

## SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 3,6-DISUBSTITUTED-1,2,4-TRIAZOLO-1,3,4-THIADIAZOLE DERIVATIVES

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**ABSTRACT.** Twelve novel triazolothiadiazole derivatives were synthesized from 4-amino-5-substituted-4*H*-1,2,4-triazole-3-thiols with various aromatic carboxylic acids by cyclization in the presence of phosphorous oxychloride. All the newly synthesized compounds were characterized by FTIR, <sup>1</sup>H NMR, mass spectroscopy and elemental analysis. The antimicrobial activities of the title compounds were examined by disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctinia solani*. The bioassay indicated all synthesized triazolothiadiazole derivatives possessed moderate to good antibacterial and antifungal activities against the tested organisms. Especially, compounds **2e** and **2k** exhibited excellent antibacterial and antifungal activities among these triazolothiadiazole derivatives.

**KEY WORDS:** Triazolothiadiazole, Triazole, Thiadiazole, Antimicrobial activity

### INTRODUCTION

Since Kanaoka reported the triazole[3,4-*b*]-1,3,4-thiadiazole derivatives by condensing the triazole and thiadiazole molecules in 1956 [1], the triazolothiadiazole derivatives have received considerable interest due to their diverse biological activities such as fungicidal [2], bactericidal [3], insecticidal [4], herbicidal [5], anti-inflammatory [6], anticonvulsant [7], anticancer [8] and analgesic [9] activities. Thus, many chemists reported synthesis and antimicrobial activity of some 1,2,4-triazolothiadiazole derivatives in recent years [10–15]. Especially, Karegoudar synthesized some new 1,2,4-triazolothiadiazoles bearing 2,3,5-trichlorophenyl moiety and studied their antimicrobial activity [16], which revealed that these compounds showed good antibacterial and antifungal activities.

Therefore, with the purpose of broadening the class of compounds exhibiting good antimicrobial activity, we introduce the fluorophenyl, chlorophenyl moieties into the triazole ring and the phenyl, nitrophenyl, *tert*-butylphenyl moieties into the thiadiazole ring to investigate their antimicrobial activities. Herein we report the synthesis and biological activities of some new 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives. Structures and properties of the obtained compounds were demonstrated by means of elemental analysis, FT-IR, <sup>1</sup>H NMR, ESI-MS spectroscopy.

### EXPERIMENTAL

#### General

Melting points were determined using X-4 digital melting-point apparatus and are uncorrected. Elemental analysis (C, H, N) was performed with a Perkin Elmer 2400 elemental analyzer. Infrared spectra were recorded on a Nicolet FTIR 5700 spectrophotometer with KBr pellets.

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Electrospray ionization mass spectra (ESI-MS) were performed with a Finnigan LCQ Advantage Max spectrometer.  $^1\text{H}$  NMR spectra were measured on an Avance III<sup>TM</sup> 300 MHz NB Digital NMR spectrometer in  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  solution with TMS as internal standard. Substituted benzoyl hydrazines were synthesized by our group according to the method reported in the literature [17]. Hydrazine hydrate, phosphorus oxychloride and substituted benzoic acid were purchased from Shanghai Chemical Reagent Company Ltd. (Shanghai, China). Other reagents were of analytical grade purity and used without further purification.

*General synthetic procedure for 4-amino-5-substituted-4H-1,2,4-triazole-3-thiols (1a–1d)*

Substituted benzoyl hydrazines (0.02 mol) and KOH (0.06 mol) were dissolved in ethanol (25 mL). To this,  $\text{CS}_2$  (0.2 mol) was added dropwise with stirring at room temperature and maintained for 12 h. The yellow potassium dithiocarbazine salts were formed, filtered and washed with absolute ethanol three times. The intermediate potassium dithiocarbazines were dissolved in hot water (25 mL) and hydrazine hydrate (0.2 mol) was added. This mixture was heated and refluxed for 3 h, then poured into ice-water and acidified with concentrated hydrochloric acid. The precipitates were filtered, washed with water and recrystallized from ethanol to obtain 4-amino-5-substituted-4H-1,2,4-triazole-3-thiols (**1a–1d**).

