Synthesis and antibacterial activity of some new 1,2,4-triazole derivatives bearing carbohydrazide moiety

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RESEARCH ARTICLE

ABSTRACT

In this study, a series of 1,2,4-triazol-3-carbohydrazide derivatives and compound of 1,2,4-triazole-3-(4*H*)-thion have been synthesized. Structures and purity of the new compounds were confirmed by the use of their chromatographic and spectral data besides microanalysis. Four different bacterial stains for the study of the biological activity of compounds 6g, 7c, 7g and 7i; two Gram-positive strains, and two Gram-negative strains have been used. Compound 6g was found to be the most active of the four tested compounds against *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*, with an inhibition zone diameter of 16, 9, and 10 mm, respectively. Calculating the minimal inhibitory concentration value (MIC) for the positive drugs who formed an inhibition zone in the agar well diffusion method, we found that both compounds 6g and 7i were the most active of the four tested compounds against *Pseudomonas aeruginosa* and *Bacillus cereus* with an MIC value of 0.5 μg/mL for both bacteria. These results suggest that these two compounds could be considered as potential antibacterial agents against a range of bacteria.

1. Introduction

Antibiotic resistance is becoming a serious threat to public health and there is an urgent need for new and improved antimicrobial agents [1-5]. The occurrence of 1,2,4-triazole system in numerous biologically active molecules has been recognized to possess activities, such as analgesic [6], antibacterial [7-9], antifungal [10,11], anti-inflammatory [12], antitumor [13,14], antitrypanosomal [15], antiproliferative [16] and antibiotics properties [17].

Literature survey reveals that important chemotherapeutics, such as ribavirin (antiviral) [18,19], fluconazole (antifungal) [20,21], alprazolam (tranquilizer) [22], trapidil (hypotensive) [19], vorozole and letrozole (nonsteroidal competitive aromatase inhibitor) [19,21,23,24] (Figure 1), that consist of substituted 1,2,4-triazole ring.

In previous work, we report the synthesis of a variety of 1,5-dialkyl-1H-1,2,4-triazoles bearing different precursors such as N-alkylphthalimides [25], D-mannopentitol-1-yl-1,2,4-triazoles [26], benzotriazoles [27], 3'-triazolo-thymidines [28],

1,4-disubstituted piperazines [29]. Additionally, we have synthesized a series of 1,2,4-triazoles derivatives as CYP enzyme inhibitors [30].

On the basis of these pharmacological activity of 1,2,4-triazole derivatives, as well as the antimicrobial activity of some carbohydrazide compounds prompted us to synthesize a series of new 1,5-disubstituted 1,2,4-triazole derivatives bearing carbohydrazide moiety as antibacterial agents, via the cycloaddition of ethyl cyanoacetate with different reactive cumulenes.

2. Experimental

2.1. Materials and methods

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Merck, or Fluka and were used without purification. Reaction progress was monitored by thin layer chromatography (TLC) on Alugram SIL G UV254 (Macherey-Nagel).
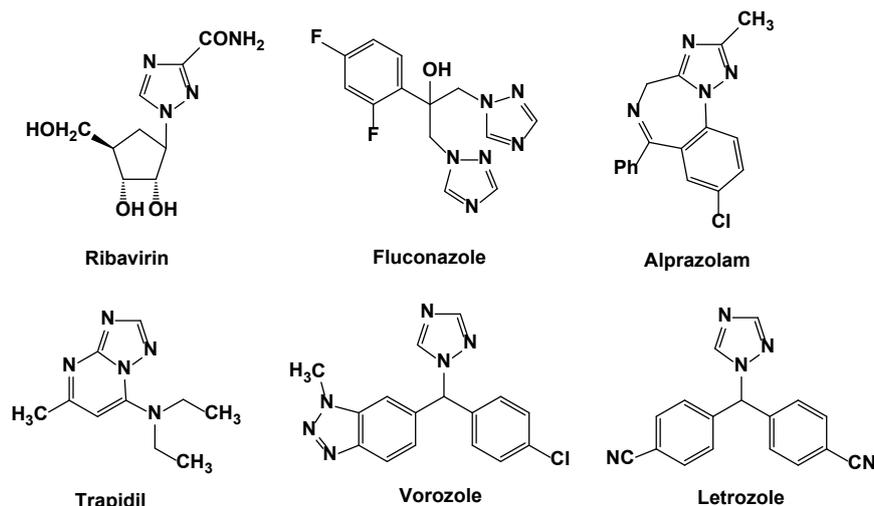
Figure 1. Drugs bering 1,2,4-triazole ring used in medicine.

Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. All new compounds were analyzed for C, H, and N using a 2400 CHN Elemental Analyzer by Perkin Elmer. The observed results agreed with the calculated percentages to within ±0.4%. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 instrument. Chemical shifts are given in parts per million (ppm), and tetramethylsilane (TMS) was used as internal standard for spectra obtained in CDCl$_3$. Mass spectra (ESI) were measured on a TSQ Quantum (Thermo Electron Corporation) instrument with a RP18 100-3 column (Macherey Nagel).

2.2. Synthesis

2.2.1 General procedure for synthesis of compounds 6 and 7

Compound **4** (0.092 g, 0.5 mmol) or compound **5** (0.099 g, 0.5 mmol), reacted with substituted sulfonyl chlorides (**0.6 mmol)** in chloroform (20 mL) in the presence of pyridine (5 drops) and stirred for 48 h at room temperature. Then residue was evaporated to dryness and the residue was partitioned between CHCl$_3$ (40 mL) and water (3×40 mL). The organic phase was evaporated to dryness (Na$_2$SO$_4$). The product was then filtered and washed with diethyl ether followed by recrystallization from CH$_2$Cl$_2$ / ether to afford compounds **6** or **7** (Scheme 1 and 2).

Benzene sulfonic acid N'[2-(1-ethyl-5-methyl-1H-[1,2,4]tria zole-3-yl)-acetyl]-hydrazide (**6a**): Color: Dark brown crystals. Yield: 63 %. M.p.: 175-177 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.26 (t, 3H, CH$_2$CH_3), 2.33 (s, 3H, -CH$_3$C=N), 3.29 (s, 2H, CH$_2$C=O), 4.02 (q, 2H, CH_2CH$_3$), 7.49 (t, 2H, Ar-H), 7.59-7.64 (t, 1H, Ar-H), 7.79 (d, 2H, Ar-H), 9.92 (bs, 1H, NH), 10.23 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 11.5 (C$_5$'), 15.1 (C$_1$''), 33.6 (C$_3$'), 43.0 (C$_1$'), 125.9, 128.0, 129.2, 133.3 (Ar), 151.2 (C-3), 155.8 (C-5), 167.1 (C=O). MS (ESI, *m/z*): 324 (M + H)$^+$. Anal. calcd. for C$_{13}$H$_{17}$N$_5$O$_3$S: C, 48.29; H, 5.30; N, 21.66. Found: C, 48.55; H, 5.44; N, 21.89%.

4-Chlorobenzene sulfonic acid N'[2-(1-ethyl-5-methyl-1H-[1,2,4]triazole-3-yl)-acetyl]-hydrazide (**6b**): Color: Milky crystals. Yield: 68 %. M.p.: 160-162 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.28 (t, 3H, CH$_2$CH_3), 2.34 (s, 3H, CH$_3$C=N), 2.54 (s, 2H, CH$_2$C=O), 4.03 (q, 2H, CH_2CH$_3$), 7.56 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 10.00 (bs, 1H, NH), 10.26 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 11.6 (C$_5$'), 15.2 (C$_1$''), 33.8 (C$_3$'), 42.8 (C$_1$'), 129.3, 130.0, 138.2, 138.4 (Ar), 152.0 (C-3), 156.3 (C-5), 167.4 (C=O). MS (ESI, *m/z*): 358, 360 (M + H)$^+$. Anal. calcd. for

C$_{13}$H$_{16}$ClN$_5$O$_3$S: C, 43.64; H, 4.51; N, 19.57. Found: C, 43.85; H, 4.72; N, 19.80(%).

3-Trifluoromethyl-benzene sulfonic acid N'[2-(1-ethyl-5-methyl-1H-[1,2,4]triazole-3-yl)-acetyl]-hydrazide (**6c**): Color: White crystals. Yield: 72 %. M.p.: 206-208 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.27 (t, 3H, CH$_2$CH_3), 2.32 (s, 3H, CH$_3$C=N), 3.28 (s, 2H, CH$_2$C=O), 4.01 (q, 2H, CH_2CH$_3$), 7.76 (t, 1H, Ar-H), 8.01-8.10 (m, 3H, Ar-H), 10.25 (bs, 1H, NH), 10.35 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 11.6 (C$_5$'), 15.2 (C$_1$''), 33.8 (C$_3$'), 42.8 (C$_1$'), 124.4 , 124.5, 130.0, 130.1, 130.8, 132.2, 140.9 (Ar), 152.1 (C-3), 156.3 (C-5), 167.5 (C=O). MS (ESI, *m/z*): 392 (M + H)$^+$. Anal. calcd. for C$_{14}$H$_{16}$F$_3$N$_5$O$_3$S: C, 42.96; H, 4.12; N, 17.89. Found: C, 42.77; H, 4.21; N, 18.09(%).

