To a solution of bispropanehydrazide **1** (1.33 g, 5 mmol) in methanol (30 ml), 4-chlorophenyl isocyanate (1.54 ml, 1.84 g, 12 mmol) was added drop-wise and the mixture was heated under reflux for 10 min. The crystalline precipitate was isolated by filtration and recrystallized from DMF–H_2O mixture to afford **3** as white solid (2.73 g, 95 %), m.p. 218–219 °C; IR (KBr) ν/cm^{-1}: 1660 (C=O), 3062, 3287, 3308 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.44 (t, 4H, J = 7.2 Hz, CH_2CO), 3.58 (t, 4H, J = 7.2 Hz, CH_2N), 6.63 (t, 1H, J = 7.2 Hz, $H_{(4)}Ar$), 6.73 (d, 2H, J = 7.2 Hz, $H_{(2,6)}Ar$), 7.19 (t, 2H, J = 7.2 Hz, $H_{(3,5)}Ar$), 7.29 (d, 4H, J = 8.85 Hz, $H_{(2,6)}Ar'$), 7.48 (d, 4H, J = 8.85 Hz, $H_{(3,5)}Ar'$), 8.14 (s, 2H, NH), 8.89 (s, 2H, NH), 9.77 (s, 2H, NHAr′). MS (ESI, 20 V) *m/z* (%): 572 ($[M+H]^+$, 30). Anal. Calcd. For $C_{26}H_{27}C_{l2}N_7O_4$ (572.443): C, 54.55; H, 4.75; N, 17.13 %. Found: C, 54.51; H, 4.68; N, 17.09 %.

*2,2′-(3,3′-(Phenylazanediyl)bis(propanoyl))bis(N-methylhydrazine-1-carbothioamide) (**4**)* To a solution of bispropanehydrazide **1** (1.33 g, 5 mmol) in methanol (30 ml), methyl isothiocyanate (0.82 ml, 0.88 g, 12 mmol) was added drop-wise and the reaction mixture was heated under reflux for 20 min. The crystalline precipitate was isolated by filtration, washed with methanol, and recrystallized from methanol to afford **4** as white solid (1.60 g, 78 %), m.p. 163–164 °C; IR (KBr) ν/cm^{-1}: 1558 (C=O), 1682 (C=S), 2986, 3167, 3283 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (t, 4H, J = 7.2 Hz, $COCH_2$), 2.86 (d, 6H, J = 4.4 Hz, $NHCH_3$), 3.54 (t, 4H, J = 7.2 Hz, CH_2N), 6.63 (t, 1H, J = 7.2 Hz, $H_{(4)}Ar$), 6.73 (d, 2H, J = 8.4 Hz, $H_{(2,6)}Ar$), 7.19 (t, 2H, J = 8.4 Hz, $H_{(3,5)}Ar$), 7.85 (s, 2H, NH), 9.19 (s,

2H, NH), 9.75 (s, 2H, *NH*CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.8 (CH_3), 31.1 (*CH_2*CO), 46.1 (CH_2N), 111.9, 115.8, 129.2, 146.9 (C-Ar), 170.5 (C=O), 182.1 (C=S). Anal. Calcd. For $C_{16}H_{25}N_7O_2S_2$ (411.545): C, 46.7; H, 6.12; N, 23.82 %, Found: C, 46.66; H, 6.09; N, 23.80 %.

*2,2′-(3,3′-(Phenylazanediyl)bis(propanoyl))bis(N-phenylhydrazine-1-carbothioamide) (**5**)* Synthesis and characteristics have been described earlier [27].

*2,2′-(3,3′-(Phenylazanediyl)bis(propanoyl))bis(N-(2,6-dimethylphenyl)hydrazine-1-carbothioamide) (**6**)* To a solution of bispropanehydrazide **1** (1.33 g, 5 mmol) dissolved in methanol (30 ml), 2,6-dimethylphenyl isothiocyanate (1.81 ml, 1.96 g, 12 mmol) was added drop-wise and the reaction mixture was heated under reflux for 8 h. Then it was cooled down to room temperature and water was added. The precipitate was isolated by filtration and recrystallized from DMF–H_2O mixture to afford **6** as white solid (1.63 g, 55 %), m.p. 122–123 °C; IR (KBr) ν/cm^{-1}: 1599 (C=O), 1679 (C=S), 2972, 3211, 3293 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.14 (s, 12H, CH_3), 2.47 (t, 4H, $J = 7.2$ Hz, $COCH_2$), 3.59 (t, 4H, $J = 7.2$ Hz, CH_2N), 6.61 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.73 (t, 2H, $J = 8.2$ Hz, $H_{(2,6)}$Ar), 6.99–7.12 (m, 6H, HAr′), 7.17 (t, 2H, $J = 8.2$ Hz, $H_{(3,5)}$Ar), 9.17 (s, 2H, NH), 9.46 (s, 2H, NH), 10.02 (s, 2H, NHAr′). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ (ppm): 17.9 (4 CH_3), 31.5 (CH_2CO), 46.1 (CH_2N), 111.7, 115.6, 126.8, 127.5, 129.2, 136.6, 136.9, 146.9 (C-Ar), 169.9 (C=O), 181.1 (C=S). Anal. Calcd. For $C_{30}H_{37}N_7O_2S_2$ (591.790): C, 60.89; H, 6.30; N, 16.57 %. Found: C, 60.83; H, 6.29; N, 16.54 %.

*N-Phenyl-5-(2-(phenyl(2-(5-(phenylamino)-1,3,4-oxadiazol-2-yl)ethyl)amino)ethyl)-1,3,4-oxadiazol-2-amine (**7**)* A reaction mixture of bisphenylsemicarbazide **2** (1.51 g, 3 mmol) and $POCl_3$ (10 ml) was heated under reflux for 24 h. The solid product formed upon pouring the reaction mixture drop-wise into ice–water mixture (20 ml) was collected by filtration. Filtrate was neutralized with aqueous ammonia; the crystals were filtered off and washed with water. Combined precipitate was recrystallized from water to afford **7** as brown solid (0.75 g, 54 %)., m.p. 128–129 °C; IR (KBr) ν/cm^{-1}: 1585 (C=O), 1680 (C=S), 2973, 3211, 3287 (NH); ^{1}H NMR (700 MHz, DMSO-d_6) δ (ppm): 3.02 (t, 4H, $J = 6.6$ Hz, CH_2CN), 3.46 (t, 4H, $J = 6.6$ Hz, CH_2N), 6.58 (t, 1H, $J = 7.5$ Hz, $H_{(4)}$Ar), 6.99 (d, 2H, $J = 7.5$ Hz, $H_{(2,6)}$ Ar), 7.11 (t, 2H, $J = 7.5$ Hz, $H_{(3,5)}$ Ar), 7.26–7.64 (m, 10H, HAr′), 10.39 (s, 2H, 2NH). ^{13}C NMR (175 MHz, DMSO-d_6) δ (ppm): 33.2 (CH_2C), 46.4 (CH_2N), 112.1, 115.8, 118.3, 121.4, 128.6, 129.9, 139.5, 147.9 (C-Ar), 155.2 (CO), 170.5 (CNH). Anal. Calcd. For $C_{26}H_{25}N_7O_2$ (467.5224): C, 66.79; H, 5.39; N, 20.97 %. Found: C, 66.76; H, 5.35; N, 20.94 %.