*4-Amino-5-(2-chlorophenyl)-4H-1,2,4-triazole-3-thiol (1a)*. White crystals, yield 65%, m.p. 165–167 °C; IR (KBr):  $\nu$  3326 (s), 3256 (s), 2622 (w), 1627 (m), 1561 (m), 1461 (s), 1302 (s), 1077 (m), 1026 (s), 952 (s), 757 (s), 726 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.39 (s, 1H, S–H), 7.41–7.57 (m, 4H, Ar–H), 4.82 (s, 2H,  $\text{NH}_2$ ) ppm; ESI-MS  $m/z$ : 226.95 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_8\text{H}_7\text{ClN}_4\text{S}$ : C, 42.39; H, 3.11; N, 24.72%. Found: C, 42.15; H, 3.08; N, 24.98%.

*4-Amino-5-(2-fluorophenyl)-4H-1,2,4-triazole-3-thiol (1b)*. White crystals, yield 61%, m.p. 166–168 °C; IR (KBr):  $\nu$  3314 (s), 3226 (m), 2629 (w), 1621 (s), 1457 (s), 1323 (s), 1238 (s), 1055 (s), 949 (s), 765 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  10.75 (s, 1H, S–H), 7.55–7.63 (m, 2H, Ar–H), 7.23–7.34 (m, 2H, Ar–H), 4.86 (s, 2H,  $\text{NH}_2$ ) ppm; ESI-MS  $m/z$ : 210.90 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_8\text{H}_7\text{FN}_4\text{S}$ : C, 45.70; H, 3.36; N, 26.65%. Found: C, 45.35; H, 3.32; N, 26.84%.

*4-Amino-5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol (1c)*. White crystals, yield 75%, m.p. 186–188 °C; IR (KBr):  $\nu$  3320 (s), 3238 (s), 2631 (w), 1626 (m), 1575 (m), 1461 (s), 1236 (s), 1032 (s), 926 (s), 731 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.93 (1H, S–H), 7.92 (d, 2H,  $J = 7.5$  Hz, Ar–H), 7.48 (d, 2H,  $J = 7.5$  Hz, Ar–H), 5.02 (s, 2H,  $\text{NH}_2$ ) ppm; ESI-MS:  $m/z$  226.89 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_8\text{H}_7\text{ClN}_4\text{S}$ : C, 42.39; H, 3.11; N, 24.72%. Found: C, 42.16; H, 3.09; N, 24.96%.

*4-Amino-5-(4-fluorophenyl)-4H-1,2,4-triazole-3-thiol (1d)*. Yellow crystals, yield 68%, m.p. 202–204 °C; IR(KBr):  $\nu$  3319 (s), 3240 (s), 2625 (w), 1632 (m), 1615 (m), 1452 (s), 1325 (m), 1216 (m), 1161 (m), 1064 (s), 955 (m), 742 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.72 (s, 1H, S–H), 7.76 (d, 2H,  $J = 8.1$  Hz, Ar–H), 7.15 (d, 2H,  $J = 8.1$  Hz, Ar–H), 4.93 (s, 2H,  $\text{NH}_2$ ) ppm; ESI-MS:  $m/z$  210.36 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_8\text{H}_7\text{FN}_4\text{S}$ : C, 45.71; H, 3.36; N 26.65%. Found: C, 45.38; H, 3.31; N, 26.83%.

*General synthetic procedure for 3,6-disubstituted-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (2a–2l)*

To a solution of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiols (**1a–1d**) (3.0 mmol) in phosphorus oxychloride (25 mL), the substituted benzoic acid (4.5 mmol) was added dropwise. The reaction mixture was stirred for 3 h under reflux. Then the mixture was poured into ice-

water and adjusted to pH = 8 with NaOH solution. The precipitates were formed and filtered, washed three times with ethanol. The products were recrystallized from absolute ethanol and dried to obtain 3,6-disubstituted-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**2a–2l**).