2,5-Dichlorobenzene sulfonic acid N'[2-(1-ethyl-5-methyl-1H-[1,2,4]triazole-3-yl)-acetyl]-hydrazide (**6g**) [31].

2-Thiophene sulfonic acid N'[2-(1-ethyl-5-methyl-1H-[1,2,4] triazole-3-yl)-acetyl]-hydrazide (**6i**): Color: Brown crystals. Yield: 73 %. M.p.: 211-213 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.28 (t, 3H, CH$_2$CH_3), 2.33 (s, 3H, CH$_3$C=N), 2.54 (s, 2H, CH$_2$C=O), 4.02 (q, 2H, CH_2CH$_3$), 7.09-7.95 (m, 3H, Thiophene-H), 9.99 (bs, 1H, NH), 10.24 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 11.6 (C$_5$'), 15.2 (C$_1$''), 33.8 (C$_3$'), 42.8 (C$_1$'), 128.0, 133.8, 134.3, 139.3 (Thiophene-C), 152.0 (C-3), 156.3 (C-5); 167.4 (C=O). MS (ESI, *m/z*): 330 (M + H)$^+$. Anal. calcd. for C$_{11}$H$_{15}$N$_5$O$_3$S$_2$: C, 40.11; H, 4.59; N, 21.26; Found: C, 40.30; H, 4.74; N, 21.51(%).

7-Methoxy-4-methyl-2-oxo-2H-chromene-6-sulfonic acid N'[2-(1-ethyl-5-methyl-1H-[1, 2, 4]triazole-3-yl)-acetyl]-hydrazide (**6k**): Color: White crystals. Yield: 63 %. M.p.: 222-224 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.21 (t, 3H, CH$_2$CH_3), 2.26 (s, 3H, CH$_3$C=N), 2.42 (s, 3H, CH$_3$-Coumarin), 3.27 (s, 2H, CH$_2$C=O), 3.92 (s, 3H, OCH$_3$), 4.01 (q, 2H, CH_2CH$_3$), 6.34 (s, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 9.65 (bs, 1H, NH), 10.16 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 11.5 (C$_5$'), 15.6 (C$_1$''), 18.5 (CH$_3$-Coumarin), 33.6 (C$_3$'), 42.8 (C$_1$'), 57.8 (O-CH$_3$), 101.6, 112.3, 112.5, 124.2, 127.7, 151.9, 153.5, 156.3 (Ar), 158.2 (C-3), 159.9 (C-5), 160.7, 167.6 (C=O). MS (ESI, *m/z*): 436 (M + H)$^+$. Anal. calcd. for C$_{18}$H$_{21}$N$_5$O$_6$S: C, 49.65; H, 4.86; N, 16.08. Found: C, 49.87; H, 4.99; N, 16.29 (%).

