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Der Pharma Chemica, 2012, 4 (3):1064-1073

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ISSN 0975-413X
CODEN (USA): PCHHAX

Antimicrobial Activity of Newly Synthesized Thiadiazoles, 5-benzyl-2H-tetrazole and Their Nucleosides

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ABSTRACT

A number of new substituted tetrazole and their hydrazone 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) and antifungal activity against four fungal strains namely, *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium marneffeii* and *Trichophyton mentagrophytes*. The synthesized compounds displayed different degrees of antimicrobial activity.

Key Words: Tetrazoles, Thiadiazoles, Sugar hydrazones, Antimicrobial activity, Antifungal 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. Tetrazole 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, coordination chemistry, explosives, and pharmaceutical fields [1,2]. 5-Substituted 1,2,3,4-tetrazoles are reported to possess antibacterial [3,4], antifungal [5], antiviral [6,7], Analgesic [8,9], anti-inflammatory [10,11], antiulcer [12,13], anticonvulsant [14], anticancer [15], antidiabetic [16], hypoglycemic [17], antiproliferative [18], and antihypertensive [19] activities. The tetrazole function is metabolically stable [20] this feature and a close similarity between the acidic character of the tetrazole group and carboxylic acid group [21] have inspired medicinal chemists to synthesize substituted tetrazoles as potential medicinal agents. Also 1,3,4-Thiadiazole ring containing compounds have 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 [22], antimicrobial [23], anticonvulsant [24], and antihypertensive [25]. Furthermore, 1,3,4-thiadiazoles exhibit broad spectrum of biological activities, possibly due to the presence of toxophoric N_2C_2S moiety [26]. They find applications as antibacterials, antitumor agents, pesticides, herbicides, dyes, lubricants, and analytical reagents [27]. Several megalol 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 [28]. Several 2,5-disubstituted-1,3,4-thiadiazole derivatives [29], 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 [30].

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 [31,32], the present work reports the synthesis and antimicrobial activity of new substituted 1,3,4-thiadiazole derivatives and 5-phenyl-tetrazole sugar hydrazone.

MATERIALS AND METHODS

Synthetic methods, analytical and spectral data

Melting points were determined using a Büchi apparatus. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker, Bremen, Germany). ¹H-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 F245. 5-benzyl-2H-tetrazole (**2**) [33] was prepared according to the reported procedure.

Chemistry

General procedure for the synthesis of compounds 3 and 4

To a well stirred solution of the tetrazol derivative **2** [33] (1.60 g, 10 mmol) and potassium hydroxide (0.56 g, 10 mmol) in absolute ethanol (15 ml) was added to 3-chloropropane-1,2-diol or 2-(2-chloroethoxy)ethanol (10 mmol). The solution was heated under reflux with stirring for 5 h. The solvent was removed under reduced pressure and ether (20ml) was added to afford a precipitate which was crystallized from ethanol to give **3** or **4** respectively.

3-(5-benzyl-2H-tetrazol-2-yl)propane-1,2-diol (3)

Yield 82%, mp 115-116 °C; IR (KBr, cm^{-1}): 3430 (OH), 1658 (C=N). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.61 (m, 1H, CH), 3.88-4.30 (m, 4H, 2CH₂), 4.11 (s, 2H, CH₂), 4.52 (m, 2H, 2OH), 7.22-7.35 (m, 5H, Ar-H) ppm; EI-MS: *m/z* 234 [M⁺]; Anal. Calcd. For C₁₁H₁₄N₄O₂; C, 56.40; H, 6.02; N, 23.92. Found: C, 56.42; H, 6.12; N, 24.11.

2-[2-(5-benzyl-2-H-tetrazol-2-yl)ethoxy]ethanol (4)

Yield 85%, mp 117-118 °C; IR (KBr, cm^{-1}): 3450 (OH), 1660 (C=N). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.51-3.92 (m, 4H, 2CH₂), 4.12 (s, 2H, CH₂), 4.96 (1H, m, OH), 5.27 (t, 2H, *J* = 6.2 Hz, CH₂), 5.57 (t, 2H, *J* = 6.2 Hz, CH₂), 7.20-7.34 (m, 5H, Ar-H). EI-MS: *m/z* 248 [M⁺]; Anal. Calcd. For C₁₂H₁₆N₄O₂; C, 58.05; H, 6.50; N, 22.57. Found: C, 58.15; H, 6.55; N, 22.61.