*N-(4-Chlorophenyl)-5-(2-((2-(5-((4-chlorophenyl)amino)-1,3,4-oxadiazol-2-yl)ethyl)(phenyl)amino)ethyl)-1,3,4-oxadiazol-2-amine (**8**)* Was prepared from bis-chlorophenylsemicarbazide **3** (1.72 g, 3 mmol) by following the same synthesis procedure as for **7** to give **8** as brown solid (0.78 g, 73 %), m.p. 271–272 °C; IR

(KBr) ν/cm^{-1}: 3184 (NH), 1695 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.09 (t, 4H, $J = 6.9$ Hz, $COCH_2$), 3.51 (t, 4H, $J = 6.9$ Hz, CH_2N), 6.75–6.89 (m, 5H, HAr), 7.19–7.42 (m, 8H, HAr′), 9.84 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 32.7 (CH_2C), 47.8 (CH_2N), 112.3, 116.2, 119.8, 128.7, 129.5, 138.22, 138.7, 146.8 (C-Ar), 154.90 (CO), 168.80 (CNH). Anal. Calcd. For $C_{26}H_{23}Cl_2N_7O_2$ (536.413): C, 58.22; H, 4.32; N, 18.28 %. Found: C, 58.18; H, 4.30; N, 18.29 %.

*N-Methyl-5-(2-((2-(5-(methylamino)-1,3,4-thiadiazol-2-yl)ethyl)(phenyl)amino)ethyl)-1,3,4-thiadiazol-2-amine (**9**)* To conc. H_2SO_4 (5 ml) bismethylthiosemicarbazide **4** (0.82 g, 2 mmol) was added in portions and the reaction mixture was stirred at room temperature for 15 min. Afterwards, it was poured drop-wise into ice–water mixture and neutralized with Na_2CO_3. The crystalline precipitate was isolated by filtration, washed with water and recrystallized from methanol–water mixture to afford **9** as light brown solid (0.49 g, 65 %), m.p. 167–168 °C; IR (KBr) ν/cm^{-1}: 1598 (C=N), 3190 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.04 (t, 4H, $J = 7.0$ Hz, $COCH_2$), 2.86 (d, 6H, $J = 4.9$ Hz, $NHCH_3$), 3.63 (t, 4H, $J = 7.0$ Hz, CH_2N), 6.68 (t, 1H, $J = 7.0$ Hz, $H_{(4)}Ar$), 6.76 (d, 2H, $J = 8.4$ Hz, $H_{(2,6)}Ar$), 7.22 (t, 2H, $J = 8.4$ Hz, $H_{(3,5)}Ar$), 7.51 (q, 2H, $NHCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 27.3 (CH_3), 31.1 (CH_2C), 49.8 (CH_2N), 112.3, 116.3, 129.3, 146.6, 154.8 (CS), 169.4 (CNH). Anal. Calcd. For $C_{16}H_{21}N_7S_2$ (375.515): C, 51.18; H, 5.64; N, 26.11 %, Found: C, 51.16; H, 5.65; N, 26.09 %.

*N-Phenyl-5-(2-(phenyl(2-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)ethyl)amino)ethyl)-1,3,4-thiadiazol-2-amine (**10**)* Was prepared from bisphenylthiosemicarbazide **5** (1.61 g, 3 mmol) by following the same synthesis procedure as for **9** to give **10** as white solid (1.18 g, 47 %), m.p. 154–155 °C; IR (KBr) ν/cm^{-1}: 1600 (C=N), 3196 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.15 (t, 4H, $J = 6.6$ Hz, CH_2C), 3.72 (t, 4H, $J = 6.6$ Hz, CH_2N), 6.71 (t, 1H, $J = 7.2$ Hz, $H_{(4)}Ar$), 6.79–6.89 (m, 2H, $H_{(2,6)}Ar$), 6.98 (t, 2H, $J = 7.2$ Hz, $H_{(3,5)}Ar$), 7.24 (t, 2H, $J = 8.0$ Hz, $H_{(4)}Ar'$), 7.32 (t, 4H, $J = 8.0$ Hz, $H_{(3,5)}Ar'$), 7.59 (d, 4H, $J = 8.0$ Hz, $H_{(2,6)}Ar'$), 10.32 (s, 2H, 2NH). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ (ppm): 27.1 (CH_2C), 49.8 (CH_2N), 112.9, 115.4, 117.2, 121.7, 129.0, 129.4, 140.6, 146.3 (C-Ar), 156.8 (CS), 164.4 (CNH). Anal. Calcd. For $C_{26}H_{25}N_7S_2$ (499.654): C, 62.50, H, 5.04; N, 19.62 %. Found: C, 62.45; H, 5.01; N, 19.60 %.

*5,5′-((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-(4-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one) (**11**)* A mixture of bischlorophenylsemicarbazide **3** (1.72 g, 3 mmol) and 20 % aqueous KOH solution (25 ml) was heated under reflux for 4 h and cooled to room temperature. Afterwards conc. HCl was added to pH 4. The crystalline precipitate was isolated by filtration, washed with water, and recrystallized from DMF–H_2O mixture to afford **11** as white solid (0.98 g, 61 %), m.p. 314–315 °C; IR (KBr) ν/cm^{-1}: 1588 (C=N), 1707 (C=O), 3172 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.56 (t, 4H, $J = 6.9$ Hz, CH_2CN), 3.30 (t, 4H, $J = 6.9$ Hz; CH_2N), 6.58 (t, 1H, $J = 8.7$ Hz, $H_{(4)}Ar$), 7.39 (d, 2H, $J = 8.7$ Hz, $H_{(3,5)}Ar$), 7.42 (d, 2H, $J = 8.7$ Hz, $H_{(2,6)}Ar$), 7.55 (d, 4H, $J = 8.7$ Hz, $H_{(3,5)}Ar'$),

7.58 (d, 4H, $J = 8.7$ Hz, $H_{(2,6)}Ar'$), 11.82 (s, 2H, NNH). MS (ESI, 20 V) *m/z* (%): 537 ($[M+H]^+$, 40). Anal. Calcd. For $C_{26}H_{23}Cl_2N_7O_2$ (536.413): C, 58.22; H, 4.32; N, 18.28 %. Found: C, 58.19; H, 4.30; N, 18.17 %.

*5,5′-((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione) (**12**)* Was prepared from bismethylthiosemicarbazide **4** (1.24 g, 3 mmol) by following the same synthesis procedure as for **11** to give **12** as white solid (1.02 g, 90 %), m.p. 275–276 °C; IR (KBr) ν/cm^{-1}: 1339 (C=S), 1599 (C=N), 3093 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.89 (t, 4H, $J = 7.2$ Hz, $COCH_2$), 3.38 (s, 6H, CH_3), 3.68 (t, 4H, $J = 7.2$ Hz, CH_2N), 6.64 (t, 1H, $J = 7.2$ Hz, $H_{(4)}Ar$), 6.72 (d, 2H, $J = 8.0$ Hz, $H_{(2,6)}Ar$), 7.18 (t, 2H, $J = 8.4$ Hz, $H_{(3,5)}Ar$), 13.57 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.9 (CH_3-N); 29.7 (CH_2C); 46.7 (CH_2N); 111.7, 116.2, 129.4, 146.5 (C-Ar), 150.9 (CN); 166.5 (C=S). Anal. Calcd. For $C_{16}H_{21}N_7S_2$ (375.515): C, 51.18; H, 5.64; N, 26.11 %. Found: C, 51.10; H, 5.60; N, 26.09 %.

*5,5′-((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione) (**13**)* Synthesis and characteristics have been described earlier [27].