*3-(2-Chlorophenyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2a)*. White needle crystals, yield 38%; m.p. 163–165 °C; IR (KBr):  $\nu$  3057(m), 1623 (m), 1520 (m), 1457 (s), 1380 (m), 1242 (m), 1062 (m), 964 (m), 762 (s), 688 (s)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.81–7.91 (m, 3H, Ar–H), 7.62–7.27 (m, 6H, Ar–H) ppm; ESI-MS:  $m/z$  313.12 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_{15}\text{H}_9\text{ClN}_4\text{S}$ : C, 57.60; H, 2.90; N, 17.91%. Found: C, 57.28; H, 2.87; N, 18.07%.

*3-(2-Chlorophenyl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(2b)*. Yellow crystals, yield 43%; m.p. 167–169 °C; IR (KBr):  $\nu$  3103 (m), 3030 (w), 1604 (m), 1529 (s), 1466 (s), 1348 (s), 1055 (m), 962 (m), 856 (m), 752 (m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$ (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.38–8.42 (m, 2H, Ar–H), 8.08–8.13 (m, 2H, Ar–H), 7.82 (d, 1H,  $J = 7.2$  Hz, Ar–H), 7.51–7.64 (m, 3H, Ar–H) ppm; ESI-MS:  $m/z$  358.18 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_{15}\text{H}_8\text{ClN}_5\text{O}_2\text{S}$ : C, 50.36; H, 2.25; N, 19.57%. Found: C, 50.74; H, 2.21; N, 19.79%.

*6-(4-(tert-butyl)phenyl)-3-(2-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2c)*. White powder, yield 41%; m.p. 133–135 °C; IR (KBr):  $\nu$  3056 (m), 2961 (s), 1607 (s), 1467 (s), 1400 (m), 1235 (s), 1115 (m), 970 (m), 841 (m), 765 (m), 706 (m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$ (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05 (d, 2H,  $J = 6.9$  Hz, Ar–H), 7.80–7.85 (m, 2H, Ar–H), 7.46–7.62 (m, 4H, Ar–H), 1.36 (s, 9H, t-Bu–H) ppm; ESI-MS:  $m/z$  369.19 [ $\text{M}^+$ ]; anal. calcd. for  $\text{C}_{19}\text{H}_{17}\text{ClN}_4\text{S}$ : C, 61.86; H, 4.65; N, 15.19%. Found: C, 61.54; H, 4.61; N, 15.35%.

*3-(2-Fluorophenyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2d)*. White needle crystals, yield 49%, m.p. 190–191 °C; IR (KBr):  $\nu$  3056 (s), 1621 (m), 1467 (s), 1388 (m), 1230 (m), 1082 (m), 984 (m), 764(m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.06–8.12 (t, 1H,  $J = 8.1$  Hz, Ar–H), 7.93 (d, 2H,  $J = 7.6$  Hz, Ar–H), 7.52–7.61 (m, 4H, Ar–H), 7.34–7.39 (m, 2H, Ar–H) ppm; ESI-MS:  $m/z$  297.07 [ $\text{M}+1$ ] $^+$ ; anal. calcd. for  $\text{C}_{15}\text{H}_9\text{FN}_4\text{S}$ : C, 60.80; H, 3.06; N, 18.91%. Found: C, 60.58; H, 3.02; N, 19.09%.

*3-(2-Fluorophenyl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2e)*. White crystals, yield 59%; m.p. 254–257 °C; IR (KBr):  $\nu$  3058 (m), 1618 (m), 1530 (s), 1473 (s), 1390 (m), 1349 (s), 1106 (m), 969 (m), 857 (m), 755 (m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.40–8.44 (m, 2H, Ar–H), 8.08–8.16 (m, 3H, Ar–H), 7.56–7.63 (m, 1H, Ar–H), 7.27–7.41 (m, 2H, Ar–H) ppm; ESI-MS:  $m/z$  342.30 [ $\text{M}+1$ ] $^+$ ; anal. calcd. for  $\text{C}_{15}\text{H}_8\text{FN}_5\text{O}_2\text{S}$ : C, 52.78; H, 2.36; N, 20.52%. Found: C, 52.46; H, 2.31; N, 20.68%.

