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Synthesis and biological activities of 3,6-disubstituted-1,2,4-triazolo(3,4-b)-1,3, 4-thiadiazoles

Subedar Prasad[a,*], Avanish Kumar Pandey[a], Dhirendra Kumar[a], Akhilesh Prasad[a] & Chitrasen Gupta[b]
[a]Department of Chemistry, S. M. M. T.D. College, Ballia, India
[b]Department of Chemistry, Kutir Post Graduate College, Chakkey, Jaunpur, India
*E-mail: subedarprasadmmtd@gmail.com

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4-Amino-5aryl/heteroaryl substituted 3-mercapto-1,2,4-triazoles, have been prepared from the corresponding aromatic carboxylic acid through a multistep sequence. These trizoles have been made to react with various aromatic acids to yield 3,6-disubstituted-1,2,4-triazole{3,4-b}-1,3,4 thiadiazoles. Elemental analysis IR, ^{1}H NMR and mass spectral data has elucidated the structures of all newly synthesized compounds. These compounds have shown significant pharmacological activities and are found to be highly active against various fungi and bacteria.

Keywords: Triazoles, Triazolo thiadiazoles, Pharmacological activities

Extensive work has been done on a 1,2,4 triazoles and 1,3,4- thiadiazoles which represent one of the most biologically active compound possesing a wide spectrum of activities[1-3] and their pharmalogical application as diuretic[4-5], hypoglycemic[5-6], antiviral[7], carcinostatic[8], antitubercular[9], antibacterial[10], antiinflammatory[11] and antifungal[12]. A triazole thiadiazole system may be viewed as a cyclic analog of two very important components thiosemicarbazide[13] and biguanide[14] which often display diverse biological activities. A part of our research work is aimed at developing new biologically active nitrogen and sulphur containing heterocycles. We report the synthesis of some new 3,6-disubstituted-1,2,4-triazolo [3,4-b]-1,3,4-thiadizole (Scheme-1b) and 4-amino-3-aryl/aralkyl/heteroaryl substituted-5-mercapto-1,2,4-triazole 3(a-c) were prepared using method[15] (Scheme 1a). A series of triazolo thiadizoles (4-8) were obtained by condensation of triazoles with aromatic acid in presence of phosphorus oxychloride. The structure of the compounds were supported by their analytical and other spectral data.

Experimental Details

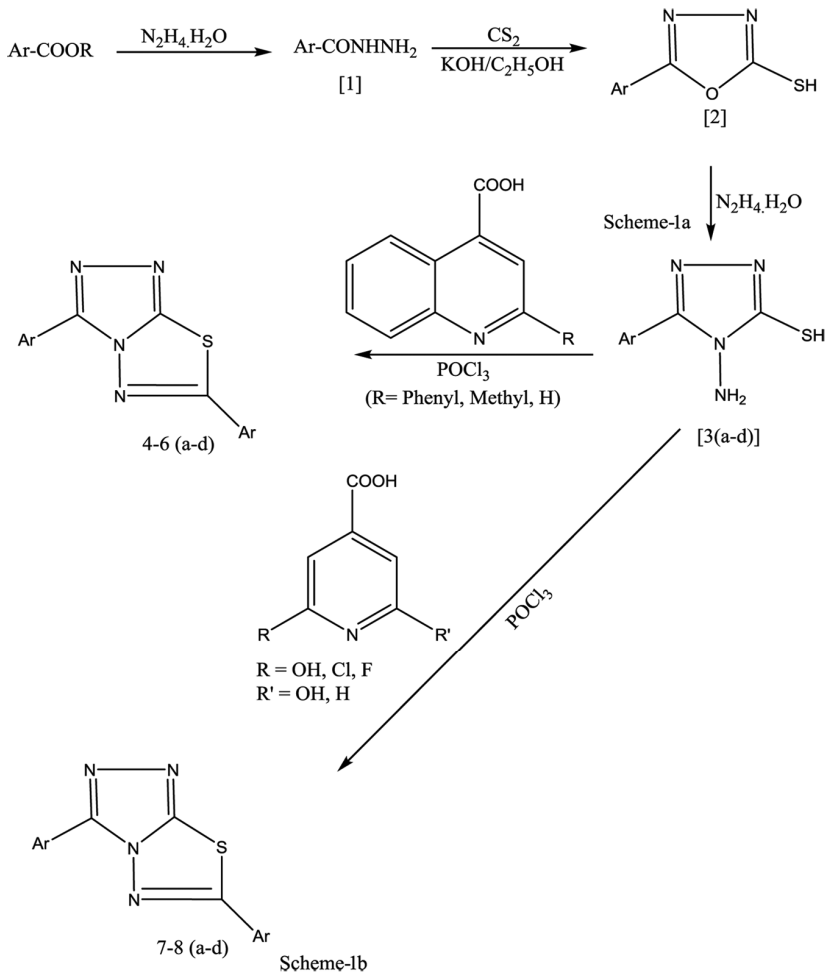
T.L.C. was used to access the completion of the reaction and purity of the compounds synthesized. Melting points were taken in open glass capiliary tubes using Thiele's tube containing liquid paraffin. IR spectra in KBr were recorded on a Perkin Elmer 337 grating spectrophoto meter and ^{1}H NMR were recorded by Bracker spectrophotometers (400 MHz) in DMSO-ds6/$CDCl_3$ using TMS as an internal standard. Mass spectra were recorded in Finnigan Mat 8230 mass spectrophotometers and elemental analysis were recorded on Thermo Anniga Flash A 1112 CHNS analyser. The purity of compounds were checked an silica gel-G coated plates by using ethyl acetate and petroleum ether (1:1) as the eluent and observed in UV light.

