http://dx.doi.org/10.5012/bkcs.2011.32.12.4227

Antimicrobial Activity of Newly Synthesized 2,5-Disubstituted 1,3,4-Thiadiaozle Derivatives

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Received August 26, 2011, Accepted October 3, 2011

A number of new 2,5-disubstituted 1,3,4-thiadiazole and their *S*- or *N*-substituted derivatives as well as the corresponding sugar hydrazone derivatives were synthesized and tested for their antimicrobial activity against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes). The synthesized compounds displayed different degrees of antimicrobial activities or inhibitory actions.

Key Words : 1,3,4-Thiadiazole, Sugar hydrazones, Antimicrobial activity

Introduction

The problem of multi-drug resistant microorganisms has reached on alarming level around the world and for the treatment of microbial infections; the synthesis of new anti-infectious compounds has become an urgent need. 1,3,4-Thiadiazole ring containing compounds represent an important class of heterocyclic nitrogen compounds and their derivatives are characterized with a broad spectrum of biological activity in both agrochemical and pharmaceutical fields. Many 1,3,4-thiadiazoles derivatives have been used as "privileged" scaffolds to produce substances of interest in numerous therapeutic areas, such as anti-inflammatory,[1,2] antimicrobial,[3-5] anticonvulsant,[6-9] and antihypertensive.[10-12] Furthermore, 1,3,4-thiadiazoles exhibit broad spectrum of biological activities, possibly due to the presence of toxophoric N_2C_2S moiety.[13] They find applications as antibacterials, antitumor agents, pesticides, herbicides, dyes, lubricants, and analytical reagents.[14-18]

Several megazol analogues belonging to a new class of 1,3,4-thiadiazole-2-arylhydrazone derivatives have been previously designed and synthesized as attractive antichagasic drug candidates.[19] Several 2,5-disubstituted-1,3,4-thiadiazole derivatives,[20,21] have been shown to possess potential antibacterial activity, a new series of *N*-substituted piperazinyl quinolones carrying a 5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole moiety were designed and synthesized as potential antibacterial agents.[22]

Owing to the above facts and as continuation of our program of identification of new active leads that may be valuable in designing new, potent, selective and less toxic antimicrobial agents,[23-25] the present work reports the synthesis and antimicrobial activity of new substituted 1,2,4-triazolo[3,4-*b*][1,3,4]-thiadiazole derivatives.

Experimental Section

Melting points were determined using a Büchi apparatus. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker, Bremen, Germany). ^{1}H-NMR spectra were recorded with a Varian Gemini spectrometer at 300 MHz with TMS as internal standard. Chemical shifts were reported in δ scale (ppm) relative to TMS as a standard, and the coupling constants (*J* values) are given in Hz. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA). Elemental analyses (C, H and N) were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. The elemental analyses were found to agree favorably with the calculated values. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}.

***N*-(5-Mercapto-1,3,4-thiadiazol-2-yl)benzamide (2).** To a well stirred solution of 5-amino-1,3,4-thiadiazole-2-thiol (**1**), (1.33 g, 0.01 mole) and triethyl amine (1 mL) in DMF (15 mL) a benzoyl chloride was added (1.4 g, 0.01 mole). The reaction mixture was stirred at room temperature for 8 h and then water (20 mL) was added.[26] The precipitated solid was filtered off, washed with water, and crystallized from ethanol to afford **2** as a white solid. Yield 81%, mp 198-199 ^{o}C; IR (KBr): ν 3275 (NH), 1672 (CON), 1618 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.72 (m, 3H, Ar-H), 7.88 (m, 2H, Ar-H), 9.15 (s, 1H, NH), 14.02 (s, 1H, SH) ppm; MS *m/z* 237 (M^+); Anal. Calcd. for $C_9H_7N_3OS_2$: C, 45.55; H, 2.97; N, 17.71. Found: C, 45.31; H, 2.59; N, 17.52.

***N*-(4-(2-Cyanoethyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)benzamide (3).** To a solution of **2** (2.37 g, 0.01 mole) and triethyl amine (0.01 mole) in 15 mL EtOH acrylonitrile was added (0.01 mole) and the mixture was heated under reflux for 6 h. The solvent was evaporated under reduced pressure and remained solid washed with cold ethanol and crystallized from ethanol to afford **3** as white crystals. Yield 77%, mp 175-176 ^{o}C; IR (KBr): ν 3278 (NH), 2208 (CN), 1675 (CON), 1608 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.55 (t, 2H, *J* = 6.2 Hz, CH_2), 4.88 (t, 2H, *J* = 6.2 Hz, CH_2), 6.74 (m, 3H, Ar-H), 7.89 (m, 2H, Ar-H), 9.12 (s, 1H, NH); MS *m/z* 290 (M^+); Anal. Calcd. for $C_{12}H_{10}N_4OS_2$: C,

49.64; H, 3.47; N, 19.30. Found: C, 49.49; H, 3.29; N, 19.07.