*6-(4-(tert-butyl)phenyl)-3-(2-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2f)*. Light brown crystals, yield 36%; m.p. 175–177 °C; IR (KBr):  $\nu$  3056 (m), 2964 (m), 1610 (m), 1466 (s), 1369 (m), 1226 (m), 1110 (m), 957 (m), 830 (m), 765 (m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.09 (t, 1H,  $J = 8.4$  Hz, Ar–H), 7.84 (d, 2H,  $J = 8.4$  Hz, Ar–H), 7.52–7.62 (m, 3H, Ar–H), 7.30–7.38 (m, 2H, Ar–H), 1.37 (s, 9H, t-Bu–H) ppm; ESI-MS:  $m/z$  353.18 [ $\text{M}+1$ ] $^+$ ; anal. calcd. for  $\text{C}_{19}\text{H}_{17}\text{FN}_4\text{S}$ : C, 64.75; H, 4.86; N, 15.90%. Found: C, 64.93; H, 4.81; N, 15.99%.

*3-(4-Chlorophenyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2g)*. White crystals, yield 44%; m.p. 212–214 °C; IR (KBr):  $\nu$  3050 (m), 1616 (m), 1524 (m), 1463 (s), 1387 (m), 1088 (m), 973 (m), 830 (m), 767(m), 686(m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.38 (d, 2H,  $J = 8.7$  Hz, Ar–H), 7.95–7.98 (m, 2H, Ar–H), 7.53–7.61 (m, 5H, Ar–H) ppm; ESI-MS:  $m/z$

312.92[M<sup>+</sup>]; anal. calcd. for C<sub>15</sub>H<sub>9</sub>ClN<sub>4</sub>S: C, 57.60; H, 2.90; N, 17.91%. Found: C, 57.39; H, 2.85; N, 18.10%.

*3-(4-Chlorophenyl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2h)*. White crystals, yield 33%; m.p. 157–159 °C; IR (KBr):  $\nu$  3072 (m), 1615 (s), 1534 (s), 1414 (m), 1342 (s), 1315 (m), 1267 (m), 1183 (s), 1112 (m), 1069 (s), 1016 (m), 969 (s), 943 (s), 837 (s), 702 (s) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.87 (d, 4H, *J* = 6.7 Hz, Ar-H), 7.53 (d, 4H, *J* = 6.7 Hz, Ar-H) ppm; ESI-MS: *m/z* 358.18 [M<sup>+</sup>]; anal. calcd. for C<sub>15</sub>H<sub>8</sub>ClN<sub>5</sub>O<sub>2</sub>S: C, 50.36; H, 2.25; N, 19.57%. Found: C, 50.21; H, 2.20; N, 19.74%.

*6-(4-(tert-butyl)phenyl)-3-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2i)*. White crystals, yield 43%; m.p. 237–239 °C; IR (KBr):  $\nu$  3051 (m), 2962 (s), 1609 (m), 1463 (s), 1093 (m), 971 (m), 832 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.36 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.87 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.51–7.60 (m, 4H, Ar-H), 1.38 (s, 9H, t-Bu-H) ppm; ESI-MS: *m/z* 369.16[M<sup>+</sup>]; anal. calcd. for C<sub>19</sub>H<sub>17</sub>ClN<sub>4</sub>S: C, 61.86; H, 4.65; N, 15.19%. Found: C, 61.57; H, 4.62; N, 15.38%.

*3-(4-Fluorophenyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2j)*. Light brown crystals, yield 33%; m.p. 164–166 °C; IR (KBr):  $\nu$  3057 (m), 1604 (m), 1535 (m), 1469 (s), 1230 (s), 1155 (m), 970 (m), 834 (m), 762 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR(300 MHz, CDCl<sub>3</sub>):  $\delta$  8.39–8.44 (m, 2H, Ar-H), 7.93 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.55–7.63 (m, 3H, Ar-H), 7.22–7.28 (m, 2H, Ar-H) ppm; ESI-MS: *m/z* 297.07 [M+1]<sup>+</sup>; anal. calcd. for C<sub>15</sub>H<sub>9</sub>FN<sub>4</sub>S: C, 60.80; H, 3.06; N, 18.91%. Found: C, 60.69; H, 3.01; N, 19.02%.