4-Chlorobenzene sulfonic acid N'[2-(1,5-diethyl-1H-[1,2,4]triazole-3-yl)-acetyl]-hydrazide (**7b**): Color: White crystals. Yield: 76 %. M.p.: 110-112 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.18 (t, 3H, C-CH$_2$CH_3), 1.26 (t, 3H, N-CH$_2$CH_3), 2.70 (q, 2H, C-CH_2CH$_3$), 3.34 (s, 2H, CH$_2$C=O), 4.03 (q, 2H, N-CH_2CH$_3$), 7.55 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 10.07 (bs, 1H, NH), 10.31 (bs, 1H, NH).
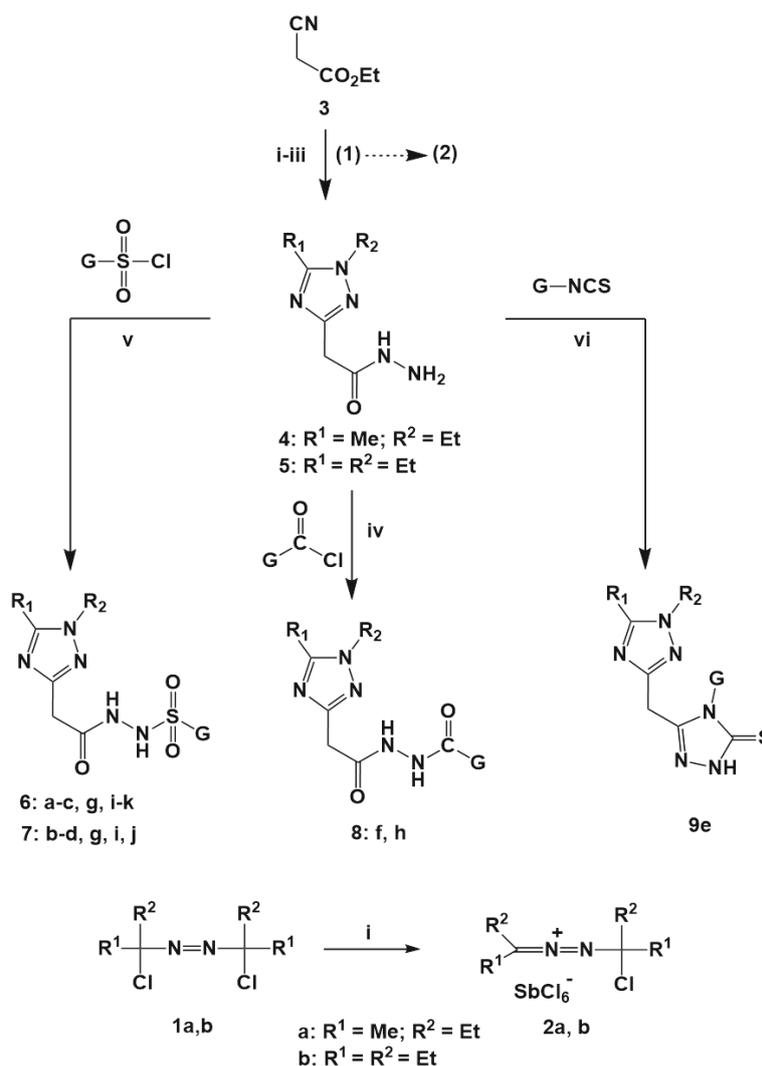
Reagents and conditions: (i) SbCl$_5$; CH$_2$Cl$_2$, -60°C to 23°C, 7 h; (ii) aq. NaHCO$_3$, NH$_3$, MeCN, 0 °C, 2 h; (iii) NH$_2$NH$_2$; (iv), (v) CHCl$_3$, pyridine; (vi) tolune, pyridine.

Scheme 1

^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 12.2 (C$_5$'''), 15.4 (C$_1$''), 18.7 (C$_5$''), 33.9 (C$_3$'), 42.6 (C$_1$'), 129.3, 130.0, 138.2, 138.4 (Ar), 156.4 (C-3), 156.5 (C-5), 167.4 (C=O). MS (ESI, *m/z*): 372, 374 (M + H)$^+$. Anal. calcd. for C$_{14}$H$_{18}$ClN$_5$O$_3$S: C, 45.22; H, 4.88; N, 18.83. Found: C, 45.41; H, 4.97; N, 19.11(%).

3-Trifluoromethyl-benzene sulfonic acid N'[2-(1,5-diethyl-1H-[1,2,4]triazole-3-yl)-acetyl] -hydrazide (**7c**) [31].