Preparation of Arylhydrazide (1)

The methyl/ethyl ester of substituted aromatic acid (0.1 mol) and hydrazine (0.1 mol) were heated and the solution was refluxed for 35 min. 25 mL of ethanol was added to the refluxing mixture as a solvent in order to homogenize the solution. The resulting mixture was further allowed to reflux for 6.5 h. Excess ethanol was distilled out and the contents were allowed to cool. The crystal formed was flitered, washed thoroughly with water and dried.

General procedure for the preparating 2-aryl-substituted-5-mercapto-1,3,4-oxiadiazole (2)

To compound **1** (0.1 mol in 40 mL ethanol), KOH (0.1 mol), absolute ethanol (60 ml) and CS_2 (0.2 mol) were added and refluxed for about 6 h till evolution of hydrogen sulphide was ceased. The reation mixture was cooled at room temperature and diluted with water. On acidification with dilute hydrochloric acid the required oxadiazoles were precipitated. The



Scheme 1 — Synthesis of triazolo thiodizoles (4-8)

precipitate was filtered thoroughly, washed with cold water and then recrystalized from ethanol.

Preparation of 3-substitutes-4-amino-5-mercapto-1,2,4-triazole(3) (3a-d)

A mixture of 2 (0.1 mol) and hydrazine hydrate (0.2 mol) in dry Pyridine (20 mL) was refluxed for 4.5 h. The reaction mixtures was cooled at room temperature and neutralized with dilute HCL. The solid obtained was filtered thoroughly, washed with cold water and recrystallized from ethanol.

4-Amino-3-(3,4-dimethoxyphenyl)-5-mercapto-1,2,4-triazole (3a): Yield 60%; m.p. 212°C; IR (KBr) v (cm^{-1}): 3291 (NH stretching), 1613 (C=N stretching); 3130 (aromatic CH stretching), 2586 (SH), 2934, 2840 (methyl CH stretch), 1269 (asymmetric C-O-C stretching), 1284 (N-N=C), 1582, 1552, 1479 (C=C ring stretching); ^{1}H NMR (ppm): 13.8 (s,1H, SH),7.1 (d,1H, C-5 of Ar), 7.56 (s, 1H, C-2 of Ar), 7.7(d, ^{1}H, C-6 of Ar), 5.78 (s, 2H, NH_2), 3.82 (s, 6H, OCH_3); MS m/z; 252 M^+, Anal. Calcd. (%) for $C_{10}H_{12}N_4O_2S$: C, 47.61; C, 4.79; N, 22.21; S, 12.71. Found: C,47.69; H, 4.81; N, 22.16; S, 12.73.

4-Amino-3-(3,5-dimethoxyphenyl)-5-mercapto-1,2,4-triazole (3b): Yield 62%, m.p. 200°C; IR (KBr) v (cm^{-1}): 3286 (NH stretching), 1610 (C=N stretching); 3090 (aromatic CH stretching), 2580 (SH) , 2934,2 847 (methyl CH stretch), 1264 (asymmetric C-O-C stretching), 1018 (symmetric C-O-C stretching), 1280 (N-N=C), 1588, 1548, 1486, 1455 (C=C ring stretching); ^{1}H NMR (ppm) 13.90 (s, 1H, SH), 7.2 (d, 2H, C-2 & C-6 of Ar) , 6.7(d, 1H, C-4 of Ar), 5.84 (s, 2H, NH_2), 3.84 (s, 6H, OCH_3); MS m/z; 252 M^+; Anal. Calcd.(%) for $C_{10}H_{12}N_4O_2S$; C, 47.61; H, 4.79; N, 22.21; S,12.71; Found: C, 47.50; H, 4.84; N, 22.26; S, 12.68.

4-Amino-3-(3,4,5-trimethoxyphenyl)-5-mercapto-1,2,4-triazole (3c): Yield 60%; m.p. 206°C; IR (KBr) v (cm^{-1}); 3271 (NH stretching) 1607 (C=N

stretching), 3092 (aromatic CH stretching), 1571, 1558, 1480, 1451 (C=C stretching), 2585 (SH), 2935, 2838 (methyl CH stretch), 1261 (asymmetric C-O-C strtching), 1037 (symmetric C-O-C strtching) 1287 (N-N=C); 1H NMR (ppm);13.90 (s,1H,SH), 7.36 (s, 2H, C-2 & C-6 of Ar), 5.82 (s,2H, NH_2), 3.76 (s, 9H, OCH_3); MS m/z: 282 M+; anal. Calcd. (%) for $C_{11}H_{14}N_4O_3S$; C, 46.80; H, 5.00; N, 19.85; S, 11.36 Found: C, 46.73; H, 4.97; N, 19.91, S, 11.33