***N*-(4-(2-(2*H*-Tetrazol-5-yl)ethyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)benzamide (4).** A mixture of **3** (0.01 mol), sodium azide (0.01 mol), and NH_4Cl (0.01 mol) in DMF (10 mL) was heated for 6 h at 120 °C. The solvent was removed under reduced pressure and the residue was dissolved in (50 mL) water and acidified with dil. HCl to pH 3. The solution was cooled in ice bath to give a precipitate which was crystallized from aqueous ethanol to afford **4** as a white solid. Yield 78%, mp 172-173 °C; IR (KBr): ν 1665 (CON), 1615 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.47 (t, 2H, J = 6.2 Hz, CH_2), 4.91 (t, 2H, J = 6.2 Hz, CH_2), 6.73 (m, 3H, Ar-H), 7.89 (m, 2H, Ar-H), 9.25 (s, 1H, NH-amide), 11.45 (s, 1H, NH-tetrazole) ppm; MS *m/z* 333 (M^+); Anal. Calcd. for $C_{12}H_{11}N_7OS_2$: C, 43.23; H, 3.33; N, 29.41. Found: C, 43.03; H, 3.19; N, 29.17.

***N*-(4-(3-Hydrazino-3-iminopropyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)benz-amide (5).** A mixture of **3** (2.9 g 0.01 mol), 15 mL ethanol, and $N_2H_4{\cdot}H_2O$ (1 mL, 0.02 mol) was refluxed for 5 h and the solvent was removed under reduced pressure. The remaining precipitate was collected, dried, and crystallized from ethanol to afford the amidrazone **5** as a whit solid. Yield 80%, mp 201-202 °C; IR (KBr): ν 3476 (NH_2), 3315 (NH), 1672 (C=O), 1605 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.45 (t, 2H, J = 6.2 Hz, CH_2), 4.85 (t, 2H, J = 6.2 Hz, CH_2), 6.75 (m, 3H, Ar-H), 7.87 (m, 2H, Ar-H), 8.78 (s, 2H, NH_2), 9.12-9.14 (bs, 2H, 2NH), 10.14 (s, 1H, NH) ppm; MS *m/z* 322 (M^+); Anal. Calcd. for $C_{12}H_{14}N_6OS_2$: C, 44.70; H, 4.38; N, 26.07. Found: C, 44.55; H, 4.34; N, 25.95.

***N*-(4-(2-(5-Mercapto-1,3,4-thiadiazol-2-yl)ethyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)benzamide (6).** To a solution of **5** (3.22 g, 0.01 mol) in *n*-BuOH (50 mL) CS_2 was added (5 mL). The mixture was heated under reflux for 15 h. The solvent was evaporated and the residue was washed with water, dissolved in 10 mL EtOH and left overnight at room temperature. The precipitate was filtered off, washed with water, and crystallized from ethanol. Yield 74%, mp 238-240 °C; IR (KBr): ν 3275 (NH), 1672 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.57 (t, 2H, J = 6.2 Hz, CH_2), 4.87 (t, 2H, J = 6.2 Hz, CH_2), 6.75 (m, 3H, Ar-H), 7.90 (m, 2H, Ar-H), 9.25 (s, 1H, NH), 14.02 (s, 1H, SH) ppm; MS *m/z* 381 (M^+); Anal. Calcd. for $C_{13}H_{11}N_5OS_4$: C, 40.93; H, 2.91; N, 18.36. Found: C, 40.83; H, 2.82; N, 18.09.

***N*-[5-(Methylthio)-1,3,4-thiadiazol-2-yl]benzamide (7).** To a solution of **2** (2.37 g, 0.01 mol) and KOH (0.01 mol) in a mixture of water (25 mL) and ethanol (10 mL), methyl iodide was added (0.01 mol). The solution was stirred at room temperature for 4 h. The resulting precipitate was filtered off and crystallized from ethanol to give **7**. Yield 79%, mp 156-157 °C; IR (KBr): ν 3268 (NH), 1669 (CON), 1612 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.54 (s, 3H, SCH_3), 6.79 (m, 3H, Ar-H), 7.41 (m, 2H, Ar-H), 9.11 (s, 1H, NH) ppm; MS *m/z* 251 (M^+); Anal. Calcd. for $C_{10}H_9N_3OS_2$: C, 47.79; H, 3.61; N, 16.72. Found: C, 47.53; H, 3.13; N, 16.48.