Ethyl-2-(5-benzyl-2H-tetrazol-2-yl)acetate (5)

Compound **2** (1.60 g, 0.01 mol) was dissolved in a solution of sodium (0.01g. atom) in 350 ml. of absolute ethanol. The solution was stirred at reflux and the ethyl bromoacetate (1.6 ml, 0.01 mol) was added in portions. The refluxing was continued for 15 h. The reaction mixture was then filtered hot and concentrated under reduced pressure. The crude product was filtered and crystallized from aqueous ethanol. Yield 86%, mp 63-64 °C; IR (KBr, cm^{-1}): 1716 (CO), 1241 (N=N=N-), 1160 (tetrazole ring) cm^{-1} . ¹H-NMR (300 MHz, DMSO-*d*₆): 1.21 (t, 3H, *J* = 7.2 Hz, CH₃), δ 4.32- 5.95 (s, 4H, 2CH₂), 4.20 (q, 2H, *J* = 7.2 Hz, CH₂) and 7.22-7.35 (m, 5H, Ar-H) ppm; EI-MS: *m/z* 246 [M⁺]; Anal. Calcd. For C₁₂H₁₄N₄O₂; C, 58.52; H, 5.50; N, 22.75. Found: C, 58.08; H, 4.53; N, 22.79.

2-(5-benzyl-2H-tetrazol-2-yl)acetohydrazide (6)

Ethyl acetate **5** (2.46 g, 0.01 mol) and 99% hydrazine hydrate (0.015 mol), in 100 ml. of ethanol, was reflux for 8 h. The reaction mixture was concentrated and cooled to crystallize out hydrazide. The hydrazide was filtered and recrystallized from ethanol. Yield 90%, mp 110-111 °C; IR (KBr, cm^{-1}): 3316 (NH), 3205 (NH₂), 1620 (CON), 1284 (N=N=N-), 1143 (tetrazole ring) cm^{-1} . ¹H-NMR (300 MHz, DMSO-*d*₆): δ 4.45 (brs, 2H, NH₂), 4.33-5.83 (s, 4H, 2CH₂), 7.22-7.35 (m, 5H, Ar-H), 9.65 (brs, 1H, NH), ppm; EI-MS: *m/z* 250 [M⁺]; Anal. Calcd. For C₁₀H₁₂N₆O: C, 48.01; H, 5.21; N, 36.18. Found: C, 47.58; H, 5.70; N, 36.20.

Sugar-2-(5-benzyl-2H-tetrazol-2-yl)acetohydrazide 7-9

General procedure: To a well stirred solution of the monosaccharide **7-9** (1.8 g, 10 mmol) in water (2ml), and glacial acetic acid (0.3 ml) was added 2-(5-benzyl-2H-tetrazol-2-yl)acetohydrazide (**6**), (2.50 g, 10 mmol) in ethanol (15 ml

). The mixture was heated under reflux for 3h 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.

D-Gulucose-2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (7)

Yield 85%, mp 130-131 °C; IR (KBr, cm^{-1}): 3450-3380 (OH), 3150 (NH), 1620 (C=N), 1138 (tetrazole ring); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 3.32 (m, 2H, H-6', H-6''), 3.36 (m, 1H, H-5'), 4.21 (m, 1H, H-4'), 4.35 (m, 1H, H-3'), 4.41 (t, 1H, $J = 5.8$ Hz, H-2'), 4.61 (s, 2H CH_2), 4.90 (m, 1H, OH), 4.96 (d, 1H, $J = 6.3$ Hz, OH), 5.21 (m, 1H, OH), 5.35 (t, 1H, $J = 4.5$ Hz, OH), 5.44 (t, 1H, $J = 4.5$ Hz, OH), 5.76 (s, 2H, CH_2), 7.22-7.35 (m, 5H, Ar-H), 8.96 (d, 1H, $J = 7.8$ Hz, H-1'), 9.61 (s, 1H, NH) ppm; El-MS: m/z 394 [M^+]; Anal. Calcd. For $\text{C}_{17}\text{H}_{22}\text{N}_6\text{O}_6$: C, 51.77; H, 5.60; N, 21.31. Found: C, 51.77; H, 5.51; N, 21.32

D-Galactose-2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (8)

Yield 87%, mp 132-133 °C; IR(KBr, cm^{-1}): 3440-3370 (OH), 3300(NH), 1577(C=N), 1140 (tetrazol ring); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 3.31 (m, 2H, H-6', H-6''), 3.35 (m, 1H, H-5'), 4.22 (m, 1H, H-4'), 4.34 (m, 1H, H-3'), 4.42 (t, 1H, $J = 5.8$ Hz, H-2'), 4.60 (s, 2H CH_2), 4.91 (m, 1H, OH), 4.95 (d, 1H, $J = 6.3$ Hz, OH), 5.20 (m, 1H, OH), 5.34 (t, 1H, $J = 4.5$ Hz, OH), 5.45 (t, 1H, $J = 4.5$ Hz, OH), 5.76 (s, 2H, CH_2), 7.21-7.34 (m, 5H, Ar-H), 8.96 (d, 1H, $J = 7.8$ Hz, H-1'), 9.61 (s, 1H, NH) ppm; El-MS: m/z 394 [M^+]; Anal. Calcd. For $\text{C}_{17}\text{H}_{22}\text{N}_6\text{O}_6$: C, 51.77; H, 5.60; N, 21.31. Found: C, 51.78; H, 5.52; N, 21.30.