4-Amino-3-(4-pyridinyl)-5-mercapto-1,2,4-triazole (3d): Yield 65%, m.p. 262°C; IR (KBr) v (cm^{-1}); 3271 (NH stretching), 1607 (C=N stretching), 3080 (aromatic CH stretching), 1571, 1558, 1480, 1451 (C=C ring stretching), 2585 (SH), 2935, 2838 (methyl CH stretch), 1261 (asymmetric C-O-C stretching), 1037 (symmetric C-O-C stretching), 1287 (N-N=C); ^{1}H NMR (ppm); 14.10 (s, 1H, SH), 8.0 (d, 2H, C-3 & C-H of Ar), 8.72 (d, 2H, C-2 & C-6 of Ar), 5.84 (s, 2H, NH_2), MS m/z 193M^+; Anal. Calcd. (%) for $C_7H_7N_5S$; C, 43.51; H, 3.65, N, 36.24; S, 16.59, Found. C, 43.42; H, 3.68; N, 36.17 ; S, 16.57.

General method for the syntheis of 3-aryl/heteroary1-6-(2-substituted-4-quinolinyl)-1,2,4-triazolo [3,4-b]-1,3,4-thiadiazoles. 4(a-d)

A mixture of respective triazole (0.02 mol), 2-phenyl-quinoline-4-carboxylic acid (0.02 mol), and phosphorous oxychloride (10 mL) was heated under reflux for 5-6 h. The recation mixture was cooled to room temperature and the mixture was gradually poured onto crushed ice with stirring. Finely powdered potassium carbonate and calculated amount of solid potassium hydroxide were added till the pH of the mixture was raised to 8, to remove the excess of phosphorous oxychloride. The mixture was allowed to stand overnight from which a solid gets separated.It was filtered, washed thoroughly with cold water, dried and recrystallized from hot ethanol.Similarly other compounds were prepared.

3-(3,4-dimethoxyphenyl)-6-(2-phenyl-4-quinolinyl)1,2,4-triazolo[3,4-b]1,3,4-thiadiazole (4a): Yield 55%, m.p: 221-225°C; IR (KBr) v (cm^{-1}): 3075 (aromatic CH stretching), 1068 (C=N stretching), 1591, 1572, 1490, 1451 (C=C ring stretch), 2940, 2880 (methyl CH stretch), 1260 (asymmetric C-O-C stretching), 1014 (symmetric C-O-C stretching), 1280 (N-N=C); ^{1}H NMR d (ppm): 7.14-8.2 (m, Ar-H), 3.80 (s, 6H, OCH_3); MS m/z: 465 M^+; Anal. Calcd.(%) for $C_{26}H_{19}N_5O_2S$: C, 67.08; H, 4.11; N, 15.04; S, 6.89. Found: C, 67.10; H, 4.16; N, 15.12;S, 6.95.

3-(3,5-dimethoxyphenyl)-6-(2-phenyl-4-quinolinyl)1,2,4-triazolo [3,4-b]1,3,4-thiadiazole (4b): Yield 49%, m.p: 230°C; IR (KBr) v (cm^{-1}): 3080 (aromatic CH stretching), 1610 (C=N stretching), 1580, 1572, 1481 (C=C ring stretch), 2946, 2846 and 2841 (methyl CH stretch), 1262 (asymmetric C-O-C stretching), 1020 (symmetric C-O-C stretching), 1280 (N-N=C); ^{1}H NMR d (ppm) : 6.5-8.0 (m, Ar-H), 3.85 (s, 6H, OCH_3); MS m/z: 465 M^+; Anal. Calcd.(%) for $C_{26}H_{19}N_5O_2S$: C, 67.08; H, 4.11; N, 15.04; S, 6.89. Found: C, 66.85; H, 4.13; N, 15.18; S, 6.80.

3-(3,45-trimethoxyphenyl)-6-(2-phenyl-4-quinolinyl)1,2,4-triazolo [3,4-b]1,3,4-thiadiazole (4c): Yield 45%, m.p: 182°C; IR (KBr) v (cm^{-1}): 092 (aromatic CH stretching), 1610 (C=N stretching), 1585, 1550, 1480, 1450 (C=C ring stretch), 2955, 2840 (methyl CH stretch), 1255 (asymmetric C-O-C stretching), 1025 (symmetric C-O-C stretching), 1275 (N-N=C); ^{1}H NMR d (ppm): 7.2-8.3 (m,12H Ar-H), 3.84 (s, 9H, OCH_3); MS m/z: 495 M^+; Anal. Calcd.(%) for $C_{27}H_{21}N_5O_3S$: C, 65.44; H, 4.27; N, 14.13; S, 6.47. Found: C, 65.60; H,4.16; N, 14.15; S, 6.43.