***N*-(5-Hydrazino-1,3,4-thiadiazol-2-yl)benzamide (8).** A mixture of **7** (2.51 g, 0.01 mol), ethanol (15 mL), and $N_2H_4{\cdot}H_2O$ (0.02 mol) was refluxed for 5 h and the solvent was removed under reduced pressure. The remaining precipitate was collected, dried, and crystallized from ethanol to afford the hydrazone **8** as **a** whit solid. Yield 84%, mp 198-199 °C; IR (KBr): ν 3440 (NH_2), 3270 (NH), 1668 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.76 (s, 2H, NH_2), 6.81 (m, 3H, Ar-H), 7.52 (m, 2H, Ar-H), 10.12 (bs, 2H, 2NH) ppm; MS *m/z* 235 (M^+); Anal. Calcd. for $C_9H_9N_5OS$: C, 45.95; H, 3.86; N, 29.77. Found: C, 45.88; H, 3.71; N, 29.53.

***N*-(3-Thioxo-2,3-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)benzamide (9).** To a solution of **8** (2.35 g, 0.01 mol) in ethanol (50 mL) a solution of KOH (0.01 mol) in water (2 mL) and CS_2 (5 mL) were added. The solution was heated under reflux for 15 h. The solvent was evaporated and the residue was dissolved in water, filtered off, and acidified with dil. HCl. The precipitate was filtered off, washed with water, and crystallized from ethanol. Yield 72%, mp 177-178 °C; IR (KBr): ν 3272 (NH), 1670 (CON), 1612 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.75 (m, 3H, Ar-H), 7.90 (m, 2H, Ar-H), 9.56 (bs, 1H, NH), 14.10 (s, 1H, SH) ppm; MS *m/z* 277 (M^+); Anal. Calcd. for $C_{10}H_7N_5OS_2$: C, 43.31; H, 2.54; N, 25.25. Found: C, 43.16; H, 2.33; N, 25.08.

***N*-[5-(Sugarhydrazono)-1,3,4-thiadiazol-2-yl]benzamide 10-12.** To a well stirred solution of the respective monosaccharide (0.01 mol) in water (2 mL), and glacial acetic acid (0.2 mL) *N*-(5-hydrazino-1,3,4-thiadiazol-2-yl)benzamide **8**, (0.01 mol) in ethanol was added (10 mL). The mixture was heated under reflux for 3 h, the resulting solution was concentrated and left to cool. The precipitate formed was filtered off, washed with water and ethanol, then dried and crystallized from ethanol.

***N*-{[5-(D-Galacto-2',3',4',5',6'-pentahydroxyhexyliden-1'-yl)hydrazino]-1,3,4-thiadiazol-2-yl}benzamide (10).** Yield 80%, mp 189-190 °C; IR (KBr): ν 3468-3390 (OH), 3285 (NH), 1670 (CON), 1612 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.47 (m, 2H, H-6', H-6''), 3.54 (m, 1H, H-5'), 4.21 (m, 1H, H-4'), 4.35 (m, 1H, H-3'), 4.40 (t, 1H, J = 5.8 Hz, H-2'), 4.61 (m, 1H, OH), 4.75 (d, 1H, J = 6.3 Hz, OH), 4.91 (m, 1H, OH), 4.97 (t, 1H, J = 4.5 Hz, OH), 5.38 (t, 1H, J = 4.5 Hz, OH), 6.81 (m, 3H, Ar-H), 7.21 (d,1H, J = 7.8 Hz, H-1'), 7.50 (m, 2H, Ar-H), 9.12 (bs, 1H, NH), 11.12 (s, 1H, NH) ppm; MS *m/z* 397 (M^+); Anal. Calcd. for $C_{15}H_{19}N_5O_6S$: C, 45.33; H, 4.82; N, 17.62. Found: C, 45.17; H, 4.59; N, 17.39.