*3-(4-Fluorophenyl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2k)*. Brick-red powder, yield 39%; m.p. 270–272 °C; IR (KBr):  $\nu$  3068 (m), 1604 (m), 1525 (s), 1463 (s), 1345 (s), 1225 (m), 1156 (m), 1104 (m), 969 (m), 848 (s), 684 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.38–8.47 (m, 4H, Ar-H), 8.16 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.25–7.38 (m, 2H, Ar-H) ppm; ESI-MS: *m/z* 341.92[M+1]<sup>+</sup>; anal. calcd. for C<sub>15</sub>H<sub>8</sub>FN<sub>5</sub>O<sub>2</sub>S: C, 52.78; H, 2.36; N, 20.52%. Found: C, 52.57; H, 2.33; N, 20.65%.

*6-(4-(tert-butyl)phenyl)-3-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (2l)*. Light brown crystals, yield 32 %; m.p. 162–164 °C; IR (KBr):  $\nu$  3031 (m), 2962 (s), 1608 (m), 1540 (m), 1477 (s), 1235 (s), 1158 (m), 832 (m), 725 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.40–8.44 (m, 2H, Ar-H), 7.87 (d, 2H, *J* = 6.7 Hz, Ar-H), 7.58 (d, 2H, *J* = 6.7 Hz, Ar-H), 7.25 (t, 2H, *J* = 8.7 Hz, Ar-H), 1.38 (s, 9H, t-Bu-H) ppm; ESI-MS: *m/z* 353.18 [M+1]<sup>+</sup>; anal. calcd. for C<sub>19</sub>H<sub>17</sub>FN<sub>4</sub>S: C, 64.75; H, 4.86; N, 15.90%. Found: C, 64.54; H, 4.81; N, 16.01%.

#### Biological activity tests

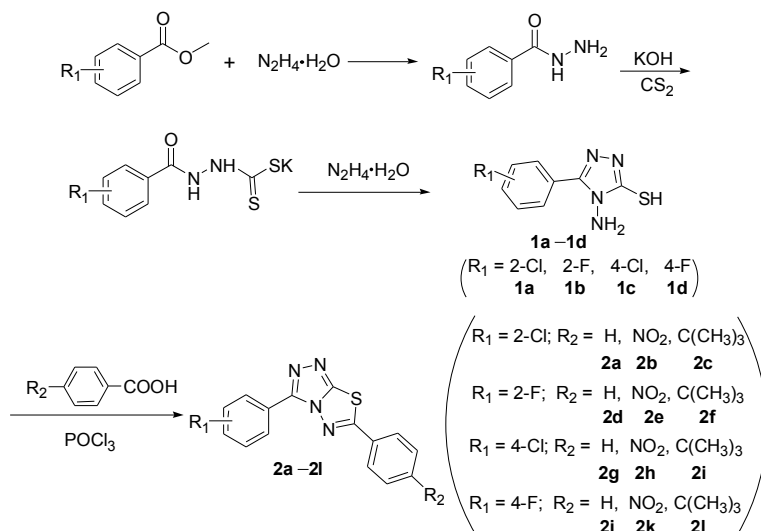
The newly synthesized compounds (**2a–2l**) were tested for their *in vitro* antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* by the disc diffusion method [18, 19] at a concentration of 50 mg L<sup>-1</sup>. Muller-Hinton agar (Hi-Media) was employed as culture medium and DMSO was used as solvent control for antimicrobial activity. Norfloxacin and Triadimefon were used as standard for antibacterial and antifungal activities, respectively. The inhibition zones were measured in mm at the end of an incubation period of 24 h at 37 °C for bacteria and 72 h at 24 °C for fungi. Generally, the results were taken in duplicate. Results with difference higher than 5% were neglected and repeated. In addition, the relative inhibition percentage of the tested compounds with respect to standard drug was calculated according the formula  $RI = D_i / D_s \times 100\%$ , where

$RI$  is the relative inhibition percentage,  $D_t$  is the diameter of inhibition zone for the tested compounds,  $D_s$  is the diameter of inhibition zone for the standard drug.