3-(4-pyridinyl)-6-(2-phenyl-4-quinolinyl)1,2,4-triazolo [3,4-b]1,3,4-thiadiazole (4d): Yield 55%, m.p.: 210°C; IR (KBr) v (cm^{-1}): 3075 (aromatic CH stretching), 1611 (C=N stretching), 1580, 1562 (C=C ring stretch), 1284 (N-N=C); ^{1}H NMR d (ppm): 7.2-8.3 (m, 13H Ar-H), 8.70 (d, 2H, C-3 & C-5 of Ar); MS m/z: 406 M^+; Anal. Calcd.(%) for $C_{23}H_{14}N_6S$: C, 67.96; H, 3.47; N, 20.68; S, 7.89. Found: C, 68.10; H, 3.42; N, 2.83; S, 7.81.

3-(3,4-dimethoxyphenyl)-6-(2-phenyl-4-quinolinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (5a): Yield 55%, m.p.: 168°C; IR (KBr) v (cm^{-1}): 3082 (aromatic CH stretching), 1612 (C=N stretching), 1595, 1482, 1450 (C=C ring stretch), 2965, 2840 (methyl CH stretch), 1262 (asymmetric C-O-C stretching), 1022 (symmetric C-O-C stretching), 1285 (N-N=C); ^{1}H NMR (ppm): 7.20-8.0 (m, Ar-H), 3.80 (s, 3H, OCH_3): 2.78 (s, 3H, CH_3); MS m/z: 404 M^+; Anal. Calcd. (%) For $C_{21}H_{17}N_5O_2S$: C, 62.52; H, 4.25; N, 17.36; S, 7.95. Found: C, 62.60; H, 4.24; N, 17.32; S, 7.99.

3-(3,5-dimethoxyphenyl)-6-(2-methyl-4-quinolinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (5b): Yield 50%, m.p. 182°C; IR (KBr) v (cm^{-1}): 1610 (C=N stretching) 3075 (aromatic CH stretching), 1575, 1558, 1475 (C=C ring stretch), 2940, 2842 (methyl CH

stretch), 1260 (asymmetric C-O-C stretching), 1025 (symmetric C-O-C stretching), 1285 (N-N=C); ^{1}H NMR (ppm): 6.72-8.02 (m, Ar-H), 3.80 (s, 6H, OCH_3); 2.81 (s, 3H, CH_3); MS m/z: 403 M^+; Anal. Calcd. (%) for $C_{21}H_{17}N_5O_2S$: C, 62.52; H, 4.25; N, 17.36; S, 7.95. Found: C, 62.44; H, 4.21; N, 17.32; S, 7.98.

3-(3,4,5-trimethoxyphenyl)-6-(2-methyl-4-quinolinyl)-1,2,4-triazolo[3,4-b]-1,2,3,4-thiadiazole (5c): Yield 53%, m.p. 180°C; IR (KBr) v (cm^{-1}): 3090 (aromatic CH stretching) 1610 (C=N stretching), 1582, 1560, 1485 (C=C ring stretch), 2962, 2850 (methyl CH stretch), 1262 (asymmetric C-O-C stretching), 1022 (symmetric C-O-C stretching), 1275 (N-N=C); ^{1}H NMR (ppm): 7.28-8.01 (m, Ar-H), 3.86 (s, 9H, OCH_3 of Ar); MS m/z: 433 M^+; Anal. Calcd. (%) for $C_{22}H_{19}N_5O_3S$: C, 60.96; H,4.42; N,16.16; S,7.40. Found: C, 61.09; H, 4.39; N, 16.21; S,7.38.

3-(4-pyridinyl)-6-(2-methyl-4-quinolinyl)1,2,4-triazole[3,4-b]-1,3,4-thiadiazole (5d): Yield 48%, m.p. 204°C; IR (KBr) v (cm^{-1}): 3080 (aromatic CH stretching), 1611 (C=N stretching), 1580, 1560 (C=C ring stretch) 1285 (N-N=C); ^{1}H NMR (ppm): 7.32-8.1 (m, 5H of Ar), 8.40 (d, 2H, C-3 & C-5 of Ar); 8.80 (d, 2H, C-2 & C-6 of Ar); 2.8 (s, 3H, CH_3); MS m/z:344 M^+; Anal. Calcd. (%) for $C_{18}H_{12}N_6S$: C, 62.80; H, 3.40; N, 24.53; S, 9.33. Found: C, 68.82; H, 3.49; N, 24.45; S, 9.20.

3-(3,4-dimethoxyphenyl)-6-(4-quinolinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (4b)*:* Yield 45% m.p. 238°C; IR (KBr) v (cm^{-1}): 3073 (aromatic CH stretching), 1607 (C=N stretching), 1590, 1568 (C=C ring stretching), 2942, 2855 (methyl CH stretch), 1265 (asymmetric C-O-C stretching), 1025 (symmetric C-O-C stretching), 1275 (N-N=C); ^{1}H NMR (ppm): 7.16-7.5 (m, Ar-H), 8.02 (d, 1H, C-8 of Ar), 8.88 (d,1H, C-2 of Ar), 3.80 (s, 6H, OCH_3); MS m/z: 389 M^+; Anal. Calcd. (%) for $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98; S, 8.23. Found: C, 61.56; H, 3.91; N, 18.14; S, 8.08.