*5,5′-((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-(2,6-dimethylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione) (**14**)* Was prepared from bisdimethylthiosemicarbazide **6** (1.77 g, 3 mmol) by following the same synthesis procedure as for **11**, except that the reaction duration was 1 h, to afford **14** as white solid (1.48 g, 89 %), m.p. 160–161 °C; IR (KBr) ν/cm^{-1}: 1291 (C=S), 1600 (C=N), 3085 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.92 (s, 12H, CH_3), 2.35–2.56 (m, 4H, CH_2CN), 3.40–3.61 (m, 4H, CH_2N), 6.59–7.44 (m, 11H, HAr+Ar′),14.00 (s, 2H, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 17.3 (4 CH_3), 31.1 (CH_2C), 40.4 (CH_2N), 111.8, 117.1, 128.8, 129.4, 129.7, 135.7, 136.0, 146.5 (C-Ar), 155.2 (CN), 166.6 (C=S). Anal. Calcd. For $C_{30}H_{33}N_7S_2$ (555.760): C, 64.83; H, 5.98; N, 17.64 %. Found: C, 64.79; H, 5.95; N, 17.61 %.

*General procedure for synthesis of N,N-bis({2-[5-substituted thio)-4-phenyl-4H-1,2,4-triazol-3-yl]ethyl})anilines **15–26*** *Method A*. To a solution of bistriazolethione **13** (0.40 g, 0.8 mmol) in DMF (5 ml), NaH (0.048 g, 2 mmol) was added, the reaction mixture was stirred at room temperature until evolution of hydrogen stopped (approx. 10 min), and haloalkane (2 mmol) was added drop-wise. The reaction mixture was stirred at 50 °C for 20 h. Afterwards, cold water (20 ml) was added, the precipitate formed was isolated by filtration, and recrystallized from 2-propanol.

Method B. To a solution of bistriazolethione **13** (0.40 g, 0.8 mmol) in DMF (5 ml), KOH powder (0.11 g, 2 mmol), K_2CO_3 (0.3 g, 2,2 mmol), and haloalkane (2 mmol) were added. The reaction mixture was stirred at 35–40 °C for 24 h. Afterwards cold water (30 ml) was added, the precipitate formed was isolated by filtration, and recrystallized from 2-propanol (unless indicated otherwise).

Method C. To a solution of **13** (0.40 g, 0.8 mmol) in DMF (5 ml), triethylamine (0.20 g, 0.28 ml, 2 mmol) and haloalkane (2 mmol) were added. The reaction mixture was stirred at room temperature for 4 h. Afterwards cold water (30 ml) was added, the precipitate formed was isolated by filtration, and recrystallized from 2-propanol.

N,N-Bis(2-(5-(ethylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(15)*** Prepared according to *method A* from bistriazolethione **13** and iodoethane (0.31 g, 0.16 ml). White solid (0.24 g, 53 %), m.p. 195–196 °C; IR (KBr) ν/cm^{-1}: 1596 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.25 (t, 6H, $J = 7.2$ Hz, CH_3), 2.11 (s, 3H, CH_3), 2.68 (t, 4H, $J = 7.2$ Hz, CH_2C), 3.04 (q, 4H, $J = 7.2$ Hz, $J = 14.8$ Hz, SCH_2CH_3), 3.35 (t, 4H, $J = 7.2$ Hz, NCH_2), 5.94 (d, 2H, $J = 8.4$ Hz, $H_{(2,6)}$Ar), 6.48 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.87 (t, 2H, $J = 7.2$ Hz, $H_{(3,5)}$Ar), 7.36–7.41 (m, 4H, $H_{(3,5)}$Ar′), 7.53–7.60 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 14.8 (CH_3), 22.5 (CH_2C), 26.5 (CH_2), 47.6 (CH_2N), 110.8, 115.7, 127.4, 129.0, 129.9, 129.9, 132.9, 145.9 (C-Ar), 149.8 (CN), 153.5 (CS). Anal. Calcd. For $C_{30}H_{33}N_7S_2$ (555.760): C, 64.83; H, 5.98; N, 17.64 %. Found: C, 64.53; H, 6.03; N, 17.60 %.

N,N-Bis(2-(5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(16)*** Prepared according to *method A* from bistriazolethione **13** and 2-iodopropane (0.34 g, 0.20 ml). White solid (0.26 g, 56 %), m.p. 185–186 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.25 (d, 12H, 2 CH_3, $J = 6.8$ Hz), 2.11 (s, 3H, CH_3), 2.69 (t, 4H, $J = 7.6$ Hz, CH_2C), 3.34 (t, 4H, $J = 7.2$ Hz, NCH_2), 3.57 (sep, 2H, $J = 6.8$ Hz, CH), 5.94 (d, 2H, $J = 7.8$ Hz, $H_{(2,6)}$Ar), 6.48 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.87 (t, 2H, $J = 7.8$ Hz, $H_{(3,5)}$Ar), 7.33–7.40 (m, 4H, $H_{(3,5)}$Ar′), 7.53–7.61 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.6 (CH_2C), 23.0 (CH_3), 38.4 (CH), 47.6 (CH_2N), 110.8, 115.7, 127.5, 129.0, 129.8, 129.9, 133.0, 145.9 (C-Ar), 149.2 (CN), 153.5 (CS). Anal. Calcd. For $C_{32}H_{37}N_7S_2$ (583.813): C, 65.83; H, 6.39; N, 16.79 %, Found: C, 65.74; H, 6.32; N, 16.75 %.

N,N-Bis(2-(5-(isobutylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(17)*** Prepared according to *method A* from bistriazolethione **13** and 1-bromo-2-methylpropane (0.34 g, 0.20 ml). White solid (0.24 g, 50 %), m.p. 154–155 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.91 (d, 12H, $J = 6.4$ Hz, CH_3), 1.86 (sep, 2H, $J = 6.8$ Hz, CH), 2.69 (t, 4H, $J = 7.4$ Hz, CH_2-C), 2.96 (d, 4H, $J = 6.8$ Hz, SCH_2), 3.36 (t, 4H, $J = 7.4$ Hz, NCH_2), 5.95 (d, 2H, $J = 8.0$ Hz, $H_{(2,6)}$Ar), 6.50 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.89 (t, 2H, $J = 7.8$ Hz, $H_{(3,5)}$Ar), 7.35–7.41 (m,4H, $H_{(3,5)}$Ar′), 7.56–7.63 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.3 (CH_3), 22.5 (CH_2C), 27.8 (CH), 40.5 (CH_2), 47.6 (CH_2N), 110.7, 115.6, 127.4, 129.0, 129.9, 129.9, 132.9, 145.9 (C-Ar), 150.0 (CN), 153.5 (CS). Anal. Calcd. For $C_{34}H_{41}N_7S_2$ (611.866): C, 66.74; H, 6.75; N, 16.02 %. Found: C, 66.71; H, 6.70; N, 15.97 %.

N,N-Bis(2-(5-(butylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(18)*** Prepared according to *Method A* from bistriazolethione **13** and 1-iodobutane (0.34 g, 0.23 ml). White solid (0.25 g, 57 %), m.p. 145–146 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N), 2929 (NC); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (t, 6H, CH_3, $J = 7.2$ Hz), 1.31 (sxt, 4H, CH_2, $J = 7.6$ Hz), 1.57 (qui, 4H, SCH_2CH_2, $J = 7.6$ Hz), 2.67 (t, 4H, $J = 7.6$ Hz, CH_2C), 3.03 (t, 4H, $J = 7.2$ Hz, SCH_2), 3.34 (t, 4H, $J = 7.2$ Hz, NCH_2), 5.94 (d, 2H, $J = 8.4$ Hz, $H_{(2,6)}$Ar), 6.48 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.87 (t, 2H, $J = 7.2$ Hz, $H_{(3,5)}$Ar), 7.32–7.42 (m, 4H, $H_{(3,5)}$Ar′), 7.51–7.63 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.3 (CH_3), 20.9 (CH_2), 22.5 (CH_2C), 30.9 (CH_2), 31.8 (CH_2), 47.6 (CH_2N), 110.8, 115.7, 127.4, 129.0, 129.9, 129.9, 132.9, 145.9 (C-Ar), 149.9 (CN), 153.5 (CS). Anal. Calcd. For $C_{34}H_{41}N_7S_2$ (611.866): C, 66.74; H, 6.75; N, 16.02 %. Found: C, 66.68; H, 6.69; N, 15.97 %.