D-Mannose 2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (9)

Yield 80%, mp 135-136 °C; IR(KBr, cm^{-1}): 3423-3380 (OH), 3220 (NH), 1138 (tetrazol ring), 1570 (C=N). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 3.30 (m, 2H, H-6', H-6''), 3.34 (m, 1H, H-5'), 4.20 (m, 1H, H-4'), 4.34 (m, 1H, H-3'), 4.40 (t, 1H, $J = 5.8$ Hz, H-2'), 4.60 (s, 2H CH_2), 4.92 (m, 1H, OH), 4.95 (d, 1H, $J = 6.3$ Hz, OH), 5.20 (m, 1H, OH), 5.36 (t, 1H, $J = 4.5$ Hz, OH), 5.45 (t, 1H, $J = 4.5$ Hz, OH), 5.77 (s, 2H, CH_2), 7.22-7.35 (m, 5H, Ar-H), 8.95 (d, 1H, $J = 7.8$ Hz, H-1'), 9.62 (s, 1H, NH) ppm; El-MS: m/z 394 [M^+]; Anal. Calcd. For $\text{C}_{17}\text{H}_{22}\text{N}_6\text{O}_6$: C, 51.77; H, 5.60; N, 21.31. Found: C, 51.69; H, 5.58; N, 21.29.

O-Acetylsugar-2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (10-12)

General procedure: To a solution of sugar hydrazone **7-9** (3.94 g, 10 mmol) in pyridine (7ml) was added acetic anhydride (1 g, 10 mmol) was stirred at room temperature for 8 h. The resulting solution was poured on to 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 recrystallized from ethanol.

2,3,4,5,6-Penta-O-acetyl-D-gulcopentitolyl-2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (10)

Yield 75%, mp 109-110 °C; IR (KBr, cm^{-1}): 3310 (NH), 1720 (C=O), 1680 (CON), 1615 (C=N), 1138 (tetrazol ring); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 1.97-2.44 (s, 15H, 5 CH_3), 3.95 (dd, 1H, $J = 11.2, 2.8$ Hz, H-6'), 4.11 (dd, 1H, $J = 11.2, 3.2$ Hz, H-6''), 4.34 (m, 1H, H-5'), 4.36 (t, 1H, $J = 7.5$ Hz, H-4'), 4.61 (s, 2H, CH_2), 5.15 (dd, 1H, $J = 3.4, 7.5$ Hz, H-3'), 5.26 (t, 1H, $J = 7.8$ Hz, H-2'), 5.76 (s, 2H, CH_2), 8.95 (d, 1H, $J = 7.8$ Hz, H-1'), 7.22-7.34 (m, 5H, Ar-H), 9.59 (brs, 1H, NH) ppm; El-MS: m/z 604 [M^+]; Anal. Calcd. For $\text{C}_{26}\text{H}_{32}\text{N}_6\text{O}_{11}$: C, 51.65; H, 5.34; N, 13.90. Found: C, 51.75; H, 5.23; N, 13.96.

2,3,4,5,6-Penta-O-acetyl-D-galactopentitolyl-2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (11). Yield 69%, mp

112-113 °C; IR (KBr, cm^{-1}): 3315 (NH), 1720 (C=O), 1712 (C=N), 1667 (CON), 1140 (tetrazol ring) $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 1.95-2.45 (s, 15H, 5 CH_3), 3.96 (dd, 1H, $J = 11.2, 2.8$ Hz, H-6'), 4.13 (dd, 1H, $J = 11.2, 3.2$ Hz, H-6''), 4.33 (m, 1H, H-5'), 4.35 (t, 1H, $J = 7.5$ Hz, H-4'), 4.62 (s, 2H, CH_2), 5.17 (dd, 1H, $J = 3.4, 7.5$ Hz, H-3'), 5.27 (t, 1H, $J = 7.8$ Hz, H-2'), 5.77 (s, 2H, CH_2), 8.94 (d, 1H, $J = 7.8$ Hz, H-1'), 7.22-7.34 (m, 5H, Ar-H), 9.61 (brs, 1H, NH) ppm; El-MS: m/z 604 [M^+]; Anal. Calcd. For $\text{C}_{26}\text{H}_{32}\text{N}_6\text{O}_{11}$: C, 51.65; H, 5.34; N, 13.90. Found: C, 51.67; H, 5.38; N, 13.93.

2,3,4,5,6-Penta-O-acetyl-D-mannopentitolyl-2-(5-benzyl-2H-tetrazole-2-yl)acetylhydrazone (12).