3-(3,5-dimethoxyphenyl)-6-(4-quinolinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (6b): Yield 47% m.p. 246°C; IR (KBr) v (cm^{-1}): 3062 (aromatic CH stretching), 1583, 1566 (C=C ring stretching), 2962, 2855 (methyl CH stretch), 1615 (C=N stretching), 1260 (asymmetric C-O-C stretching), 1022 (symmetric C-O-C stretching), 1281 (N-N=C); ^{1}H NMR (ppm): 6.72-7.9 (m, Ar-H), 8.04 (d,1H, C-8 of Ar), 8.7 (d,1H, C-2 of Ar), 3.82 (s, 6H, OCH_3); MS m/z: 389 M^+; Anal Calcd. (%) for $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98; S, 8.23. Found: C, 61.80; H, 3.84; N, 18.01; S, 8.24.

3-(3,4,5-trimethoxyphenyl)-6-(2-methyl-4-quinolinyl)-1,2,4-triazolo[3,4-b]-1,2,3,4-thiadiazole (6c): Yield 49% m.p. 232°C; IR (KBr) v (cm^{-1}): 3076 (aromatic CH stretching) 1613 (C=N stretching), 1588, 1573, (C=C ring stretch), 2972, 2845 (methyl CH stretch), 1262 (asymmetric C-O-C stretching), 1016 (symmetric C-O-C stretching), 1283 (N-N=C); ^{1}H NMR (ppm): 7.31-7.75 (m, Ar-H), 8.05 (d, 1H, C-8 of Ar); 8.7 (d, 1H, C-2 of Ar), 3.85 (s, 9H, OCH_3); MS m/z: 410 M^+; Anal. Calcd. (%) for $C_{21}H_{17}N_5O_2S$: C, 60.13; H, 4.09; N, 16.17; S, 7.64. Found: C, 61.05; H, 4.10; N, 16.61; S,7.62.

3-(4-pyridinyl)-6-(4-quinolinyl)1,2,4-triazole[3,4-b]-1,3,4-thiadiazole (6d): Yield 53%, m.p. 281°C; IR (KBr) v (cm^{-1}): 3081 (aromatic CH stretching), 1613 (C=N stretching), 1578, 1563 (C=C ring stretch); 1287 (N-N=C); ^{1}H NMR (ppm): 7.3-7.7 (m, 4H of Ar), 7.94 (d, 2H, C-2 & C-6 of Ar); 8.7 (d, 2H, C-2 & C-6 of Ar); 8.04 (d, 1H, C-8 of Ar), 8.8 (d, 1H, C-2 of Ar) MS m/z: 330 M^+; Anal. Calcd.: (%) for $C_{17}H_{10}N_6S$: C, 61.80; H, 3.05; N, 25.44; S, 9.71. Found: C, 61.73; H, 3.07; N, 25.33; S, 9.76.

3-(3,4-dimethoxyphenyl)-6-(2,6-dihydroxy-4-pyridinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (7a): Yield 48%, m.p.: 279°C; IR (KBr) v (cm-1): 3432 (OH stretching), 3071 (aromatic CH stretching), 1613 (C=N stretching), 1591, 1540, 1482, 1454 (C=C ring stretching), 2963, 2934 (methyl CH stretch), 1260 (asymmetric C-O-C stretching), 1026 symmetric C-O-C stretching), 1292 (N-N=C); ^{1}H NMR (ppm): 7.10 (d, 1H, C-5 of Ar), 7.53 (s, 1H, C-2 of Ar), 7.65 (d, 1H, C-6 of Ar), 7.24 (s, 2H, C-3 & C-5 of Ar), 6.10 (s, 2H, OH), 3.8 (s, 6H, OCH_3); MS m/z: 371 M^+; Anal. Calcd.: (%) For $C_{16}H_{13}N_5O_2S$: C, 51.75; H, 3.53; N, 18.86; S, 8.63. Found : C, 51.64; H, 3.56; N, 18.81; S, 8.59.

3-(3,5-dimethoxy phenyl) -6- (2,6-dihydroxy-4-pyridinyl) -1,2,4-triazolo [3,4-b]-1,3,4-thiadiazole (7b): Yield 54%, m.p. 292°C; IR (KBr) v (cm^{-1}): 3440 (OH stretching) 3072, 3032 (aromatic CH stretching), 1607 (C=N stretching), 1592, 1535, 1478, 1445 (C=C ring stretching), 2972, 2932 (methyl CH stretch), 1261 (asymmetric C-O-C stretching), 1018 (symmetric C-O-C stretching), 1282 (N-N=C); ^{1}H NMR (ppm): 6.73 (d, 1H, C-4 of Ar), 7.22 (d, 1H, C-2 & C-6 of Ar), 7.26 (s, 2H, OH), 3.83 (s,6H, OCH_3): MS m/z: 371 M^+; Anal. Calcd. (%) For $C_{16}H_{13}N_5O_2S$: C, 51.75; H, 3.53; N,

18.86; S, 8.63. Found: C, 51.60; H, 3.48; N, 18.98; S, 8.67.