4-Nitrobenzene sulfonic acid N'[2-(1,5-diethyl-1H-[1,2,4] triazole-3-yl)-acetyl]-hydrazide (**7d**): Color: White crystals. Yield: 81 %. M.p: 145-147 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.17 (t, 3H, C-CH$_2$C*H$_3$*), 1.26 (t, 3H, N-CH$_2$C*H$_3$*), 2.69 (q, 2H, C-C*H$_2$*CH$_3$), 3.33 (s, 2H, CH$_2$C=O), 4.02 (q, 2H, N-C*H$_2$*CH$_3$), 8.04 (d, 2H, Ar-H), 8.30 (d, 2H, Ar-H), 10.41 (bs, 1H, NH), 10.45 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 12.2 (C$_5$'''), 15.4 (C$_1$''), 18.7 (C$_5$''), 33.9 (C$_3$'), 42.6 (C$_1$'), 124.4, 129.7, 145.4, 149.9 (Ar), 150.2 (C-3), 156.3 (C-5), 167.6 (C=O). MS (ESI, *m/z*): 383 (M + H)$^+$. Anal. calcd. for C$_{14}$H$_{18}$N$_6$O$_5$S: C, 43.97; H, 4.74; N, 21.98. Found: C, 44.26; H, 4.94; N, 22.22(%).
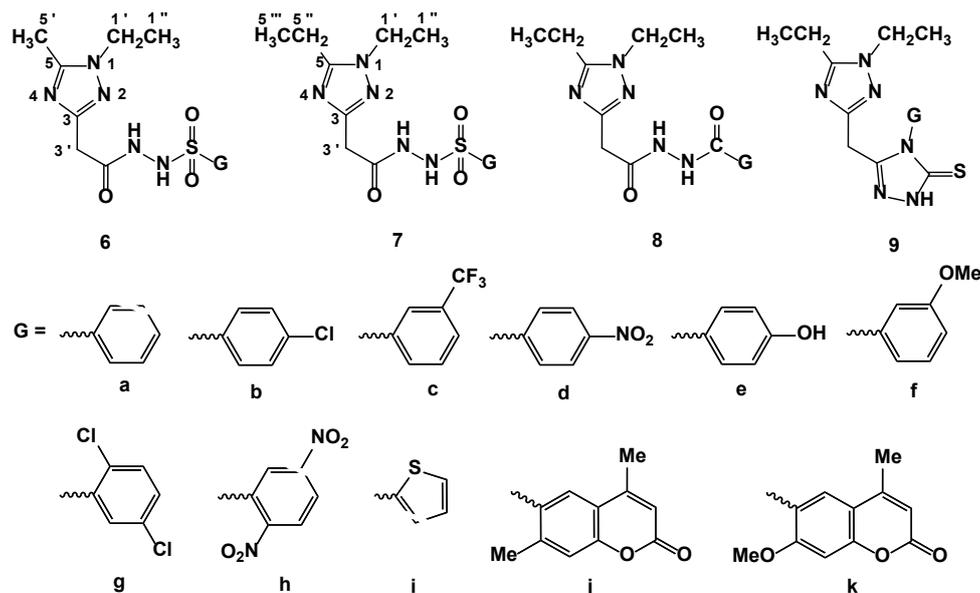
2,5-Dichlorobenzene sulfonic acid N'[2-(1,5-diethyl-1H-[1,2, 4] triazole-3-yl)-acetyl]-hydrazide (**7g**) [31].

2-Thiophene sulfonic acid N'[2-(1,5-diethyl-1H-[1,2,4]tria zole-3-yl)-acetyl]-hydrazide (**7i**) [31].

4,7-Dimethyl-2-oxo-2H-chromene-6-sulfonic acid N'[2-(1,5-diethyl-1H-[1,2,4]triazole-3-yl)-acetyl]-hydrazide (**7j**): Color: Yellow crystals. Yield: 71 %. M.p.: 225-227 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.09 (t, 3H, C-CH$_2$C*H$_3$*), 1.21 (t, 3H, N-CH$_2$C*H$_3$*), 2.44 (s, 3H, CH$_3$-Coumarin), 2.61 (q, 2H, C-C*H$_2$*CH$_3$), 3.33 (s, 2H, CH$_2$C=O), 2.73 (s, 3H, CH$_3$-Coumarin), 3.94 (q, 2H, N-C*H$_2$*CH$_3$), 6.48 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 10.12 (bs, 1H, NH), 10.26 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 12.2 (C$_5$'''), 15.4 (C$_1$''), 18.3 (CH$_3$-Coumarin), 18.6 (C$_5$''), 20.7 (CH$_3$-Coumarin), 33.6 (C$_3$'), 42.5 (C$_1$'), 114.9, 117.4, 120.3, 127.6, 133.7, 143.4, 153.1, 156.3 (Ar), 155.6 (C-3), 156.4 (C-5), 159.7, 167.8 (C=O). MS (ESI, *m/z*): 434 (M + H)$^+$. Anal. calcd. for C$_{19}$H$_{23}$N$_5$O$_5$S: C, 52.65; H, 5.35; N, 16.16. Found: C, 52.89; H, 5.56; N, 16.37(%).

2.2.2. General procedure for synthesis of compounds 8

Compound **5** (0.099g, 0.5 mmol), reacted with substituted carbonyl chlorides (0.6 mmol) in chloroform (20 mL) in the presence of pyridine (5 drops) and stirred for 48 h at room temperature.

Scheme 2

Then residue was evaporated to dryness and the residue was partitioned between CHCl₃ (40 mL) and water (3×40 mL). The organic phase was evaporated to dryness (Na₂SO₄). The product was then filtered and washed with diethyl ether followed by recrystallization from CH₂Cl₂/ether to afford compounds **8** (Scheme 1 and 2).