***N*-{[5-(D-Manno-2',3',4',5',6'-pentahydroxyhexyliden-1'-yl)hydrazino]-1,3,4-thiadiazol-2-yl}benzamide (11).** Yield 75%, mp 187-188 °C; IR (KBr): ν 3450-3385 (OH), 3281 (NH), 1672 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.48 (m, 2H, H-6', H-6''), 3.52 (m, 1H, H-5'), 4.20 (m, 1H, H-4'), 4.35 (m, 1H, H-3'), 4.41 (t, 1H, J = 5.8 Hz, H-2'), 4.61 (m, 1H, OH), 4.75 (d, 1H, J = 6.3 Hz, OH), 4.92 (m, 1H, OH), 4.97 (t, 1H, J = 4.5 Hz, OH), 5.36

(t, 1H, J = 4.5 Hz, OH), 6.78 (m, 3H, Ar-H), 7.20 (d,1H, J = 7.8 Hz, H-1'), 7.48 (m, 2H, Ar-H), 9.14 (bs, 1H, NH), 11.10 (s, 1H, NH) ppm; MS *m/z* 397 (M^+); Anal. Calcd. for $C_{15}H_{19}N_5O_6S$: C, 45.33; H, 4.82; N, 17.62. Found: C, 45.22; H, 4.50; N, 17.43.

***N*-{[5-(D-Ribo-2',3',4',5'-tetrahydroxypentyliden-1'-yl) hydrazino]-1,3,4-thiadiazol-2-yl}benzamide (12).** Yield 77%, mp 192-193 °C; IR (KBr): ν 3465-3395 (OH), 3280 (NH), 1671 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.47 (m, 2H, H-5', H-5"), 4.32 (m, 1H, H-4'), 4.46 (m, 1H, H-3'), 4.58 (t, 1H, J = 5.8 Hz, H-2'), 4.68 (m, 1H, OH), 4.77 (d, 1H, J = 6.3 Hz, OH), 4.92 (t, 1H, J = 4.5 Hz, OH), 5.36 (t, 1H, J = 4.5 Hz, OH), 6.79 (m, 3H, Ar-H), 7.22 (d,1H, J = 7.8 Hz, H-1'), 7.49 (m, 2H, Ar-H), 9.12 (bs, 1H, NH), 11.14 (s, 1H, NH) ppm; MS *m/z* 367 (M^+); Anal. Calcd. for $C_{14}H_{17}N_5O_5S$: C, 45.77; H, 4.66; N, 19.06. Found: C, 45.60; H, 4.48; N, 18.89.

General procedure for the synthesis of per-*O*-acetyl-sugar hydrazone derivatives 13-15. To a solution of **10-12** (0.01 mol) in pyridine (7 mL) was added acetic anhydride (0.01 mol) and stirred at room temperature for 5 h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with a saturated solution of $NaHCO_3$ followed by water and then dried. The products were crystallized from ethanol.

***N*-{5-[(2',3',4',5',6'-Penta-*O*-acetyl-D-galactohexylidin-1'-yl)hydrazino]-1,3,4-thiadiazol-2-yl}benzamide (13).** Yield 80%, mp 160-161 °C; IR (KBr): ν 1740 (C=O), 3282 (NH), 1674 (CON), 1614 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.92, 1.95, 2.02, 2.06, 2.10 (5s, 15H, $5CH_3$), 3.98 (dd, 1H, J = 11.2 Hz, J = 2.8 Hz, H-6'), 4.08 (dd, 1H, J = 11.2 Hz, J = 3.2 Hz, H-6"), 4.20 (m, 1H, H-5'), 4.24 (t, 1H, J = 7.5 Hz, H-4'), 5.18 (dd, 1H, J = 3.4 Hz, J = 7.5 Hz, H-3'), 5.25 (t, 1H, J = 7.8 Hz, H-2'), 6.78 (m, 3H, Ar-H), 7.21 (d,1H, J = 7.8 Hz, H-1'), 7.51 (m, 2H, Ar-H), 9.11 (bs, 1H, NH-amide), 11.18 (s, 1H, NH-hydrazone) ppm; MS *m/z* 607 (M^+); Anal. Calcd. for $C_{25}H_{29}N_5O_{11}S$: C, 49.42; H, 4.81; N, 11.53. Found: C, 49.33; H, 4.63; N, 11.22.

***N*-{5-[(2',3',4',5',6'-Penta-*O*-acetyl-D-mannohexylidin-1'-yl)hydrazino]-1,3,4-thiadiazol-2-yl}benzamide (14).** Yield 82%, mp 158-159 °C; IR (KBr): ν 1742 (C=O), 3282 (NH), 1672 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.93, 1.95, 2.02, 2.07, 2.10 (5s, 15H, $5CH_3$), 3.97 (dd, 1H, J = 11.2 Hz, J = 2.8 Hz, H-6'), 4.07 (dd, 1H, J = 11.2 Hz, J = 3.2 Hz, H-6"), 4.18 (m, 1H, H-5'), 4.24 (t, 1H, J = 7.5 Hz, H-4'), 5.10 (dd, 1H, J = 3.4 Hz, J = 7.5 Hz, H-3'), 5.24 (t, 1H, J = 7.8 Hz, H-2'), 6.79 (m, 3H, Ar-H), 7.20 (d,1 H, J = 7.8 Hz, H-1'), 7.50 (m, 2H, Ar-H), 9.12 (bs, 1H, NH-amide), 11.16 (s, 1H, NH-hydrazone) ppm; MS *m/z* 607 (M^+); Anal. Calcd. for $C_{25}H_{29}N_5O_{11}S$: C, 49.42; H, 4.81; N, 11.53. Found: C, 49.27; H, 4.55; N, 11.17.