Yield 65%, mp 114-115 °C; IR (KBr, cm^{-1}): 3331 (NH), 1715 (C=O), 1660 (CON), 1710 (C=N), 1138 (tetrazol ring). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 1.95-2.46 (s, 15H, 5 CH_3), 3.96 (dd, 1H, $J = 11.2, 2.8$ Hz, H-6'), 4.09 (dd, 1H, $J = 11.2, 3.2$ Hz, H-6''), 4.31 (m, 1H, H-5'), 4.34 (t, 1H, $J = 7.5$ Hz, H-4'), 4.60 (s, 2H, CH_2), 5.18 (dd, 1H, $J = 3.4, 7.5$ Hz, H-3'), 5.27 (t, 1H, $J = 7.8$ Hz, H-2'), 5.76 (s, 2H, CH_2), 8.97 (d, 1H, $J = 7.8$ Hz, H-1'), 7.22-7.35 (m, 5H, Ar-H), 9.60 (brs, 1H, NH) ppm; El-MS: m/z 604 [M^+]; Anal. Calcd. For $\text{C}_{26}\text{H}_{32}\text{N}_6\text{O}_{11}$: C, 51.65; H, 5.34; N, 13.90. Found: C, 51.68; H, 5.35; N, 13.89.

2-phenylacetimidohydrazide (13).

A solution of the nitrile derivative **1** (1.47 g, 10 mmol), hydrazine hydrate (10 mmol) in ethanol was heated under reflux for 7 h. The solvent was removed under reduced pressure. The remaining precipitate was collected, dried, and recrystallized from ethanol to afford the amidrazone **13**. Yield 77%, mp 66-67 °C; IR (KBr, cm⁻¹): 3415 (NH₂), 3120 (NH), 1630 (C=N). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 4.64 (s, 2H, CH₂), 7.62 (bs, 2H, NH₂), 9.23-9.42 (bs, 2H, 2NH), 7.22-7.35 (m, 5H, Ar-H). EI-MS: *m/z* 149 [M⁺]; Anal. Calcd. For C₈H₁₁N₅; C, 64.40; H, 7.43; N, 28.16. Found: C, 64.52; H, 7.51; N, 28.25.

5-(benzyl-3-yl)-1,3,4-thiadiazol-2-thiol (14).

To a solution of compound **13** (1.49 g, 10mmol) in methanol (15ml) was added carbon disulfide (15mmol). The solution was heated under reflux with stirring for 10 h. The solvent was concentrated under reduced pressure and left overnight at 5 °C. The resulting precipitate was filtered off, washed with cold ethanol and crystallized from ethanol to afford the thiadiazole derivative **14**. Yield 79%, mp 284-285 °C; IR (KBr, cm⁻¹): 3440 (SH), 1612 (C=N). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.85 (s, 2H, CH₂), 7.22-7.35 (m, 5H, Ar-H), 14.91 (s, 1H, SH). EI-MS: *m/z* 208 [M⁺]; Anal. Calcd. For C₉H₈N₂S₂; C, 51.89; H, 3.87; N, 13.45. Found: C, 52.00; H, 3.99; N, 13.50.

General procedure for the synthesis of compounds 15 and 16.

To a well stirred solution of the thiadiazole derivative **14** (2.08 g, 10 mmol) and potassium hydroxide (0.56 g, 10 mmol) in absolute ethanol (15 ml) was added to 3-chloropropane-1,2-diol or 2-(2-chloroethoxy)ethanol (10 mmol). The solution was heated under reflux with stirring for 11 h. The solvent was removed under reduced pressure and ether (20ml) was added to afford a precipitate which was recrystallized from ethanol to give **15** or **16** respectively.

3-[5-(benzyl-3-yl)-1,3,4-thiadiazol-2-ylthio]propane-1,2-diol (15).

Yield 83%, mp 294-295 °C; IR (KBr, cm⁻¹): 3420 (OH), 1630 (C=N). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.61 (m, 1H, CH), 3.88-4.30 (m, 4H, 2CH₂), 4.11 (s, 2H, CH₂), 4.52-4.72 (m, 2H, 2OH), 7.22-7.35 (m, 5H, Ar-H) ppm; EI-MS: *m/z* 282 [M⁺]; Anal. Calcd. For C₁₂H₁₄N₂S₂O₂; C, 51.04; H, 5.00; N, 9.92. Found: C, 51.15; H, 5.11; N, 10.01.

2-{2-[5-(benzyl-3-yl)-1,3,4-thiadiazol-2-ylthio]ethoxy}ethanol (16).

Yield 71%, mp 287-288 °C; IR (KBr, cm⁻¹): 3442 (OH), 1620 (C=N). ¹H-NMR (300 MHz, DMSO-*d*₆): δ 3.92-3.51 (m, 4H, 2CH₂), 4.12 (s, 2H, CH₂), 4.96 (1H, m, OH), 5.27 (t, 2H, J = 6.2 Hz, CH₂), 5.57 (t, 2H, J = 6.2 Hz, CH₂), 7.22-7.35 (m, 5H, Ar-H) ppm; EI-MS: *m/z* 296 [M⁺]; Anal. Calcd. For C₁₃H₁₆N₂S₂O₂; C, 52.68; H, 5.44; N, 9.45. Found: C, 52.70; H, 5.41; N, 9.54