## RESULTS AND DISCUSSION

### Chemistry

Synthetic pathway for target compounds, 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole (**2a–2l**), is shown in Scheme 1. The substituted benzoyl hydrazines were prepared by treatment of benzoic acid esters and hydrazine hydrate in ethanol and followed by reaction with carbon disulfide in the presence of potassium hydroxide in ethanol to afford the corresponding intermediate potassium dithiocarbazinate. This salt underwent cyclization with the excess hydrazine hydrate to give 4-amino-5-substituted-phenyl-4*H*-1,2,4-triazole-3-thiols (**1a–1d**). The resultant triazoles (**1a–1d**) were further converted to 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole (**2a–2l**) by one-pot reaction with substituted aromatic carboxylic acids in the presence of phosphorus oxychloride.



Scheme 1. Synthetic pathway for the title compounds **2a–2l**.

The newly synthesized compounds were confirmed by elemental analysis, FTIR,  $^1\text{H}$  NMR, and mass spectral data. In the IR spectra, the relatively strong peaks at  $3326\text{--}3226\text{ cm}^{-1}$  were attributed to the  $\text{NH}_2$  stretching vibrations and the weak single peaks at  $2622\text{--}2631\text{ cm}^{-1}$  due to the  $\text{S-H}$  stretching vibrations for the triazole compounds **1a–1d**. However, in the title compounds **2a–2l**, their IR spectra did not exhibit the characteristic absorptions of the  $\text{NH}_2$  and  $\text{S-H}$  stretching vibrations of parent compounds **1a–1d**, which clearly indicated the fusing between compounds **1a–1d** and substituted aromatic carboxylic acids [20].

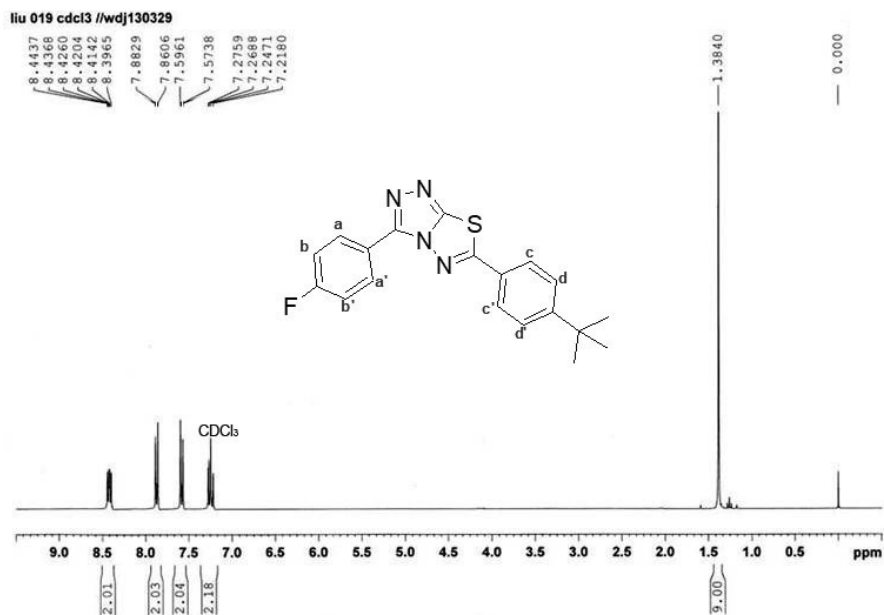


Figure 1. The  $^1\text{H}$  NMR spectrum of the compound **2I**.