N,N-Bis(2-(5-(isopentylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(19)*** Prepared according to *method A* from bistriazolethione **13** and 1-iodo-3-methylbutane (0.40 g, 0.26 ml). White solid (0.27 g, 53 %), m.p. 153–154 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (d, 12H, $J = 6.8$ Hz, 2 CH_3), 1.48 (q, 4H, $J = 7.2$ Hz, $J = 14.4$ Hz, CH_2),1.58 (sep, 2H, $J = 6.6$ Hz, CH), 2.67 (t, 4H, $J = 7.2$ Hz, CH_2C), 3.03 (t, 4H, $J = 7.2$ Hz, SCH_2), 3.33 (t, 4H, $J = 7.2$ Hz, NCH_2), 5.94 (d, 2H, $J = 8.0$ Hz, $H_{(2,6)}$Ar), 6.48 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.87 (t, 2H, $J = 7.2$ Hz, $H_{(3,5)}$Ar), 7.32–7.43 (m, 4H, $H_{(3,5)}$Ar′), 7.51–7.63 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.9 (CH_3), 22.5 (CH_2N), 26.6 (CH), 30.2 (CH_2), 37.8 (CH_2), 47.6 (CH_2N), 110.8, 115.7, 127.4, 129.0, 129.9, 129.9, 132.9, 145.9 (C-Ar), 149.9 (CN), 153.6 (CS). Anal. Calcd. For $C_{36}H_{45}N_7S_2$ (639.919): C, 67.57; H, 7.09; N, 15.32 %. Found: C, 67.49; H, 7.11; N, 15.29 %.

N,N-Bis(2-(5-(pentylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(20)*** Prepared according to *method A* from bistriazolethione **13** and 1-iodopentane (0.40 g, 0.26 ml). White solid (0.18 g, 70 %), m.p. 128–129 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.85 (t, 6H, $J = 7.2$ Hz, CH_3), 1.22–1.32 (m, 8H, CH_2CH_2), 1.61 (qui, 4H, $J = 7.2$ Hz, SCH_2CH_2), 2.69 (t, 4H, $J = 7.2$ Hz, CH_2-C), 3.04 (t, 4H, $J = 7.2$ Hz, SCH_2), 3.37 (t, 4H, $J = 7.2$ Hz, NCH_2), 5.98 (d, 2H, $J = 7.2$ Hz, $H_{(2,6)}$Ar), 6.51 (t, 1H, $J = 7.2$ Hz, $H_{(4)}$Ar), 6.90 (d, 2H, $J = 8.1$ Hz, $H_{(3,5)}$Ar), 7.37–7.41 (m, 4H, $H_{(3,5)}$Ar′), 7.54–7.63 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (175 MHz, DMSO-d_6) δ (ppm): 14.3 (CH_3), 22.1 (CH_2C), 23.1 (CH_2), 29.1 (CH_2), 30.5 (CH_2), 32.6 (CH_2), 48.1 (CH_2N), 111.3, 116.2, 127.9, 129.6, 130.4, 130.4, 133.5, 146.5 (C-Ar), 150.4 (CN), 154.06 (CS). Anal. Calcd. For $C_{36}H_{45}N_7S_2$ (639.919): C, 67.57; H, 7.09; N, 15.32 %. Found: C, 67.54; H, 7.05; N, 15.29 %.

2,2′-((((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4H-1,2,4-triazole-5,3-diyl)) bis(sulfanediyl))diacetamide ***(21)*** Prepared according to *method B* from bistriazolethione **13** and 2-chloroacetamide (0.19 g). White solid (0.41 g, 84 %), m.p.

68–69 °C; IR (KBr) ν/cm^{-1}: 1598 (C=N), 1679 (C=O), 3322 (NH_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.70 (t, 4H, $J = 7.2$ Hz, CH_2C), 3.39 (t, 4H, $J = 7.2$ Hz, NCH_2), 3.89 (s, 4H, SCH_2), 6.01 (d, 2H, $J = 8.0$ Hz, $H_{(2,6)}Ar$), 6.52 (t, 1H, $J = 7.2$ Hz, $H_{(4)}Ar$), 6.91 (t, 2H, $J = 7.2$ Hz, $H_{(3,5)}Ar$), 7.42–7.45 (m, 4H, $H_{(3,5)}Ar'$), 7.59–7.63 (m, 6H, $H_{(2,4,6)}Ar'$), 7.66 (s, 4H. NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.4 (CH_2C), 35.9 (CH_2), 47.7 (CH_2N), 110.9, 115.7, 127.2, 129.0, 129.9, 129.9, 132.7, 145.9 (C-Ar), 149.8 (CN), 168.5 (CS), 194.2 (C=O). Anal. Calcd. For $C_{30}H_{31}N_9O_2S_2$ (613.756): C, 58.71; H, 5.09; N, 20.54 %. Found: C, 58.67; H, 5.03; N, 20.51 %.

N,N-Bis(2-(5-(benzylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)ethyl)aniline ***(22)*** Prepared according to *method B* from bistriazolethione **13** and chlorophenylmethane (0.25 g). White solid (0.38 g, 69 %), m.p. 172–173 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.68 (t, 4H, $J = 7.2$ Hz, CH_2C), 3.35 (t, 4H, $J = 7.2$ Hz, CH_2N), 4.33 (s, 4H, SCH_2), 5.94 (d, 2H, $J = 8.4$ Hz, $H_{(2,6)}Ar$), 6.88 (d, 2H, $J = 8.4$ Hz, $H_{(3,5)}Ar$), 7.23–7.33 (m, 12H, HAr′ + HAr″), 7.51–7.60 (m, 8H, HAr′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.5 (CH_2C), 36.3 (CH_2), 47.7 (CH_2N), 110.8, 115.7, 128.4, 128.9, 127.4, 129.7, 129.8, 129.9, 132.8, 134.4, 137.1, 145.8 (C-Ar), 149.5 (CN), 153.7 (CS). Anal. Calcd. For $C_{40}H_{37}N_7S_2$ (679.899): C, 70.66; H, 5.49; N, 14.42 %. Found: C, 70.61; H, 5.45; N, 14.41 %.

2,2′-((((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4H-1,2,4-triazole-5,3-diyl)) bis(sulfanediyl))bis(1-phenylethan-1-one) ***(23)*** Prepared according to *method C* from bistriazolethione **13** and 2-bromo-1-phenylethanone (0.40 g). White solid (0.40 g, 68 %), m.p. 84–85 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N), 1679 (C=O), 2915; ^{1}H NMR (700 MHz, DMSO-d_6) δ (ppm): 2.70 (t, 4H, $J = 7.2$ Hz, CH_2C), 3.37 (t, 4H, $J = 7.2$ Hz, CH_2N), 4.88 (s, 4H, SCH_2), 6.01 (d, 2H, $J = 6.4$ Hz, $H_{(2,6)}Ar$), 6.52 (t, 1H, $J = 6.4$ Hz, $H_{(4)}Ar$), 6.92 (t, 2H, $J = 6.4$ Hz, $H_{(3,5)}Ar$), 7.43–7.70 (m, 16H, HAr′ + HAr″), 8.02 (d, 4H, $J = 6.4$ Hz, HAr″). ^{13}C NMR (175 MHz, DMSO-d_6) δ (ppm): 22.4 (CH_2C), 40.1 (CH_2), 47.7 (CH_2N), 110.8, 115.7, 127.3, 128.3, 128.7, 129.04, 129.9, 130.0, 132.7, 133.7, 135.2, 145.9 (C-Ar), 149.4 (CN), 153.6 (CS), 193.0 (C=O). Anal. Calcd. For $C_{42}H_{37}N_7O_2S_2$ (735.919): C, 68.55; H, 5.07; N, 13.32 %. Found: C, 68.49; H, 5.03; N, 13.28 %.