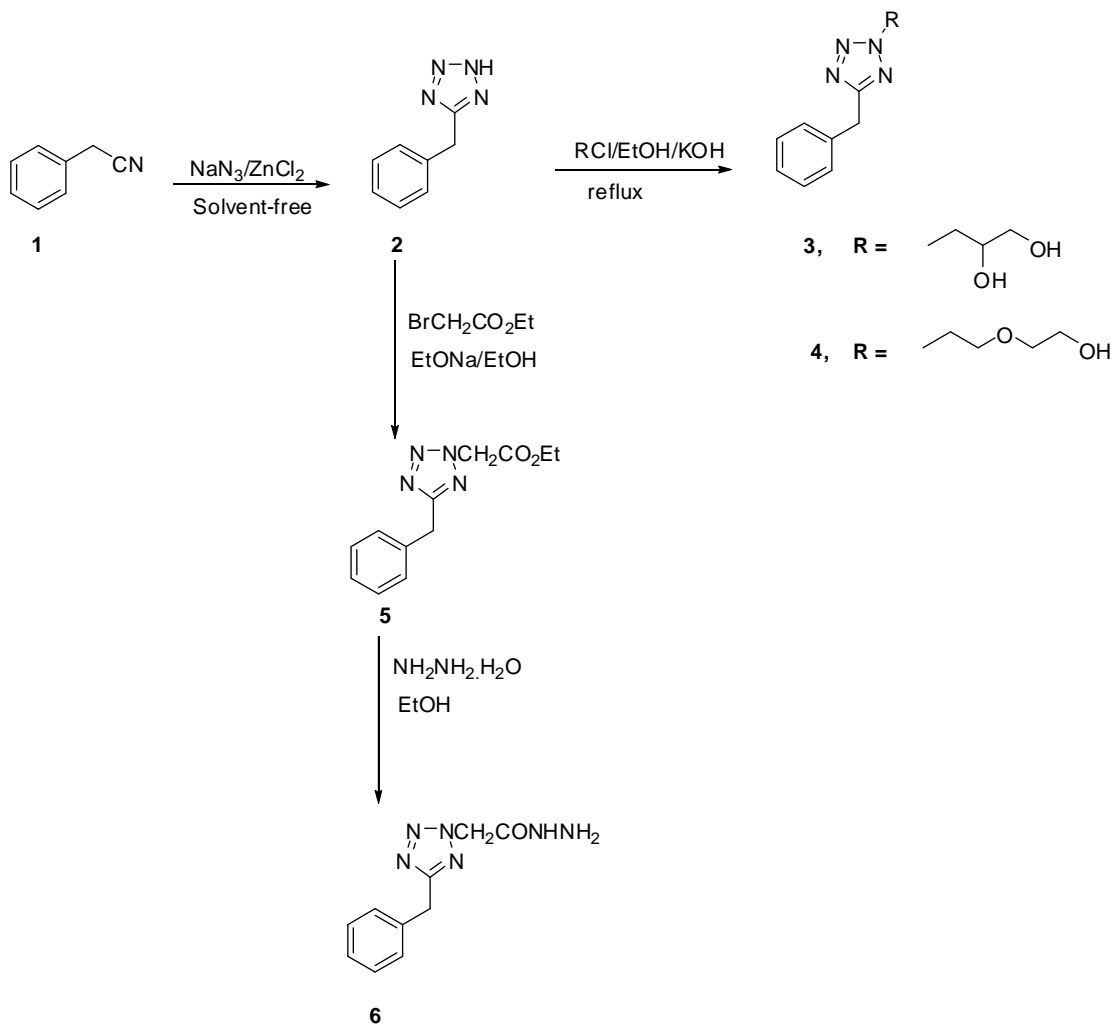
Pharmacological evaluation**Antibacterial activity**

The synthesized compounds were tested for their antimicrobial activity against three microorganisms, and the minimal inhibitory concentrations (MICs) of the tested compounds were determined by the dilution method.

Bacterial strains were supplied, 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.) for 24 h at 37°C. 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. The layer was allowed to cool and harden. With the aid of cork-borer, cups of about 10 mm diameter were produced [34].