***N*-{5-[(2',3',4',5'-Tetra-*O*-acetyl-D-ribopentylidin-1'-yl) hydrazino]-1,3,4-thiadiazol-2-yl}benzamide (15).** Yield 80%, mp 161-162 °C; IR (KBr): ν 1744 (C=O), 3278 (NH), 1670 (CON), 1612 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.91, 1.95, 2.02, 2.08 (4s, 12H, $4CH_3$), 3.97 (dd, 1H, J = 11.2 Hz, J = 2.8 Hz, H-5'), 4.07 (dd, 1H, J = 11.2 Hz, J = 3.2 Hz, H-5"), 4.24 (t, 1H, J = 7.5 Hz, H-4'), 5.10 (dd, 1H, J = 3.4 Hz, J = 7.5 Hz, H-3'), 5.24 (t, 1H, J = 8.2 Hz, H-2'), 6.77 (m, 3H, Ar-H), 7.24 (d, 1H, J = 8.2 Hz, H-1'), 7.53 (m, 2H, Ar-H), 9.16 (bs, 1H, NH-amide), 11.15 (s, 1H, NH-hydrazone) ppm; MS *m/z* 535 (M^+); Anal. Calcd. for $C_{22}H_{25}N_5O_9S$: C, 49.34; H, 4.71; N, 13.08. Found: C, 49.12; H, 4.44; N, 12.84.

***N*-{2-Acetyl-3-(polyacetoxyalkyl)-2,3-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl}benzamide 16-18.** A solution of the sugar hydrazones **10-12**, (0.01 mol) in acetic anhydride (5 mL) was boiled under reflux for 1.5 h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with a solution of sodium hydrogen carbonate followed by water and then dried. The products were crystallized from ethanol.

***N*-{2-Acetyl-3-(1',2',3',4',5'-penta-*O*-acetyl-D-galactopent-1'-yl)-2,3-dihydro-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl}benzamide (16):** Yield 75%, mp 152-153 °C; IR (KBr): ν 1741 (OCO), 3277 (NH), 1671 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.95, 1.97, 2.03, 2.06, 2.10, 2.23 (6s, 18H, $6CH_3$), 4.05 (dd, 1H, J = 11.2 Hz, J = 2.8 Hz, H-5'), 4.12 (dd, 1H, J = 11.2 Hz, J = 3.2 Hz, H-5"), 4.25 (t, 1H, J = 7.5 Hz, H-4'), 5.20 (dd, 1H, J = 3.4 Hz, J = 7.5 Hz, H-3'), 5.24 (t, 1H, J = 7.5 Hz, H-2'), 5.28 (dd, 1H, J = 7.5 Hz, J = 8.8 Hz, H-1'), 5.72 (d, 1H, J = 8.8 Hz, H-3), 6.82 (m, 3H, Ar-H), 7.53 (m, 2H, Ar-H), 9.12 (bs, 1H, NH) ppm; MS *m/z* 649 (M^+); Anal. Calcd. for $C_{27}H_{31}N_5O_{12}S$: C, 49.92; H, 4.81; N, 10.78. Found: C, 49.80; H, 4.69; N, 10.70.

***N*-{2-Acetyl-3-(1',2',3',4',5'-penta-*O*-acetyl-D-mannopent-1'-yl)-2,3-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl}benzamide (17).** Yield 77%, mp 154-155 °C; IR (KBr): ν 1744 (C=O), 3275 (NH), 1670 (CON), 1608 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.95, 1.98, 2.03, 2.05, 2.09, 2.24 (6s, 18H, $6CH_3$), 4.00 (dd, 1H, J = 11.2 Hz, J = 2.8 Hz, H-5'), 4.14 (dd, 1H, J = 11.2 Hz, J = 3.2 Hz, H-5"), 4.24 (t, 1H, J = 7.5 Hz, H-4'), 5.21 (dd, 1H, J = 3.4 Hz, J = 7.5 Hz, H-3'), 5.24 (t, 1H, J = 7.5 Hz, H-2'), 5.29 (dd, 1H, J = 7.5 Hz, J = 8.8 Hz, H-1'), 5.71 (d, 1H, J = 8.8 Hz, H-3), 6.81 (m, 3H, Ar-H), 7.52 (m, 2H, Ar-H), 9.11(bs, 1H, NH) ppm; MS *m/z* 649 (M^+); Anal. Calcd. for $C_{27}H_{31}N_5O_{12}S$: C, 49.92; H, 4.81; N, 10.78. Found: C, 49.77; H, 4.60; N, 10.63.