3-(3,4,5-trimethoxy phenyl)-6-(2,6-dihydroxy-4-pyridinyl)-1,2,4-triazolo[3,4-b]-1,2,3,4-thiadiazole (7c): Yield 52%, m.p. 285°C; IR (KBr) ν (cm^{-1}): 3452 (OH stretching), 3075 (aromatic CH stretching), 1612 (C=N stretching), 1591, 1565, 1480, 1450 (C=C ring stretching), 2956, 2932 (methyl CH stretch), 1265 (asymmetric C-O-C stretching), 1023 (symmetric C-O-C stretching), 1285 (N-N=C); ^{1}H NMR (ppm): 7.30 (s, 2H, C-2 & C-6 of Ar), 7.22 (s, 2H, C-3 & C-5 of Ar), 6.22 (s, 2H, OH), 3.88 (s, 9H, OCH_3): MS m/z: 401 M^+; Anal. Calcd. (%) For $C_{17}H_{15}N_5O_3S$: C, 50.87; H, 3.77; N, 17.45; S, 7.99. Found: C, 50.86; H, 3.77; N, 17.46; S, 7.92.

3-(4-pyridinyl)-6-(2,6-dihydroxy-4-pyridinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (7d): Yield 50%, m.p. 302°C; IR (KBr) ν (cm^{-1}): 3440 (OH stretching), 3085 (aromatic CH stretching), 1613 (C=N stretching), 1580, 1555 (C=C ring stretching), 1284 (N-N=C); ^{1}H NMR (ppm): 8.02 (d, 2H, C-3 & C-5 of Ar), 8.70 (d, 2H, C-2 & C-6 of Ar), 7.22 (s, 2H, C-3 & C-5 of Ar), 6.21 (s, 2H, OH); MS m/z: 312 M^+; Anal. Calcd.(%) For $C_{13}H_8N_6S$: C, 50.00; H, 2.58; N, 26.91; S, 10.27. Found: C, 49.91; H, 2.65; N, 26.78; S, 10.31.

3-(3,4-dimethoxyphenyl)-6-(2-chloro-4-pyridinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (8a): Yield 52%, m.p. 220°; IR (KBr) ν (cm^{-1}): 3032 (aromatic CH stretching), 1612 (C=N stretching), 1580, 1565, 1482, 1454 (C=C ring stretching), 2972, 2840 (methyl CH stretch), 255 (asymmetric C-O-C stretching), 1035 (symmetric C-O-C stretching), 1270 (N-N=C); ^{1}H NMR (ppm): 7.10 (d, 1H, C-5 of Ar), 7.55 (s, 1H, C-2 of Ar), 7.65 (d, 1H, C-6 of Ar), 8.02 (s, 1H, C-3 of Ar), 7.9 (d, 1H, C-5 of Ar'), (d, 1H, C-6' of Ar'), 3.81 (s, 6H, OCH_3); MS m/z: 375 M^{+2}; Anal. Calcd. (%) For $C_{16}H_{12}N_5O_2S$ C, 51.41; H, 3.24; N, 18.73; S, 8.58. Found: C, 51.50; H, 3.56; N, 18.62; S,8.50.

3-(3,4-dimethoxyphenyl)-6-(2-chloro-4-pyridinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (8b): Yield 48%, m.p. 222°C; IR (KBr) ν (cm^{-1}): 3082, 3047 (aromatic CH stretching), 1610 (C=N stretching), 1580, 1552, 1460, 1440 (C=C ring stretch), 2982, 2850 (methyl CH stretch), 1262 (asymmetric C-O-C stretching), 1025 (symmetric C-O-C stretching), 1286(N-N=C); ^{1}H NMR (ppm): 7.24 (d, 2H, C-2 & C-6 of Ar), 6.73 (d, 1H, C-4 of Ar), 8.00 (s, 1H, C-3 of Ar), 7.9 (d, 1H, C-5 of Ar), 8.86 (d, 1H,C-6 of Ar), 3.84 (s,6H,OCH_3) MS m/z: 373 M^{+2}; of Ar), 3.81 (s, 6H, OCH_3); MS m/z: 375 M^{+2}; Anal. Calcd. (%) For $C_{16}H_{12}N_5O_2S$: C, 51.41; H, 3.24; N, 18.73; S, 8.58. Found: C,51.50; H, 3.26; N,18.66; S, 8.54.

3-(3,4,5-trimethoxyphenyl)-6-(2-chloro-4-pyridinyl)-1,2,4-triazolo [3,4-b]-1,2,3,4-thiadiazole (8c): Yield 50%, m.p. 224°C; IR (KBr) ν (cm^{-1}): 3075 (aromatic CH stretching), 1612 (C=N stretching), 1586, 1552, 1484 (C=C ring stretch), 2956, 2852 (methyl CH stretch), 1265 (asymmetric C-O-C stretching), 1017 (symmetric C-O-C stretching), 1275 (N-N=C); ^{1}H NMR (ppm): 7.35 (s, 2H, C-2 & C-6 of Ar), 8.00 (s, 1H, C-3 of Ar), 7.9 (d, 1H, C-5 of Ar) 8.90 (d, 1H, C-6 of Ar), 3.84 (s, 9H, OCH_3): MS m/z: 403 M^+; Anal. Calcd. (%) For $C_{17}H_{14}N_5O_3S$: C, 50.56; H, 3.49; N, 17.34; S, 7.94. Found: C, 50. 47; H, 3.50; N, 17.29; S, 7.91.