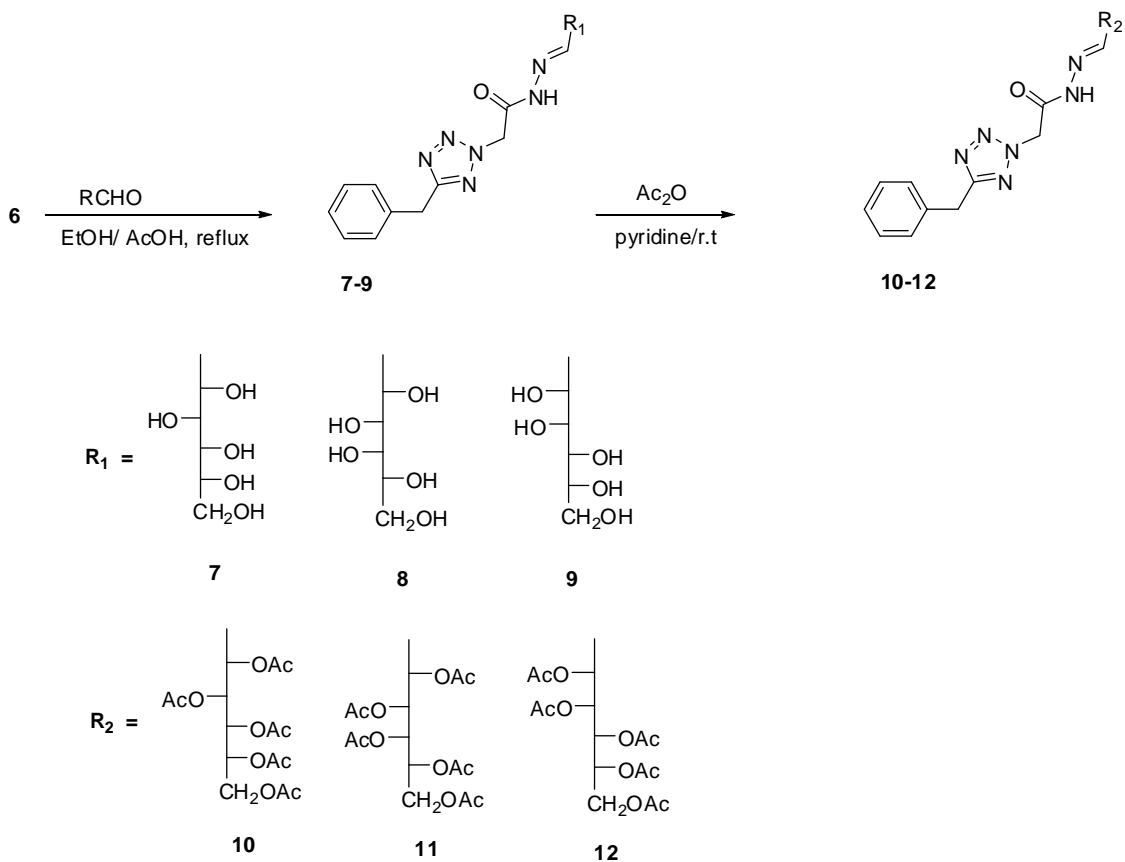
Agar diffusion technique

Antibacterial activities 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 incorporated in 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°C overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the graph of logarithm concentrations versus diameter of the inhibition zones [35].

**Scheme 1.** Synthetic route of compounds 2-6.**Table 1.** Minimum inhibitory concentrations (MIC- $\mu\text{g/mL}$) of the title compounds Negative control DMSO, no activity

Compound	Gram positive <i>Bacillus subtilis</i>	Gram negative <i>Pseudomonas aseuginosa</i>	Actinomycetes <i>Streptomyces Species</i>
2	125	75	100
3	75	100	75
4	75	100	75
5	100	100	125
6	125	125	100
7	75	100	75
8	75	100	75
9	75	100	75
10	100	100	75
11	100	100	75
12	100	100	75
13	125	100	250
14	125	250	125
15	100	100	75
16	100	100	75
Penicillin	31	45	34

^aTotally inactive (MIC > 500 $\mu\text{g/mL}$)

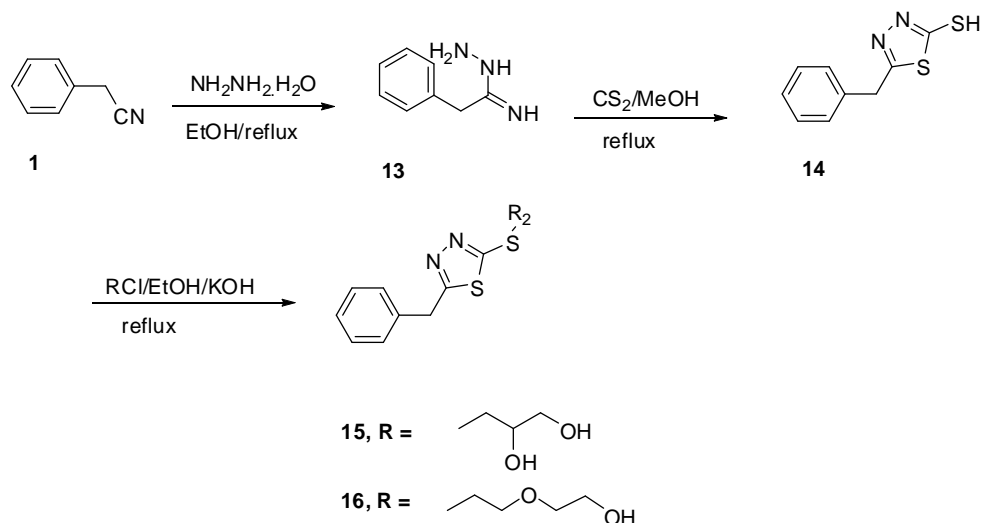


Scheme 2. Synthetic route of compounds 7-12.