2,2′-((((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4H-1,2,4-triazole-5,3-diyl)) bis(sulfanediyl))bis(1-(4-chlorophenyl)ethan-1-one) ***(24)*** Prepared according to *method C* from bistriazolethione **13** and 2-bromo-1-(4-chlorophenyl)ethanone (0.47 g). White solid (0.51 g, 79 %), m.p. 171–172 °C; IR (KBr) ν/cm^{-1}: 1589 (C=N), 1681 (C=O); ^{1} H NMR (700 MHz, DMSO-d_6) δ (ppm): 2.69 (t, 4H, $J = 7.2$ Hz, CH_2C), 3.38 (t, 4H, $J = 7.2$ Hz, CH_2N), 4.85 (s, 4H, SCH_2), 6.00 (d, 2H, $J = 7.2$ Hz, $H_{(2,6)}Ar$), 6.51 (t, 1H, $J = 7.2$ Hz, $H_{(4)}Ar$), 6.91 (t, 2H, $J = 7.2$ Hz, $H_{(3,5)}Ar$), 7.42 (dd, 4H, $J = 1.6$ Hz, $J = 6.0$ Hz, $H_{(3,5)}Ar'$), 7.56–7.67 (m, 10H, HAr′ + HAr″), 8.03 (d, 4H, $J = 6.4$ Hz, HAr″). ^{13}C NMR (175 MHz, DMSO-d_6) δ (ppm): 22.4 (CH_2C), 40.0 (CH_2), 47.66 (CH_2N), 110.8, 115.7, 127.2, 128.9, 129.0,

129.9, 130.0, 130.3, 132.7, 133.9, 138.6, 145.9 (C-Ar), 149.3 (CN), 153.7 (CS), 192.2 (C=O). Anal. Calcd. For $C_{42}H_{35}Cl_{12}N_7O_2S_2$ (804.809): C, 62.68; H, 4.38; N, 12.18 %. Found: C, 62.63; H, 4.31; N, 12.15 %.

*2,2′-((((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4H-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))bis(1-(4-nitrophenyl)ethan-1-one) (**25**)* Prepared according to *Method C* from bistriazolethione **13** and 2-bromo-1-(4-nitrophenyl)ethanone (0.49 g). Brown solid (0.60 g, 90 %), m.p. 150–151 °C; IR (KBr) ν/cm^{-1}: 1345 (NO_2), 1589 (C=N), 1681 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.59 (t, 4H, J = 7.2 Hz, CH_2C), 3.38 (t, 4H, J = 7.2 Hz, CH_2N), 5.26 (s, 4H, SCH_2), 6.02 (d, 2H, J = 7.2 Hz, $H_{(2,6)}$Ar,), 6.53 (t, 1H, J = 7.2 Hz, $H_{(4)}$Ar), 6.93 (t, 2H, J = 7.2 Hz, $H_{(3,5)}$Ar), 7.38–7.43 (m, 4H, $H_{(3,5)}$Ar′), 7.55–7.59 (m, 6H, $H_{(2,4,6)}$Ar′), 8.22–8.46 (m, 8H, HAr″). ^{13}C NMR (175 MHz, DMSO-d_6) δ (ppm): 23.1 (CH_2C), 30.5 (CH_2), 46.9 (CH_2N), 110.8, 115.9, 123.8, 128.3, 129.0, 129.7, 130.0, 130.3, 132.8, 133.5, 140.1, 145.7 (C-Ar), 150.1 (CN), 153.9 (CS), 193.9 (C=O). Anal. Calcd. For $C_{42}H_{35}N_9O_6S_2$ (825.914): C, 61.08; H, 4.27; N, 15.26 %. Found: C, 61.01; H, 4.22; N, 15.27 %.

*Diethyl 2,2′-((((phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4H-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))diacetate (**26**)* Prepared according to *Method B* from bistriazolethione **13** and 2.45 g (2.14 ml) ethyl chloroacetate (20 mmol). White solid (0.48 g, 89 %), m.p. 153–154 °C; IR (KBr) ν/cm^{-1}: 1597 (C=N), 1730 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.19 (t, 6H, J = 7.2 Hz, CH_3), 2.70 (t, 4H, J = 7.2 Hz, CH_2C), 3.08 (t, 4H, J = 7.2 Hz, NCH_2), 4.03 (s, 4H, SCH_2), 4.11 (q, 4H, J = 7.2 Hz, J = 14.0 Hz, OCH_2CH_3), 5.98 (d, 2H, J = 8.0 Hz, $H_{(2,6)}$Ar), 6.51 (t, 1H, J = 7.2 Hz, $H_{(4)}$Ar), 6.90 (t, 2H, J = 7.2 Hz $H_{(3,5)}$Ar), 7.41–7.43 (m, 4H, $H_{(3,5)}$Ar′), 7.59–7.62 (m, 6H, $H_{(2,4,6)}$Ar′). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.9 (OCH_2CH_3), 22.4 (CH_2C), 33.9 (CH_2), 47.6 (CH_2N), 61.2 (OCH_2CH_3), 110.9, 115.8, 127.2, 129.1, 130.0, 130.1, 132.6, 145.87 (C-Ar), 149.2 (CN), 153.8 (C=O), 168.0 (CS). Anal. Calcd. For $C_{34}H_{37}N_7O_4S_2$ (671.832): C, 60.78; H, 5.55; N, 14.59 %, Found: C, 60.72; H, 5.51; N, 14.56 %.

*2,2′-((((Phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4H-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))di(acetohydrazide) (**27**)* To a solution of bisphenyltriazole-sulfaneylacetate **26** (5.37 g, 8 mmol) in methanol (100 ml), hydrazine hydrate (0.62 ml, 0.64 g, 20 mmol) was added and the reaction mixture was stirred at room temperature for 12 h. Afterwards H_2O (50 ml) was added and the mixture was left to stand at 4 °C for 24 h. The precipitate was isolated by filtration and recrystallized from 2-propanol to afford **27** as white solid (3.59 g, 70 %), m.p. 105–106 °C; IR (KBr) ν/cm^{-1}: 1598 (C=N), 1674 (C=O), 3051, 3252 (NH, NH_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.71 (t, 4H, J = 7.2 Hz, CH_2C), 3.39 (t, 4H, J = 7.2 Hz, NCH_2), 3.86 (s, 4H, SCH_2), 4.28 (br.s, 4H, NH_2), 5.98 (d, 2H, J = 7.2 Hz, $H_{(2,6)}$Ar), 6.51 (t, 1H, J = 7.2 Hz, $H_{(4)}$Ar), 6.90 (t, 2H, J = 7.2 Hz $H_{(3,5)}$Ar), 7.38–7.44 (m, 4H, $H_{(3,5)}$Ar′), 7.56–7.64 (m, 6H, $H_{(2,4,6)}$Ar′), 9.37 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.5 (CH_2C), 34.2 (CH_2), 47.7

(CH_2N), 110.9, 115.8, 127.3, 129.1, 129.9, 130.1, 132.7, 145.9 (C-Ar), 149.7 (CN), 153.8 (C=O), 168.0 (CS). Anal. Calcd. For $C_{30}H_{33}N_{11}O_2S_2$ (643.786): C, 55.97; H, 5.17; N, 23.93 %, Found: C, 55.89; H, 5.15; N, 23.90 %.