***N*-{2-Acetyl-3-(1',2',3',4'-tetra-*O*-acetyl-D-ribobut-1'-yl)-2,3-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl}benzamide (18).** Yield 79%, mp 157-158 °C; IR (KBr): ν 1742 (C=O), 3278 (NH), 1669 (CON), 1610 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.95, 1.97, 2.04, 2.08, 2.29 (5s, 15H, $5CH_3$), 4.07 (dd, 1H, J = 11.2 Hz, J = 2.8 Hz, H-4'), 4.14 (dd, 1H, J = 11.2 Hz, J = 3.6 Hz, H-4"), 5.21 (dd, 1H, J = 3.6 Hz, J = 7.5 Hz, H-3'), 5.26 (dd, 1H, J = 7.5 Hz, J = 8.6 Hz, H-2'), 5.33 (dd, 1H, J = 7.5 Hz, J = 8.6 Hz, H-1'), 5.74 (d, 1H, J = 8.6 Hz, H-3), 6.82 (m, 3H, Ar-H), 7.54 (m, 2H, Ar-H), 9.15 (bs, 1H, NH) ppm; MS *m/z* 577 (M^+); Anal. Calcd. for $C_{24}H_{27}N_5O_{10}S$: C, 49.91; H, 4.71; N, 12.13. Found: C, 49.73; H, 4.58; N, 12.03.

Antimicrobial Activity.

Sample Preparation: Each of the test compounds and

standards were dissolved in 12.5% DMSO, at concentrations of 500 μg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities.

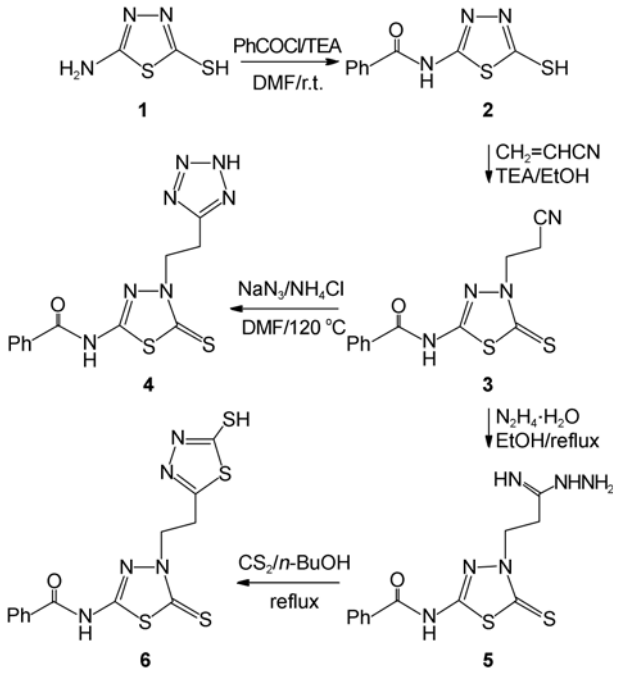
Culture of Microorganisms: Bacteria strains were supplied from Botany Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt, namely *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative) and *Streptomyces* species (Actinomycetes). The bacterial strains were maintained on MHA (Mueller-Hinton agar) medium (Oxoid, Chemical Co., UK) for 24 h at 37 ºC. The medium was molten on a water bath, inoculated with 0.5 mL of the culture of the specific microorganism and poured into sterile Petri dishes to form a layer of about 3-4 mm thickness. The layer was allowed to cool and harden. With the aid of cork-borer, cups of about 10 mm diameter were produced.[31]

Agar Diffusion Technique: The antibacterial activities of the synthesized compounds were tested against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes) using MH medium (17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract). A stock solution of each synthesized compound (500 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. Different concentrations of the test compounds in DMF were placed separately in cups in the agar medium. All plates were incubated at 37 ºC overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones.[32,33]