Table 2. Antifungal activities of the synthesized compounds (zone of inhibition in mm, #MIC in mg/mL given in parenthesis).

Compound	T. mentagrophytes	P. marneffei	A. flavus	A. fumigatus
2	< 10 (50)	< 10 (50)	< 10 (50)	< 10 (50)
3	16 (12.5)	16 (12.5)	13 (12.5)	12 (12.5)
4	17 (12.5)	17 (12.5)	15 (12.5)	13 (12.5)
5	< 10 (50)	< 10 (50)	< 10 (50)	< 10 (50)
6	< 10 (100)	< 10 (100)	< 10 (100)	< 10 (100)
7	23 (6.25)	22 (6.25)	20 (6.25)	22 (6.25)
8	22 (6.25)	23 (6.25)	21 (6.25)	20 (6.25)
9	22 (6.25)	23 (6.25)	20 (6.25)	21 (6.25)
10	< 10 (50)	< 10 (50)	< 10 (50)	< 10 (50)
11	< 10 (50)	< 10 (50)	< 10 (50)	< 10 (50)
12	< 10 (50)	< 10 (50)	< 10 (50)	< 10 (50)
13	< 10 (100)	< 10 (100)	< 10 (100)	< 10 (100)
14	< 10 (50)	< 10 (50)	< 10 (50)	< 10 (50)
15	22 (6.25)	21 (6.25)	20 (6.25)	20 (6.25)
16	21 (6.25)	23 (6.25)	21 (6.25)	22 (6.25)
Itraconazole	23 (6.25)	24 (6.25)	21 (6.25)	22 (6.25)

MIC: Minimum inhibitory concentration



Scheme 3. Synthetic route of compounds **13-16**.

Antifungal activity

We have also investigated newly synthesized compounds for their antifungal activity against four fungal strains namely, *Aspergillus flavus* (NCIM No.524), *Aspergillus fumigates* (NCIM No. 902), *Penicillium marneffeii* (recultured) and *Trichophyton mentagrophytes* (recultured). Sabouraud agar media was prepared by dissolving pepton (1.0 g), D-Glucose (4.0 g) and agar (2.0 g) in sterile water (100 mL) and the pH was adjusted to 5.7. Normal saline was used to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL of saline to get suspension of corresponding species. Agar media of 20 mL was poured in to each petri dish. Excess of suspension was decanted and the plates were dried by placing them in an incubator at 37 °C for 1 h. Using an agar, punch wells were made on these seeded agar plates, from 6.25 µg/mL to 100 µg/mL of the test compounds in DMSO was added in to each labeled disc. Controls were run using DMSO at the same concentration as used with the test compounds. The petri dishes were prepared in triplicate and maintained at 37 °C for 3 to 4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone [36,37]. The results of these studies was given in table 2 and compared with the standard Itraconazole drug.

RESULTS AND DISCUSSION

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes **1-3**. 5-benzyl-2*H*-tetrazole (**2**) [33], react with 3-chloropropane-1,2-diol or 2-(2-chloroethoxy)ethanol using potassium hydroxides in refluxing ethanol to give **3, 4** in yield 82-85% respectively. Alkylation of the compound **2** using ethyl bromoacetate in refluxing ethanol containing sodium ethoxide afforded Ethyl-2-(5-benzyl-2*H*-tetrazol-2-yl)acetate (**5**) in good yield. Compound **5** was treated with hydrazine hydrate in absolute ethanol to yield 2-(5-benzyl-2*H*-tetrazol-2-yl)acetohydrazide (**6**) (Scheme 1).

The structures suggested for all compounds 3-6 are in good agreement with their analytical and spectroscopic data. IR spectrum of compounds 3-6 showed the presence of characteristic absorption bands at 3430, 3205, 1716, 1138, cm^{-1} corresponding to OH, NH_2 , C=O, tetrazol ring respectively. $^1\text{H-NMR}$ spectra of compounds **3** and **4** showed the proton of OH at δ 4.52-4.96 ppm, compound **5** showed triplet signals at 1.21 ppm, and quartet signals at δ 4.20 ppm assignable to ethyl group in addition singlet single at δ 5.95 ppm assignable to methylene group while the spectra of compound **6** showed two D_2O exchangeable singlet signals assignable for hydrazine group at δ 9.95, 4.45 ppm, and signals of ethyl group is absent, in addition to the signals of the protons of aromatic ring in the range δ 7.22-7.35 ppm and singlet signal at δ 3.91 for CH_2 . Mass spectrum showed the signal of the molecular ion peak which is in agreement with the molecular formula.