3-Methoxy-benzoic acid N'[2-(1,5-diethyl-1H-[1,2,4]triazole-3-yl)-acetyl]-hydrazide (**8f**): Color: Brown crystals. Yield: 80 %. M.p.: 118-120 °C. ¹H NMR (300 MHz, DMSO-*d₆*, δ, ppm): 1.21 (t, 3H, C-CH₂C*H₃*); 1.31 (t, 3H, N-CH₂C*H₃*); 2.71 (q, 2H, C-C*H₂*CH₃), 3.33 (s, 2H, CH₂C=O); 3.54 (s, 3H, OCH₃), 4.02 (q, 2H, N-CH₂CH₃), 7.12-7.17(m, 1H, Ar-H), 7.43 (bs, 3H, Ar-H), 10.13 (bs, 1H, NH), 10.42 (bs, 1H, NH). ¹³C NMR (300 MHz, DMSO-*d₆*, δ, ppm): 12.3 (C₅'''), 15.5 (C₁''), 18.7 (C₅''), 34.3 (C₃'), 42.6 (C₁'), 55.7 (OCH₃), 112.8, 118.2, 119.4, 120.2, 130.1, 134.3 (Ar), 156.7 (C-3), 159.6 (C-5), 166.5, 167.8 (C=O). MS (ESI, m/z): 332 (M + H)⁺. Anal. calcd. for C₁₆H₂₁N₅O₃: C, 57.99; H, 6.39; N, 21.13. Found: C, 58.25; H, 6.50; N, 21.38(%).

3,5-Dinitro-benzoic acid N'[2-(1, 5-diethyl-1H-[1, 2, 4]tria zole-3-yl)-acetyl]-hydrazide (**8h**): Color: Brown crystals. Yield: 66 %. M.p.: 137-139 °C. ¹H NMR (300 MHz, DMSO-*d₆*, δ, ppm): 1.19 (t, 3H, C-CH₂C*H₃*), 1.29 (t, 3H, N-CH₂C*H₃*), 2.69 (q, 2H, C-C*H₂*CH₃), 3.33 (s, 2H, CH₂C=O), 4.05 (q, 2H, N-C*H₂*CH₃), 7.36-9.01 (m, 3H, Ar-H), 8.09 (bs, 2H, NH). ¹³C NMR (75 MHz, DMSO-*d₆*, δ, ppm): 12.3 (C₅'''), 15.5 (C₁''), 18.7 (C₅''), 34.3 (C₃'), 42.6 (C₁'), 122.4, 124.4, 128.2, 128.4, 135.0, 136.7 (Ar), 148.8 (C-3), 150.0 (C-5), 164.4, 167.7 (C=O). MS (ESI, *m/z*): 392 (M + H)⁺. Anal. calcd. for C₁₅H₁₇N₇O₆: C, 46.04; H, 4.38; N, 25.05. Found: C, 46.26; H, 4.51; N, 25.27(%).

2.2.3. Synthesis of 5-((1,5-diethyl-1H-1,2,4-triazol-3-yl) methyl)-4-(4-hydroxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (9e)

Compound **5** (0.099 g, 0.5 mmol), reacted with 4-isothiocyanato-phenol (0.091 g, 0.6 mmol) in toluene (20 mL) in the presence of pyridine (5 drops) and stirred for 48 h at room temperature. Then residue was evaporated to dryness and the residue was partitioned between CHCl₃ (40 mL) and water (3×40 mL). The organic phase was evaporated to dryness (Na₂SO₄). The product was then filtered and washed with diethyl ether followed by recrystallization from CH₂Cl₂ / ether to afford compounds (**9e**): Color: Brown crystals. Yield: 70 %. M.p.: 140-142 °C. ¹H NMR (300 MHz, DMSO-*d₆*, δ, ppm):

1.01 (t, 3H, C-CH₂C*H₃*), 1.22 (t, 3H, N-CH₂C*H₃*), 2.54 (q, 2H, C-C*H₂*CH₃), 3.52 (s, 2H, CH₂C=O), 3.98 (q, 2H, N-C*H₂*CH₃), 5.52 (bs, 1H, OH), 6.73 (d, 2H, Ar-H), 7.07 (d, 2H, Ar-H), 10.29 (bs, 1H, NH). ¹³C NMR (75 MHz, DMSO-*d₆*, δ, ppm): 12.3 (C₅'''), 15.4 (C₁''), 18.7 (C₅''), 34.6 (C₃'), 42.8 (C₁'), 115.2, 128.3, 130.7, 155.8 (Ar), 156.9, 157.1, 168.0, 181.7 (C=S). MS (ESI, *m/z*): 331 (M + H)⁺. Anal. calcd. for C₁₅H₁₈N₆OS: C, 54.53; H, 5.49; N, 25.44. Found: C, 54.74; H, 5.68; N, 25.66(%).