3-(4-pyridinyl)-6-(2-chloro-4-pyridinyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (8d): Yield 50%, m.p. 268°C; IR (KBr) ν (cm^{-1}): 3075 (aromatic CH stretching), 1610 (C=N stretching), 1581, 1560 (C=C ring stretching), 1284 (N-N=C); ^{1}H NMR (ppm): 8.01 (d, 2H, C-3 & C-5 of Ar), 8.6 (d, 2H, C-2 & C-6 of Ar), 8.03 (s, 1H, C-3 of Ar), 7.9 (d, 1H, C-5 of Ar), 8.91 (d, 1H, C-6 of Ar); MS m/z: 314 M^{+2}; Anal. Calcd. (%) For $C_{13}H_7N_6S$ Calculated: C, 49.61; H, 2.24; N, 26.70; S, 10.19. Found: C, 49.48; H, 2.20; N, 26.81; S, 10.18.

Results and Discussion

The IR spectrum of the cyclized product exhibits absorption band at 3265-3285 cm^{-1} due to NH functional group and the weak broad absorption band around 25 75 cm^{-1} due to –SH group which confirmed the involvement of $–NH_2$ group and –SH group of the parent amino marcapto triazole in the ring formation. In the ^{1}H NMR spectrum of synthesized compound, the peak due to $–NH_2$ and –SH, which were present in the amino marcapto triazolo were absent further conformed the involvement of these functional groups in the cyclization of triazole to triazolo thiadiazoles. Some of these synthesized compounds were found to be highly active against various fungi and bacteria which are shown in Table 1 and 2.

Biological activity

Antibacterial and antifungal activities

Applying the plate diffusion techniques[16-18], all of the newly synthesized compounds were screened

Table 1 — Antibacterial activity of compounds

Compound	*E. coli*			*P. aeruginosa*			*B. subtilis*			*S. aureus*		
	15 mg/mL ±SD*	30 mg/mL ±SD*	45 mg/mL ±SD*	15 mg/mL ±SD*	30 mg/mL ±SD*	45 mg/mL ±SD*	15mg/mL ±SD*	30 mg/mL ±SD*	45 mg/mL ±SD*	15 mg/mL ±SD*	30 mg/mL ±SD*	45 mg/mL ±SD*
4a	6.86±1.73	11.75±2.25	16.52±2.65	6.72±1.16	11.38±0.58	15.49±0.58	6.82±0.47	11.15±1.16	16.16±1.53	5.82±0.58	10.12±0.58	16.30±0.58
4b	6.08±.058	11.28±0.47	16.86±1.16	5.92±0.61	10.85±0.58	16.04±1.16	5.76±0.58	10.24±1.53	15.92±0.47	5.12±0.26	10.26±0.61	16.74±0.58
4c	5.16±1.16	9.84±1.53	14.18±2.52	6.10±0.58	10.63±0.58	14.48±0.58	4.80±0.58	9.02±1.16	14.00±1.00	5.04±0.58	10.36±1.16	16.60±0.58
4d	7.64±0.26	13.18±0.58	17.36±0.45	7.12±0.26	12.06±1.16	17.26±2.09	6.16±0.61	11.84±0.58	16.86±1.53	6.18±0.58	12.69±0.17	17.48±0.58
5a	5.16±1.73	9.12±2.52	16.67±2.89	5.31±1.16	10.26±1.53	16.11±0.58	7.06±1.71	11.10±0.61	16.38±0.45	6.89±0.58	12.06±0.17	16.10±1.16
5b	5.25±1.15	8.80±0.00	16.00±0.00	5.26±0.57	10.12±1.15	16.05±1.15	6.29±0.57	10.18±1.15	16.12±0.57	6.72±0.57	11.68±0.57	16.60±0.57
5c	5.84±0.58	8.19±0.58	14.20±1.53	4.67±0.45	9.10±0.58	15.53±1.16	5.48±1.16	9.63±0.58	15.80±1.73	6.78±0.58	11.29±0.61	16.00±2.00
5d	6.28±0.45	10.48±0.58	17.00±1.00	6.00±0.00	11.84±0.17	16.82±0.73	7.38±1.16	12.48±0.17	17.10±1.53	7.10±0.58	13.73±0.63	17.92±0.26
6a	8.28±0.26	14.48±0.58	18.78±0.45	7.82±0.26	13.27±1.16	18.71±2.09	7.68±0.61	13.06±0.58	18.80±1.53	7.21±0.58	13.19±0.17	18.12±0.58
6b	7.85±1.73	14.36±2.52	18.64±2.89	7.70±1.16	14.10±1.53	18.16±0.58	7.40±1.71	13.26±0.61	18.66±0.58	6.96±0.58	12.61±0.17	18.27±1.16
6c	7.22±1.16	13.96±0.47	16.72±0.21	6.90±0.58	12.92±1.16	16.73±2.09	7.10±1.16	12.50±1.73	16.82±0.45	6.48±0.17	12.10±1.16	17.85±1.26
6d	8.74±0.58	15.27±1.16	19.41±0.58	8.17±2.09	14.15±0.17	19.10±0.58	8.14±1.53	14.10±0.45	19.79±1.71	8.16±0.58	14.10±1.16	19.12±0.58
8a	6.39±2.09	12.23±0.17	19.21±0.58	7.02±0.26	12.48±1.16	19.11±0.58	7.12±1.53	13.84±0.58	18.63±1.16	6.98±0.58	13.70±1.16	18.58±0.26
8b	6.72±0.61	12.82±1.16	19.67±2.09	6.93±0.45	12.19±0.58	19.39±1.61	7.19±0.47	13.92±0.17	18.91±0.63	7.05±0.26	13.61±1.16	18.14±1.53
8c	6.06±2.09	11.73±1.16	18.68±0.47	6.15±1.16	12.02±2.09	18.52±0.26	6.48±1.16	12.71±0.58	16.85±0.63	6.82±0.45	12.72±1.16	17.36±0.61
8d	7.48±0.58	14.78±0.47	20.19±0.17	7.79±0.61	13.78±0.47	20.59±0.26	7.82±1.17	14.55±0.58	20.38±1.16	7.82±0.63	14.66±0.17	19.23±0.58
Vancamuan	-	-	-	-	-	-	-	12.23±1.51	-	-	21.44±058	-
Amikacin	-	-	22.65±0.57	-	23.00±1.00	-	-	-	-	-	-	