Biology

Evaluation of antioxidative activity Free radical scavenging activity of compounds was measured by DPPH using the widely used method [28]. 1 mM solution of DPPH in ethanol was prepared, and this solution (1 ml) was added to the solutions of tested compounds (1 mg ml^{-1} of DMSO). The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. The absorbance was measured at 517 nm with a spectrophotometer (UV-200-RS). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated according to the following equation:

$$\text{DPPH scavenging effect}\,(\%) = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples.

Evaluation of reducing power Compounds **2–27** (1 mg) were dissolved in 1 ml of DMSO. The obtained solutions were mixed with 2.5 ml of (pH 6.6) 0.2 M phosphate buffer and 2.5 ml of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. 2.5 ml of 10 % trichloroacetic acid were added to the mixture, which was then centrifuged for 10 min at 1000 rpm. 2.5 ml of the upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride. The absorbance was measured at 700 nm with a spectrophotometer (UV-200-RS) [29].

Rapeseed organogenesis in vitro Seeds of rapeseed (*Brassica napus* L.) 'Terra' were used for the experiments. Seeds were initially washed in ethanol for 1 min; afterwards they were washed in bleach for 20 min and rinsed with distilled water. After sterilization, seeds were placed in 9-cm Petri dishes (total amount 150) on filter paper, which was wetted with 3 ml of distilled water (control sample) or solution of azole derivative. Azoles were examined at 0.5 and 1.0 mg l^{-1} concentrations. The results of experiments were compared with the ones of the control sample. Each Petri dish contained ten seeds and was sealed with a strip of parafilm to prevent evaporation. Seeds were germinated in the dark for 7 days at room temperature (22 °C) [30]. After 7 days number of leaves was counted, height of seedlings and length of roots were measured. Each experiment was repeated three times.

Biometric measurements The length of axial organs of 30 selected rapeseed seedlings per treatment was measured. In order to assess the tolerance of seedlings towards azoles we applied a Wilkinson tolerance index (WTI): $I_t = (l_{me}/l_c) \times 100$ %, where l_{me} indicates the increase in axial organ growth in an azole solution and l_c is the increase in axial organ growth in the control sample [31].

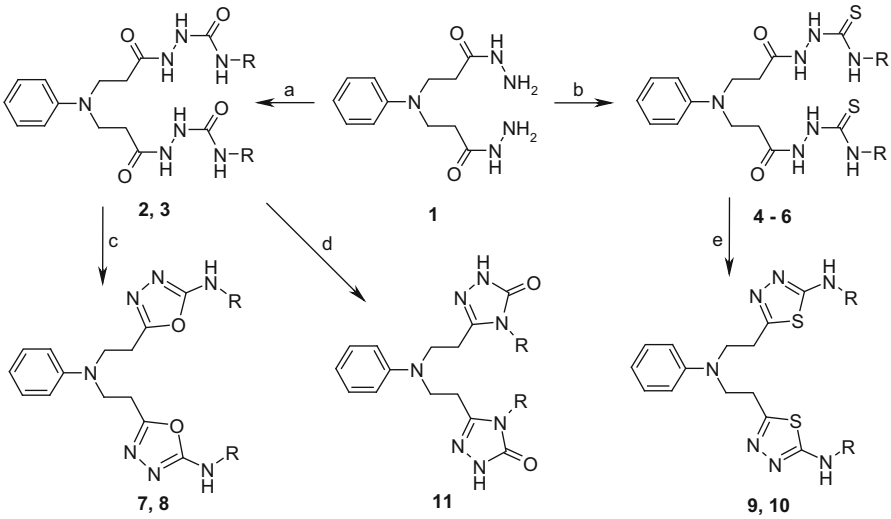
Evaluation of antimicrobial activity The following bacteria strains were used: Gram-positive cocci *Staphylococcus aureus* (ATCC 9144) Gram-negative rods *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (NCTC 6750) and fungi *Candida albicans* (ATCC 10,231). Tryptic soy agar (TSA) and tryptic soy broth (TSB) were used for bacteria cultivation and antibacterial activity tests. Antimicrobial activity of the compounds was determined by testing their different concentrations against *B. cereus*, *S. aureus*, *E. coli, P. aeruginosa* and *C. albicans* strains by the broth-dilution and spread plate methods [32, 33]. The test bacteria *S.aureus*, *E.coli, P. aeruginosa* and fungi *C. albicans* were streaked out on TSA plates and incubated at 37 °C for 24 h. A representative colony was placed in 5 ml of TSB and incubated at 37° C for 24 h. *S. aureus, E.coli, P. aeruginosa* and *C.albicans* cultures containing 10^7 CFU/ml (colony-forming units, corresponding to MC Farland's) were prepared by dilution with TSB and used for antimicrobial tests. Solutions of tested compounds in a range of concentrations, 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.3, and 3.9 μg/ml, were prepared for each sample. The test organisms (100 μL) were added to each tube and incubated at 37 °C for 24 h. At the end of this period, a small amount of the diluted mixture from each tube was pulled out and spread on TSA. The plates were incubated at 37 °C for 48 h. The growth of bacterial cells was observed on agar plates. The lowest concentration of the bacterial material at which no growth was observed was considered as the minimum bactericidal concentration (MBC) value [34]. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation. Oxytetracycline inoculated with test bacteria *S. aureus*, *E. coli,* and *P. aeruginosa* in the tubes and plates was used as a control for antibacterial activity screening. Nystatine inoculated with test fungi *C. albicans* in the tubes and plates was used as a control for antifungal activity screening. The growth of the test microorganism cells was observed on agar plates.

Results and discussion

Chemistry

Reaction of bispropanehydrazide **1** with phenyl or 4-chlorophenyl isocyanates, or methyl, phenyl or 2,6-dimethylphenyl isothiocyanate in methanol gave bishydrazinecarboxamides **2** and **3**, and bishydrazinecarbothiamide derivatives **4–6** (Scheme 1). In the reaction of bispropanehydrazide **1** with phenyl isocyanates, crystals of the bisphenylsemicarbazides **2–3** began to appear already after heating for 10 min, whereas for the reactions with phenyl isothiocyanates more prolonged heating was required. The ^{1}H NMR spectra of bisphenylsemicarbazides **2** and **3** and bisthiosemicarbazides **4–6** show a set of resonances for the protons attributable to the phenyl moiety, whereas the signals of the primary amino group present in the bispropanehydrazide **1** are absent.

Hydrazinecarboxamides undergo cyclization in phosphoryl chloride or sulfuric acid, forming 1,3,4-oxadiazole derivatives. *N*-Phenyl-5-(2-(phenyl(2-(5-(phenylamino)-1,3,4-oxadiazol-2-yl)ethyl)amino)ethyl)-1,3,4-oxadiazol-2-amine (**7**), and