2.3. Evaluation of antibacterial activity

A preliminary test for the antibacterial activity of compounds **6g**, **7c**, **7g** and **7i** was tested by two methods, the agar well diffusion method [32] and the broth dilution method [33] using different bacterial strains.

2.3.1. Test bacteria

A number of bacterial isolates were used in this study, two Gram-positive strains, *Bacillus cereus* (ATCC11778) and *Staphylococcus aureus* (ATCC25923); and two gram-negative strains, *Pseudomonas aeruginosa* (ATCC27853) and *Shigella sp* (ATCC23354).

2.3.2. Preparation of the test compounds and controls

Four compounds were selected for this biological study, named, **6g**, **7c**, **7g** and **7i**. 10 μg of each compound were dissolved in 1 mL dimethyl sulfoxide (DMSO) in small sterile plastic cups and stored in dark until use. DMSO was used as a negative control, whereas penicillin (10 μg/mL) was used as a positive control.

2.3.3. Agar well diffusion method

Mueller-Hinton agar medium was used as a growth medium. Media were dispensed in Petri dishes to produce Mueller-Hinton agar plates; plates were then inoculated with the test bacterium. The inoculum originated from a fresh bacterial culture with an O.D. of about 0.5 for all cultures. 6 mm-holes were punched in the solidified Mueller-Hinton agar plates using a cork borer; wells were then filled with 50 μL of each compound. Penicillin was used as a positive control, while DMSO was used as a negative control.

Table 1. Inhibition zone diameters in millimeters (mm) around the wells of the active compounds against different bacteria.

Compound	*P. aeruginosa*	*S. aureus*	*B. cereus*	*Shigella sp.*
6g	16	9	10	-
7c	-	-	10	-
7g	-	-	8	-
7i	15	-	10	-
Penicillin (Positive control)	-	27	10	35
DMSO (Negative control)	-	-	-	-

The plates were then incubated upright at 37 °C for 24 hours. Growth-free inhibition zones were then measured to the nearest millimeter. Each test was repeated twice.

2.3.4. Broth dilution method

The broth dilution method was used to determine the minimum inhibitory concentration (MIC) for each active compound [34]. Different volumes of nutrient broth, fresh culture, and the test chemical were mixed in the wells of a sterile microwell plate to yield the following final concentrations: 0.5, 1.0 and 2.0 µg/mL. DMSO and penicillin replaced the test chemical in the negative and the positive control experiments, respectively. MIC was expressed as the lowest dilution causing an inhibition of growth judged by lack of turbidity. A well containing the broth only (blank) was used for comparison. The plates were then incubated at 37 °C for 24 hours.

3. Results and discussion

3.1. Synthesis

The biological importance of 1,2,4-triazole prompted us to synthesize new 1,5-1*H*-1,2,4-triazole derivatives containing a carbohydrazide backbone, which would be favorable in achieving some specificity of pharmacological action in view of the development of effective clinical antibacterial drugs.

The triazolehydrazides **4** and **5** which prepared by the reaction of the reactive intermediates **2** prepared *in situ* from the dichloride **1**, were reacted *via* the cycloaddition reaction with ethyl cyanoacetate **3** to give, after spontaneous rearrangement [35,36], the triazolehydrazides **4** and **5** [27]. These compounds were used as starting materials for the synthesis of the carbohydrazide derivatives **6** and **7**, by reaction with the appropriate substituted sulfonyl chlorides in 63-81% yield. Triazole derivative **8** synthesized by reaction with appropriate substituted carbonyl chlorides in CHCl$_3$ for 18 h in 66-80% yield (Scheme 1). Next, the hydrazides **5** were used in synthesis of different 1,2,4-triazole-3-thiones. Thus, treatment of compound **5** with *p*-hydroxyphenyl isothiocyanate in toluene in the presence of pyridine as base afforded 1,2,4-triazole-3-thione **9e** (70%).

The ^{1}H NMR and ^{13}C NMR spectra of all prepared compounds are in total agreement with the suggested structures. DEPT experiments were employed to differentiate secondary and quaternary carbons from primary and tertiary carbons. Additional support of the proposed structures comes from mass spectral data; mass spectra of the prepared compounds showed the correct molecular ions (M$^{+\cdot}$), as suggested by their molecular formulas.