SD = Standard deviation # Zone of inhibition in mm

Table 2 — Antifungal activities of compounds

Compound	*C. albicans*			*A. niger*		
	15 mg/mL ±SD*	30 mg/mL ±SD*	45 mg/mL ±SD*	15 mg/mL ±SD*	30 mg/mL ±SD*	45 mg/mL ±SD*
4a	5.64±1.16	12.84±1.73	12.78±1.53	6.04±0.58	11.63±0.58	17.58±1.16
4b	5.82±0.58	11.37±0.58	17.95±1.73	6.31±1.16	11.54±1.53	16.72±0.58
4d	6.27±0.58	14.84±1.16	19.36±1.53	5.92±0.58	11.00±0.00	17.67±0.58
5a	6.18±1.16	13.82±0.58	18.75±0.61	5.62±0.47	12.62±0.58	18.54±0.27
5b	5.84±0.53	12.62±0.58	19.00±2.00	6.00±1.00	13.46±0.58	17.42±1.16
5d	5.48±0.58	12.00±1.00	17.48±1.53	6.25±0.58	13.00±1.00	16.72±0.27
6a	6.48±0.58	13.92±1.73	18.00±1.00	6.40±0.61	12.85±0.58	19.10±1.53
6b	6.14±0.58	13.66±0.47	17.82±0.27	6.32±0.61	13.00±1.00	19.00±0.00
6d	5.78±0.58	11.00±1.00	19.24±1.53	6.14±0.58	12.00±1.00	19.10±0.58
7d	5.34±0.58	10.00±2.00	17.28±1.53	5.14±0.58	9.48±0.58	16.84±0.58
8a	7.14±0.47	13.72±0.58	19.28±0.47	6.64±.058	11.84±1.73	18.42±1.16
8b	6.82±0.58	12.88±0.61	19.36±0.61	7.23±0.58	13.00±0.00	18.38±0.27
8c	5.66±0.47	11.62±0.58	17.28±1.73	6.12±0.47	10.68±0.58	15.62±1.16
8d	7.26±0.58	14.00±2.00	21.86±1.53	7.38±0.27	14.26±0.58	20.66±0.58
Irriscofulrin			23.97±1.12		22.14±1.98	

*SD= Standard Deviation

in vitro for antibacterial activity against *Eschrichia coli (E. coli)*, *Pseudomonas arruginosa (P. arruginosa) (Gramneqative), Saphy lococcus aureus (S. aureus), Bacilus subtilis (B. sutilis)* gram positive at 15 µg/mL, 30 µg/ml and 45 µg/mL concentrations, respectively. Under identical conditions the positive control antibiotics Amikacin at 45 µg/mL showed zone by inhibition 25-25 mm for gram-negative organism and Vancomycin at 100 µg/mL showed zone of inhibition 24 mm for gram positive organism. Similarly, the antifungal screening of the compounds were carried out in vitro by paper dice method against two fungi, *Aspergillus niger (A.niger)* and *Candid albicans (C. albicans)* by using Girisefulvin (45 µg/mL) as the positive control, which showed 24 mm and 25 mm, respectively) as the zone inhibition.

Conclusion

3,6-disubstituted-1,2,4-triazole{3,4-b}-1,3,4-thiadia-zoles were synthesized and characheized. These compounds have shown significant pharmacological activities and are found to be highly active against various fungi and bacteria.

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