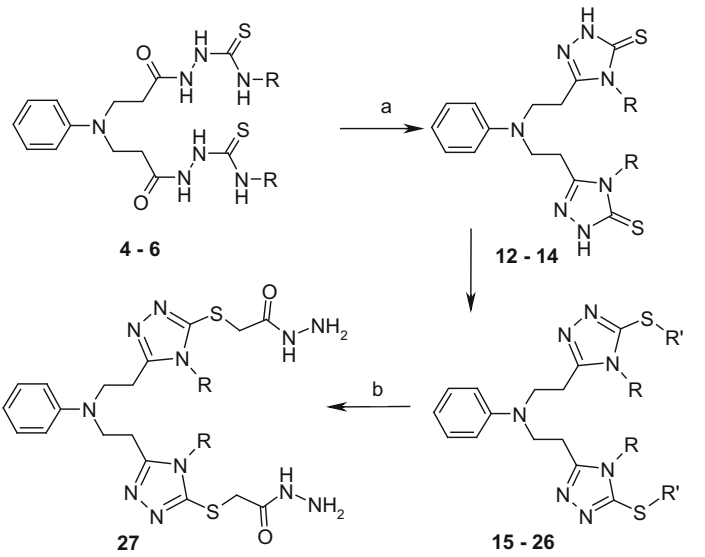


Scheme 1 Synthesis of compounds **2**–**10**. R: **2**, **5**, **7, 10** C_6H_5–; **3**, **8**, **11** 4-Cl-C_6H_4–; **4**, **9** CH_3–; **6**, 2,6-$(CH_3)_2$-C_6H_3–. Reagents and conditions: (*a*) MeOH, RC_6H_4CNO, reflux, 10–15 min (59–95 %); (*b*) MeOH, RC_6H_4CNS, reflux, 20 min–8 h (55–78 %); (*c*) $POCl_3$, reflux 24 h (54–73 %); (*d*) KOH, reflux, 4 h (61–90 %); (*e*) MeOH, H_2SO_4, rt, 15–30 min (65–79 %). Reagents and conditions: (*a*) MeOH, RC_6H_4CNS, reflux, 20 min–8 h (55–78 %); (*b*) MeOH, RC_6H_4CNO, reflux, 10–15 min (59–95 %); (*c*) $POCl_3$, reflux 24 h (54–73 %); (*d*) MeOH, H_2SO_4, rt, 15–30 min (65–79 %); (*e*) KOH, reflux, 4 h (61–90 %); (*f*) MeOH, NH_2NH_2 H_2O, rt, 12 h (70 %)

its chloro analogue **8** were synthesized by heating bisphenylsemicarbazides **2** and **3** under reflux in phosphoryl chloride. Thiadiazole derivatives **9** and **10** were synthesized from bisthiosemicarbazides **4** and **5** by stirring their mixture in concentrated sulfuric acid at room temperature for 15 min. The formation of oxa(thia)diazole ring in compounds **7**–**10** has been confirmed by the absence of the proton signals attributable to the CONHNH group in the ^{1}H NMR spectra in comparison with the spectra for **2**–**5**.

Cyclization of bissemicarbazides **3**–**6** in basic medium resulted in formation of 5,5′-((phenylazanediyl)bis(ethane-2,1-diyl))bis(4-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one) (**11**), and thio derivatives **12**–**14** (Scheme 2). The resonances of hydrogen atoms of the methylene group in the ^{1}H NMR spectra of **11**–**14** are found shifted down-field in comparison with the corresponding signals in the spectra of the starting bissemicarbazides **3**–**6** due to the significant deshielding effect of the neighboring triazole ring.

Depending on the pH of the medium, triazolethiones can exist in thione form or thiole one. The products of triazolethione alkylation and acylation reactions have been isolated from the reaction mixtures only under basic conditions when thione-thiol equilibrium of triazolethiones is shifted towards the formation of thioles. Alkylation reactions of bistriazolethione **13** with haloalkanes, in DMF in the presence of NaH at room temperature, furnished compounds **15**–**20** in 50–70 % yield. Alkylation of **13** with 2-chloroacetamide, chlorophenylmethane or ethyl



Scheme 2 Synthesis of compounds **12–27**. R: **5**, **13, 15–27** C_6H_5–; **4**, **12** CH_3–; **6**, **14** 2,6-$(CH_3)_2$-C_6H_3–; R′: **15** CH_3CH_2–; **16** $(CH_3)_2CH$–; **17** $(CH_3)_2CHCH_2$–; **18** $CH_3CH_2CH_2CH_2$–; **19** $(CH_3)_2CHCH_2CH_2$–; **20** $CH_3CH_2CH_2CH_2CH_2$–; **21** NH_2COCH_2–; **22** $C_6H_5CH_2$–; **23** $C_6H_5COCH_2$–; **24** 4-$ClC_6H_4COCH_2$–; **25** 4-$NO_2C_6H_4COCH_2$–; **26** $C_2H_5OCOCH_2$–. Reagents and conditions: (*a*) KOH, reflux, 4 h (61–90 %); (*b*) MeOH, NH_2NH_2 H_2O, rt, 12 h (70 %)

chloroacetate in the presence of KOH and K_2CO_3 provided bistriazole derivatives **21**, **22** and **26**, respectively. Bisthiophenyltriazoles **23–25** were synthesized by the reaction of bistriazolethione **13** with 2-bromoacetophenones in the basic medium provided by triethylamine. The starting compound **13** is only slightly soluble, whereas the formed *S*-alkyl derivatives **15–26** dissolve well in common organic solvents. ^{1}H NMR spectra of the synthesized *S*-substituted derivatives **15–26**, the resonance attributable to the NH group proton in the heterocyclic moiety (at approx. 13.71 ppm) is absent in comparison with the spectrum of bistriazolethione **13**. Compound **26** has been characterized by the presence of additional signals derived from the ester group, which are observed in the ^{1}H NMR spectrum at 1.19 ppm (-OCH_2CH_3), and 4.11 (-OCH_2CH_3) ppm, respectively. Resonance of the NH group proton is shifted downfield in comparison with the spectrum of the starting compound **13**. Carbons of ester groups resonated at 13.9, and 61.2 ppm, respectively, in the ^{13}C NMR spectrum. The signal of CO carbon is observed at 153.8 ppm.

Ester **26**, upon treatment with hydrazine hydrate, was converted to 2,2′-((((phenylazanediyl)bis(ethane-2,1-diyl))bis(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)) bis(sulfanediyl))di(acetohydrazide) (**27**). In the ^{1}H NMR spectrum for **27**, the signals that originated from the ester functionality in **26** disappeared; instead, new signals due to the hydrazide structure were recorded at 4.28 and 9.37 ppm, respectively. Further support for the formation of the hydrazide structure has been the appearance of strong absorption vibrations indicating the presence of $NHNH_2$ at 3252–3051 cm^{-1} in the FTIR spectrum.

Biology

Evaluation of antioxidative properties

The ability of compounds **2**–**27** to act as free radical scavengers has been tested by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. The presence of the antioxidant molecule in the reaction mixture with DPPH is indicated by color change from purple to yellow. Cefazolin, a widely used first-generation cephalosporin antibiotic, with high antioxidative action, was used as a control. As seen from the results of the antioxidative activity test (Table 1), the best free radical scavenging activity has been shown by bistriazolethione **13** containing phenyl

Table 1 Antioxidative and reducing properties of compounds **2**–**27**

Compound	DPPH (%)	Absorption	
		Concentration (mg/ml)	
		0.5	1
2	13.95	1.253	0.532
3	0.39	0.100	0.187
4	10.85	0.208	1.493
5	27.86	0.539	1.423
6	32.17	1.007	1.128
7	74.37	0.074	0.108
8	52.99	0.080	0.275
9	8.68	0.004	0.058
10	28.44	0.049	0.220
11	22.46	0.019	0.092
12	18.26	0.409	1.077
13	95.09	0.019	0.275
14	19.16	0.032	0.081
15	51.45	0.114	0.171
16	50.00	0.101	0.165
17	52.60	0.093	0.145
18	44.80	0.137	0.190
19	43.35	0.084	0.145
20	48.96	0.005	0.036
21	50.62	0.002	0.017
22	3.61	0.033	0.057
23	79.97	0.025	0.057
24	87.34	0.030	0.067
25	75.66	0.190	0.235
26	2.06	0.064	0.084
27	59.02	0.844	1.165
Cefazolin	32.06	–	–

substituent. When this substituent was replaced by the methyl or dimethyl radical, antioxidative activity decreased 5 times. Activity of *S*-substituted derivatives of bistriazolethione **13** depends on the nature of the substituent. The free radical scavenging activity of compounds containing alkyl groups (**15**–**20**) was approx. 50 %, whereas that of **22** containing benzyl group was only 3.61 %. The presence of phenacyl or 4-substituted phenacyl moiety in the structure of bisthiophenyltriazoles **23**, **24**, and **25** enhanced the antioxidative activity as well. The positive antioxidative effect was reinforced by the chloro substituent (**24**). Replacement of the ester moiety in a very inactive derivative **26** by the hydrazide moiety (**27**) resulted in a 28 times higher antioxidative activity.