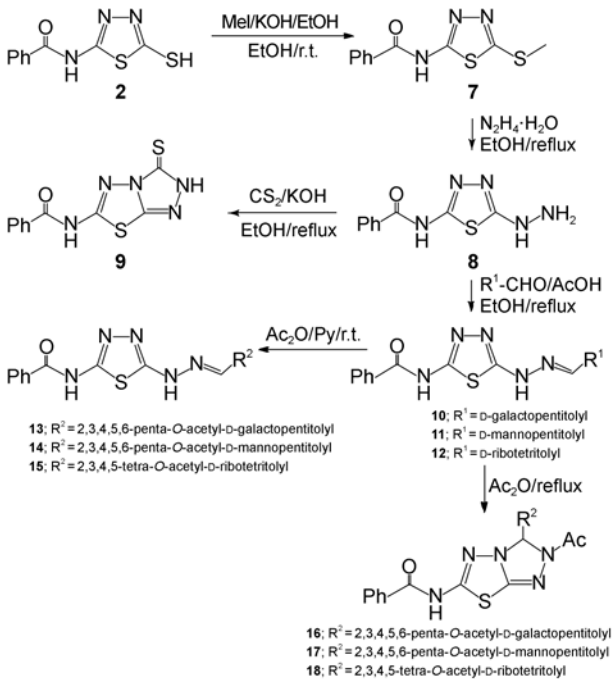
Results and Discussion

The starting material, 5-amino-1,3,4-thiadiazole-2-thiol (**1**) was synthesized by the reaction of thiosemicarbazide with carbon disulphide following previously reported procedure.[26] Benzoylation of **1** using benzoyl chloride in DMF and in the presence of triethylamine at room temperature afforded *N*-(5-mercapto-1,3,4-thiadiazol-2-yl)benzamide (**2**) in 81% yield. The IR spectrum of compound **2** showed absorption band at 1672 cm^{-1} corresponding to the carbonyl group, and its ^{1}H-NMR spectra showed the signals of the aromatic protons at δ 6.72-7.88 ppm, while the NH and SH signals appeared at δ 9.15 and 14.02 ppm, respectively. Reaction of **2** with acrylnitrile in ethanol in presence of triethyamine at reflux temperature gave the *N*-substituted nitrile derivative **3** in 77% yields. The IR spectrum showed characteristic absorption bands at 2208 and 1675 cm^{-1} corresponding to the CN and C=O groups, respectively. The ^{1}H-NMR spectrum of **3** showed the CH_2 signals each as triplet at δ 4.55 and 4.88 ppm in addition to the signals of the aromatic protons at δ 6.74-7.89 ppm. When the nitrile derivative **3** was reacted with sodium azide in DMF in the presence of ammonium chloride at 120 ºC, the corresponding tetrazole derivative **4** was afforded in 78% yield. The ^{1}H-NMR spectrum of **4** showed the CH_2 signals each as triplet at δ 4.57 and 4.91 ppm in addition to the signals of the aromatic protons at δ 6.73-7.89 ppm, and the two NH signals at δ 9.25 (NH-amide) and 11.45 (NH-tetrazole) pm. Reaction of **3** with hydrazine hydrate in ethanol at reflux temperature gave the corresponding amidrazone derivative **5** in 80% yield. The IR spectrum of **5** showed absorption bands at 3476, 3315 and 1672 cm^{-1} for the NH_2, NH, and C=O respectively. The ^{1}H-NMR as well as the mass spectra agreed with the assigned structure. Treatment of the amidrazone **5** with carbon disulphide in *n*-BuOH at reflux temperature afforded *N*-{4-[2-(5-mercapto-1,3,4-thiadiazol-2-yl)ethyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (**6**) in 74% yield. The ^{1}H-NMR spectrum of **6** showed the CH_2 signals at δ 4.57 and 4.87 ppm in addition to the signals of the aromatic protons at δ 6.75-7.90 ppm, and the NH and SH signals at δ 9.25 and 14.02 ppm respectively (Scheme 1).

Methylation of **2** with methyl iodide in EtOH-H_2O mixture in presence of KOH at room temperature afforded the corresponding *S*-methyl-1,3,4-thiadiazole derivative **7** in 79% yield. Treatment of **7** with hydrazine hydrate in ethanol at reflux temperature afforded *N*-(5-hydrazinyl-1,3,4-thiadiazol-2-yl)benzamide (**8**) in 84% yield. The ^{1}H-NMR spectrum of **7** showed the singlet peak at δ 2.54 ppm corresponding to the *S*-CH_3 group which disappeared in the ^{1}H-NMR spectrum of **8** and instead signals appeared at δ 5.76 and 10.12 ppm for the NH_2 and NH, respectively. Reaction of the hydrazine derivative **8** with CS_2 in the presence of KOH in ethanol at reflux temperature gave the 1,2,4-triazolo[1,3,4-thiadiazole] derivative **9** in 72% yield. The ^{1}H-NMR spectrum of compound **9** revealed the presence of the signals of the aromatic protons at δ 6.75-7.90 ppm in addition to the NH and SH signals at δ 9.56 and 14.10 ppm. In addition the