The  $^1\text{H}$  NMR spectra of the triazole compounds **1a–1d** showed two characteristic two proton signals at 11.93–10.75 and 5.02–4.82 ppm, which were attributed to the protons of S–H and  $\text{NH}_2$  group for the triazoles, respectively. But these two proton signals were disappeared in the  $^1\text{H}$  NMR spectra of the title compounds **2a–2I**. The chemical shifts at 8.44–7.22 ppm were assigned to aromatic protons of compounds **2a–2I**. Here, we chose the compound **2I** as an example to discuss their chemical shifts. The  $^1\text{H}$  NMR spectrum of the compound **2I** was shown in Figure 1. From Figure 1, the multiple peak at  $\delta$  8.44–8.40 ppm was assigned to  $\text{H}_a$  and  $\text{H}_{a'}$  and the two doublet peaks ( $J = 6.7$  Hz) at  $\delta$  7.87 and 7.58 ppm were due to  $\text{H}_c$  and  $\text{H}_{c'}$  and  $\text{H}_d$  and  $\text{H}_{d'}$ , respectively. The protons  $\text{H}_b$  and  $\text{H}_{b'}$  exhibited a triplet peak ( $J = 8.7$  Hz) at  $\delta$  7.25 ppm. The nine-proton singlet peak at  $\delta$  1.38 ppm was attributed to the protons of the *tert*-butyl group. These results also confirmed that the triazoles **1a–1d** had converted to target triazolothiadiazole compounds **2a–2I** by reacting with substituted aromatic carboxylic acids in phosphorus oxychloride.

The ESI mass spectra of the all synthesized compounds **1a–1d** and **2a–2I** were compared to confirm elemental compositions. Their molecular ion peaks ( $M^+$ ) for these compounds were observed in accordance with the Nitrogen Rule. For example, the electrospray ionization mass spectrum (Figure 2) of the compound **2I** displayed a peak at  $m/z$  353.18  $[\text{M}+1]^+$ , which was due to its molecular ion peak.

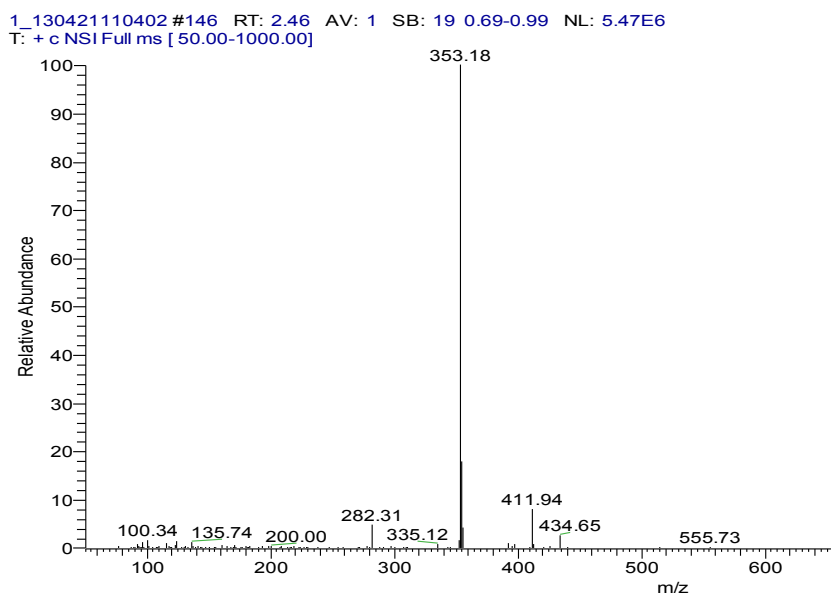


Figure 2. Electrospray ionization mass spectrum of the compound **2l**.