Evaluation of reducing properties

Reducing properties of the synthesized compounds **2**–**27** were evaluated by testing their power to reduce ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}). In this method, reducing agent forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride. The higher is the absorption, the stronger reducing properties of the molecule. The synthesized derivatives are weak reducing agents. Among them, bisthiosemicarbazides possessing methyl group (**4**) and phenyl one (**5**) were identified as the strongest reducing agents at concentration 1 mg/ml, whereas diacetamide **21** showed the weakest reducing power (Table 1).

Evaluation of growth regulating properties

The growth regulating activity of the triazolethione **13** and its *S*-substituted derivatives **15**–**20**, **22**, **26**, and **27** was screened on rapeseed cultivar 'Terra' (Table 2). The growth regulating properties of *S*-substituted triazoles depend on the

Table 2 Results of investigation of growth regulating properties of compounds **13, 15–20, 22, 26,** and **27**

Concentration	Control	Compound									
		13	**15**	**16**	**17**	**18**	**19**	**20**	**22**	**26**	**27**
0.5 mg/l	Average height of seedlings (mm)										
	27.1	25.3	30.2	26.8	30.1	31.5	31.2	28.2	28.2	30.8	26.8
	Average length of roots (mm)										
	72.9	55.5	61.1	58.7	65.0	60.6	59.2	58.4	51.5	58.9	53.8
	Average number of leaves										
	3.9	3.6	3.8	3.8	3.9	3.9	3.9	3.8	3.9	3.8	3.8
1 mg/l	Average height of seedlings (mm)										
	27.1	24.6	31.8	29.1	31.9	34.5	34.8	32.2	28.3	34.4	29.7
	Average length of roots (mm)										
	72.9	54.5	58.9	55.5	61.4	60.1	55.2	55.4	47.9	57.6	50.5
	Average number of leaves										
	3.9	3.5	3.9	3.7	3.9	3.8	3.8	3.6	3.7	3.7	4.1

nature of the substituent. Bisphenyltriazoles **18** and **19** had the best effect on growth of the seedlings at both concentrations. Bisisobuthylthiophenyltriazole **17** proved to be the best stimulator of root growth independent of concentration. However, the results were worse than those of the control sample. The tested compounds did not affect significantly the number of the leaves in comparison with the control sample.

Table 3 Minimum inhibitory concentration (MIC, μg/ml) and minimum bactericidal concentration (MBC, μg/ml) (minimum fungicidal concentration values, MFC for *C. albicans*) values for the tested compounds **1–27**

Compound	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Candida albicans*	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MFC (μg/ml)
1	125	250	250	500	500	500	125	250
2	125	250	250	500	250	500	500	500
3	250	500	250	500	250	500	125	250
4	125	250	250	500	250	500	500	500
5	125	500	250	500	250	500	125	250
6	125	250	250	500	250	500	500	500
7	125	250	250	500	250	500	250	500
8	125	250	250	250	500	500	62.5	62.5
9	62.5	125	250	500	500	500	31.25	31.25
10	62.5	125	250	500	500	500	125	250
11	125	250	250	500	500	500	31.25	31.25
12	125	250	250	500	500	500	62.5	125
13	62.5	125	250	500	500	500	125	250
14	62.5	62.5	250	500	500	500	125	250
15	125	125	500	500	250	500	3.9	7.3
16	250	250	500	500	125	250	3.9	7.3
17	250	250	500	500	125	125	3.9	7.3
18	250	250	500	500	125	125	7.3	15.6
19	125	250	250	250	125	125	3.9	7.3
20	31.25	31.25	250	250	+	+	3.9	7.3
21	125	250	250	500	125	250	3.9	7.3
22	125	250	250	250	62.5	62.5	3.9	7.3
23	125	125	250	250	62.5	125	3.9	7.3
24	125	250	250	250	62.5	62.5	3.9	7.3
25	125	125	125	125	125	250	3.9	7.3
26	62.5	125	250	250	7.3	7.3	3.9	7.3
27	125	125	125	250	62.5	62.5	3.9	7.3
C*	62.5	62.5	250	250	250	250	15.6	15.6

* Oxytetracycline was used as a control for *S. aureus, E. coli*, and *P. aeruginosa*. Nystatin was used as a control for *C. albicans*

+, growth of microorganisms

Evaluation of antimicrobial activity

The antimicrobial activity of the compounds **1–27** was screened by testing their different concentrations against gram-positive cocci *Staphylococcus aureus* (ATCC 9144), Gram-negative rods *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (NCTC 6750) and fungi *Candida albicans* (ATCC 10231) by the broth and spread-plate methods. The minimum inhibition concentration (MIC, μg/ml) and minimum bactericidal concentration values (MBC, μg/ml) (minimum fungicidal concentration values, MFC for *C. albicans*) are listed in the Table 3. A broad-spectrum antibiotic oxytetracycline was used as a control for *S.aureus, E. coli*, and *P. aeruginosa*. Nystatin was used as a control for *C. albicans*. As the screening data for antibacterial activity have shown, a number of the investigated compounds possess antibacterial properties. Bispentylthiophenyltriazole **20** has shown a very good bactericidal activity against *S. aureus* with MIC and MBC values of 31.25 μg/ml, which are lower than the ones for oxytetracycline. Antimicrobial activity of this class of compounds depends on the nature of *S*-substituent. Compounds **9**, **10**, **13**, **14**, and **26** inhibit growth of this bacteria strain at 62.5 μg/ml and their MBC values are 125 (62.5 μg/ml for bisdimethylphenyltriazolethione **14**; the same as the one for oxytetracycline). Bisphenyltriazolesulfaneylacetate **26** has proven to be exceptionally active against *P. aeruginosa* with MIC and MBC values of 7.3 μg/ml in comparison to the values for oxytetracycline of 250 μg/ml. Strain *P. aeruginosa* was susceptible to bisphenylthiotriazoles **22**, **23**, **24**, and **27** at MIC value of 62.5 μg/ml. Among tested compounds, just **20** did not suppress growth of this bacterial strain. Triazoles (**15–17** and **19–27**) have been tested to possess exceptional fungicidal activity against fungi *C. albicans* with MIC value of 3.9 μg/ml and MFC value of 7.3 μg/ml. MIC and MFC values for the precursors of these derivatives are 125 and 250 μg/ml, respectively.

Conclusions

In summary, a series of 3-((2-carboxyethyl)phenylamino)propanoic acid derivatives bearing two identical heterocyclic moieties were synthesized. Some of the synthesized compounds have been proven to possess significant biological activities. Bistriazolethione **13** has shown excellent antioxidant activity of 95 %. Just slightly lower free radical scavenging activity has been identified for *S*-alkylated triazole derivatives possessing phenacyl fragment (**23–25**) in their structure. *S*-alkylated triazole derivatives **15–27** exhibit significant antimicrobial activity. *Staphylococcus aureus* bacterial strain has been tested to be susceptible to pentylthiophenyltriazole **20** with MIC and MBC values of 31.25 μg/ml, twice lower than those of oxytetracycline. *S*-Alkylated triazole derivative containing ester moiety has exhibited excellent bactericidal activity against *Pseudomonas aeruginosa* with MBC and MIC values 7.3 μg/ml, 34 times lower than those of oxytetracycline. *S*-alkylated triazole derivatives **15–27** have displayed significant fungicidal activity against *Candida albicans* with MIC value 3.9 μg/ml.

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