Scheme 1. Synthesis of compounds **2**-**6**



Scheme 2. Synthesis of 2,5-disubstituted 1,3,4-thiadiazole derivatives.

mass spectrum showed the signal of the molecular ion peak which is in agreement with the molecular formula.

When the hydrazino **8** was allowed to react with D-galactose, D-mannose and D-ribose in an aqueous ethanolic solution and a catalytic amount of acetic acid, the corresponding sugar hydrazono-1,3,4-thiadiazole derivatives (**10-12**) were obtained in 75-80% yields. The structures of these compounds were confirmed by IR, ^{1}H-NMR and mass spectra. The IR spectra of **10-12** showed the presence of characteristic absorption bands corresponding to the hydroxyl groups in the region 3385-3468 cm^{-1}. The ^{1}H-NMR spectra showed the signals of the sugar chain protons at δ 3.47-4.58 ppm and the C-1 methine proton as doublet in the range δ 7.20-7.22 ppm. Acetylating of **10-12** with acetic anhydride in pyridine at room temperature afforded the corresponding per-*O*-acetylated derivatives **13-15** in 80-82% yields. The IR spectra of **13-15** showed characteristic absorption bands at 1670-1674 cm^{-1} and 1740-1744 cm^{-1} corresponding to the carbonyl amide and the carbonyl ester groups, respectively. The ^{1}H-NMR spectra showed the signals of the *O*-acetyl-methyl protons as singlets in the range δ 1.91-2.10 ppm. The reaction of sugar arylhydrazones with boiling acetic anhydride is well known to give either the corresponding per-*O,N*-acetyl derivatives or the respective per-*O,N*-acetyl-1,3,4-oxadiazolin derivatives.[27-30] However, reaction of the sugar hydrazones **10-12** with acetic anhydride at 100 °C gave the sugar-substituted 1,3,4-thiadiazol derivatives **16-18** in 75-79% yields. The ^{1}H-NMR spectra of **16-18** showed signals of the *O*- and *N*-acetyl-methyl protons as singlets in the range δ 1.95-2.29 ppm, while the rest of the sugar chain protons appeared in the range δ 4.00-5.33 ppm (Scheme 2).

Table 1. Minimum inhibitory concentrations (MIC in μg/mL) of the title compounds.

Compound	Gram-positive	Gram-negative	Actinomycetes
	Bacillus subtilis	*Pseudomonas aeruginosa*	*Streptomyces species*
2	125	250	125
3	250	500	250
4	100	125	125
5	125	100	125
6	75	125	75
7	500	250	500
8	100	250	125
9	75	75	500
10	125	100	125
11	250	125	125
12	100	100	125
13	250	125	250
14	500	250	250
15	125	250	125
16	150	250	100
17	250	250	500
18	125	250	125
Penicillin	31	46	33

The negative control DMSO showed no activity

Antimicrobial Activity. The antimicrobial activity of the synthesized compounds was evaluated against three microorganisms; *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative) and *Streptomyces* species (Actinomycetes). The values of minimal inhibitory concentrations (MICs) of the tested compounds are presented in Table 1. The results of the antimicrobial activity test revealed that **6** and **9** showed the highest activity against *Bacillus subtilis* with MIC values of 75 μg/mL followed by compounds **4**, **12**, and **8**. The results revealed that **9** showed the highest inhibition activity against *Pseudomonas aeruginosa*, whereas **6** were the most active among the series of tested compounds against *Streptomyces* species with MIC values of 75 μg/mL. The results also revealed that some compounds showed little activity against the microorganisms (Table 1).

Conclusions

From the results of antimicrobial activity evaluation and Structure-activity relationship it could be concluded that that the derivative containing two 1,3-4-thiadiazole rings **6** and the condensed 1,2,4-triazolo[1,3,4]thiadiazole **9** revealed high antimicrobial activity against *Bacillus subtilis*. Furthermore, the attachment ribose sugar moiety increases the activity than the presence of either the galactose or mannose derivatives. In addition, the free hydroxyl sugar moieties containing derivatives displayed higher activity against the three microorganisms than the corresponding per-*O*-acetylated analogous.

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