#### Antimicrobial activity

In the bioassay screening, the triazolothiadiazole compounds **2a–2l** were tested for their antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani*. The inhibition zones and relative inhibition percentage of these compounds against bacteria and fungi are listed in Table 1. The results showed that all tested compounds exhibited moderate to good antibacterial and antifungal activities in DMSO. Comparatively, compounds **2e** ( $R_1 = 2$ -fluorophenyl,  $R_2 = 4$ -nitrophenyl), and **2k** ( $R_1 = 4$ -fluorophenyl,  $R_2 = 4$ -nitrophenyl) revealed much significant antimicrobial activities than the other triazolothiadiazole derivatives, which could be due to the presence of fluorophenyl and nitrophenyl moieties in triazolothiadiazole. However, compounds **2c** ( $R_1 = 2$ -chlorophenyl,  $R_2 = 4$ -*tert*-butylphenyl), **2f** ( $R_1 = 2$ -fluorophenyl,  $R_2 = 4$ -*tert*-butylphenyl) and **2i** ( $R_1 = 4$ -chlorophenyl,  $R_2 = 4$ -*tert*-butylphenyl) revealed much lower activities against the tested microorganisms among all synthesized triazolothiadiazole derivatives, which probably assigned to *tert*-butylphenyl moiety in triazolothiadiazole. By the preliminary structure-activity relationship analysis, it was concluded that the introduction of the electron-withdrawing group in triazolothiadiazole can distinctly improve their antimicrobial activities and the introduction of the electron-donating group leads to the decrease of their antimicrobial activities. Especially, the triazolothiadiazole derivatives with fluorophenyl and nitrophenyl moieties at 3,6-position of the triazolothiadiazole ring showed potent antimicrobial activities against the tested microorganisms. As a result, it indicated that the electronic nature of the substituent groups at 3,6-positions in the triazolothiadiazole ring played a significant role in antimicrobial activities.

Table 1. Antimicrobial data of 1,2,4-triazolo-1,3,4-thiadiazole derivatives.

Compounds	Inhibition zone / mm (relative inhibition percentage)		Inhibition zone / mm (relative inhibition percentage)	
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. oryzae</i>	<i>R. solani</i>
<b>2a</b>	12 (37.5%)	13 (41.9%)	14 (46.7%)	12 (41.4%)
<b>2b</b>	21 (65.6%)	19 (61.3%)	20 (66.7%)	20 (69.0%)
<b>2c</b>	10 (31.3%)	10 (32.3%)	11 (36.7%)	10 (34.5%)
<b>2d</b>	16 (50.0%)	16 (51.6%)	17 (56.7%)	15 (51.7%)
<b>2e</b>	25 (78.1%)	26 (83.9%)	26 (86.7%)	24 (82.8%)
<b>2f</b>	11 (34.4%)	13 (41.9%)	12 (40.0%)	12 (41.4%)
<b>2g</b>	14 (43.8%)	12 (38.7%)	14 (46.7%)	13 (44.8%)
<b>2h</b>	22 (68.8%)	23 (74.2%)	21 (70.0%)	20 (69.0%)
<b>2i</b>	11 (34.4%)	10 (32.3%)	12 (40.0%)	11 (37.9%)
<b>2j</b>	18 (56.3%)	17 (54.8%)	18 (60.0%)	19 (65.5%)
<b>2k</b>	30 (93.8%)	28 (90.3%)	25 (83.3%)	27 (93.1%)
<b>2l</b>	13 (40.6%)	12 (38.7%)	13 (43.3%)	12 (41.4%)
Norfloxacin	32	31	–	–
Triadimefon	–	–	30	29

## CONCLUSION

In summary, some new 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives were synthesized. Their structures were confirmed by elemental analysis, IR, <sup>1</sup>H NMR, and mass spectroscopy. The biological activities of these compounds were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani* by disc diffusion method. The results showed that these triazolothiadiazole derivatives exhibited moderate to good antibacterial and antifungal activities. Especially, compounds **2e** and **2k** displayed much higher antibacterial and antifungal activities in all the synthesized triazolothiadiazole derivatives. It demonstrated that the triazolothiadiazole derivatives with fluorophenyl and nitrophenyl moieties at 3,6-position in the triazolothiadiazole ring showed potent antimicrobial activities. Therefore, it is helpful for further structural modification of the triazolothiadiazole derivatives to improve their antimicrobial activities.

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