When the hydrazide **6** was allowed to react with D-glucose, D-galactose, and D-mannose in an aqueous ethanolic solution and a catalytic amount of acetic acid, the corresponding sugar hydrazone derivatives **7-9** were obtained in 80-85% yields. The structures of these compounds were confirmed by IR, ¹H-NMR and mass spectra. The IR spectra of compounds **7-9** showed the presence of characteristic absorption bands corresponding to the hydroxyl groups in the region 3380-3420 cm⁻¹. The ¹H-NMR spectra showed the signals of the sugar chain protons at δ 3.32-3.58 ppm, and the C-1 methine proton as doublet in the range δ 7.82-7.96 ppm.

Acetylation of **7-9** with acetic anhydride in pyridine at room temperature afforded the corresponding per-*O*-acetylated derivatives **10-12** in 65-75 % yields (Scheme 2). The IR spectra of compounds **10-12** showed characteristic absorption bands at 1660-1680 cm⁻¹ and 1715-1720 cm⁻¹ corresponding to the carbonyl amide and the carbonyl ester groups, respectively. The ¹H-NMR spectra showed the signals of the *O*-acetyl-methyl protons as singlets in the range δ 2.44-2.49 ppm. In additional mass spectrum showed the signal of the molecular ion peak which is in agreement with the molecular formula.

2-phenylacetimidohydrazide (**13**) was prepared in 77% yield from reaction between 2-phenylacetonitrile (**1**) and hydrazine hydrate in refluxing ethanol. Compound **13** was reacted with carbon disulfide in methanol at reflux temperature to afford the 5-benzyl-1,3,4-thiadiazol-2-thiol (**14**) in 79% yield. Compound **14** was allowed to react with 3-chloropropane-1,2-diol or 2-(2-chloroethoxy)ethanol in the presence of potassium hydroxide in ethanol at reflux temperature, the corresponding *S*-substituted acyclic nucleoside analogues **15** and **16** were obtained respectively in 83% and 71 % yield (Scheme 3).

The structure of compounds **13-16** was investigated qualitatively by elemental and spectral analysis (IR, ¹H-NMR, and MS). IR spectrum of compound **13** revealed the disappearance of the CN group and instead characteristic absorption bands for the NH₂ and NH groups appeared at 3415-3120 cm⁻¹. Also, ¹H-NMR spectrum of compound **13** showed the NH₂ group as singlet at δ 7.62 ppm, and the NH group signals at δ 9.23-9.42 ppm. The mass spectrum showed the signal of the molecular ion peak at 149 for the molecular formula of **13**. The IR spectrum of compound **14** showed characteristic absorption bands for the SH and C=N groups appeared at 3440, 1660 cm⁻¹. ¹H-NMR spectrum showed the SH group as singlet at δ 14.91 ppm. The IR spectra for compounds **15, 16** showed characteristic absorption bands corresponding to the OH and at 3420-3442. ¹H-NMR spectrum of **15** and **16** revealed the presence of the hydroxyl group at δ 4.72- 4.96 ppm respectively, in addition to the signals protons of aromatic ring in the range δ 7.22-7.35 ppm, and δ 4.11 for CH₂ group. The mass spectrum for compounds **11-16** showed the signal of the molecular ion peak which is in agreement with the molecular.

Antimicrobial Activity

The synthesized compounds were evaluated for their antimicrobial activity 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 I. The MIC values of the most active compounds were in accordance with the results obtained in the primary screening.

The result revealed that compounds showed varying degrees of inhibition against the tested microorganisms. In general, compound **2** displayed the highest inhibition activity against *Pseudomonas aeruginosa* with MIC value of 75 µg/mL. Compounds **3, 4, 7, 8,** and **9** displayed the highest activity with MIC value 75 µg/mL at least against two of the microorganism under test. Compounds **10, 11, 12, 15,** and **16** displayed the highest inhibition activity against *Streptomyces Species* also with MIC value of 75 µg/mL. Some of the other compounds revealed moderate activity while others revealed little.

Antifungal activity

Compounds **2, 5, 11, 12** and **13** were less active against all the four fungal strains namely, *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium marneffeii* and *Trichophyton mentagrophytes*. On the other hand compounds **7, 8, 9, 15** and **16** showed most antifungal activity against all the four fungal strains. The compounds **6** and **13** were found to have very less active against four fungal organisms. Compounds **3** and **4** were showed good antifungal activity against four fungal organisms

Structure Activity Relationship (SAR) Studies

The antimicrobial activity results and structure activity relationship indicated that the attachment of acyclic sugar moieties to tetrazole ring system resulted in increase of antimicrobial activity. Furthermore, the hydrazones incorporating free hydroxyl sugar chains showed higher activity than the corresponding acetylated analogs. In addition, the acyclic C-nucleoside analogue attached to the tetrazole base showed high inhibition activity.

CONCLUSION

In conclusion, the antimicrobial screening suggest that all the newly synthesized compounds showed moderate to good activity against the tested organisms. Among the newly synthesized compounds **3**, **4**, **7**, **8** and **9** showed the most promising antibacterial and antifungal activity. Hence the fact that the compounds prepared in this study are chemically unrelated to the current medication, suggests that further work with similar analogues is clearly warranted.

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