The ^{1}H NMR spectra of compounds **6a-c**, **6i** and **6k** showed similar signal patterns. Triazole-CH$_3$ appeared as singlets at in the region δ 2.33-2.44 ppm while the protons CH$_2$C*H*$_3$ appeared at higher field in the region δ 2.21-2.28 ppm. The quartets in the region around δ 4.1 ppm were attributed to the C*H*$_2$CH$_3$, whereas the singlets in the region δ 2.54-3.34 ppm were assigned to CH$_2$C=O. The ^{1}H NMR spectra of compounds **6a-c**, **6i** and **6k** were characterized by the presence of the O=CNH which appeared as broad singlets in the region δ 9.65-

10.25 ppm, whereas the broad singlets in the region δ 10.16-10.35 ppm were assigned to NHSO$_2$. The singlets at δ 3.33 ppm for the compounds **8f** and **8h** were assigned to CH$_2$C=O. The ^{13}C NMR spectra of compounds **6a-c**, **6i** and **6k** contained the resonance signals C-3 and C-5 of the triazole ring at higher field between δ 151.2-158.2 and 155.8-159.9 ppm, respectively, whereas the signals resonated at δ 150.2-156.4 and 156.3-156.5 ppm were attributed to C-3 and C-5 of the derivatives of compound **7**, respectively.

3.2. Antibacterial activity

Four compounds **6g**, **7c**, **7g** and **7i** were selected for testing their antibacterial activity using two methods: the agar well diffusion method and the broth dilution method. Table 1 shows the results obtained from the agar well diffusion method; we found that compound **6g** was active against three strains out of four, *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923) and *Bacillus cereus* (ATCC 11778) with an inhibition zone diameter of 16, 9, and 10 mm, respectively; *Shigella sp.* (ATCC23354) was resistant to this chemical, no inhibition zone was formed. Compound **7c** was active against *B. cereus* (ATCC 11778) only with an inhibition zone diameter of 10 mm. Penicillin was used as a positive control with an inhibition zone diameter of 27, 10, and 35 mm respectively against *S. aureus*, *B. cereus* (ATCC 11778), and *Shigella sp*; and DMSO was used as negative control for the tested bacteria. Compound **7g** was active against *B. cereus* (ATCC 11778) only with an inhibition zone diameter of 8 mm, but not against *P. aeruginosa*, *S. aureus*, and *Shigella sp*.

Compound **7i** was active against *P. aeruginosa* and *B. cereus* with an inhibition zone diameter of 15 and 10 mm, respectively; *S. aureus* and *Shigella sp*. were resistant to this compound (Table 1). As shown in Table 1, compounds **6g** and **7i** were active against the Gram-negative bacterium *P. aeruginosa* (16 and 15 mm, respectively); the bacterium was resistant to penicillin, it did not show any inhibitory zone against it. *P. aeruginosa* is one of the widely distributed pathogens in nature and considered one of the agents responsible for community-acquired infections [32], it's considered an opportunistic pathogen known to have the ability to rapidly develop resistance to multiple classes of antibiotics [37]. Epidemiological studies showed that infections caused by drug-resistant *P. aeruginosa* are related with serious complications including death [38,39]. Both compounds showed similar activity against *B. cereus* showing an inhibition zone of 10 mm, the same inhibition zone was found with the positive control penicillin. *B. cereus*, a Gram positive, spore forming bacterium responsible for food poisoning due the production of toxins [40]; it's one of the causative agents of foodborne outbreaks worldwide [41-43]. Unfortunately, none of the tested compounds showed any activity against *Shigella sp.* one of the leading causes of diarrheal diseases worldwide and one of the most resistant pathogens against many antibiotics [44,45]. Regarding the bacterial strain *S. aureus*, only compound **6g** was active against it with an inhibition zone diameter of 9 mm. Different minimal inhibitory concentrations (MIC) values were registered for each compound that showed an inhibition zone in the agar well diffusion test.

Table 2. Minimal inhibitory concentration values (µg/ml) of the tested chemicals against their sensitive strains.

Compound	P. aeruginosa	S. aureus	B. cereus
6g	0.5	2	0.5
7c	NT	NT	1
7g	NT	NT	2
7i	0.5	NT	0.5
Penicillin (positive control)	NT	0.5	0.5

*NT: Not tested, because these compounds did not show any bacterial activity against the targeted bacterium using the agar well diffusion method.

The MIC values are shown in Table 2; compounds **6g** and **7i** were the most active ones of all the tested compounds against *P. aeruginosa* and *B. cereus* with an MIC value of 0.5 µg/mL against both bacteria; compound **6g** had lower activity against *S. aureus* with an MIC value of 2 µg/mL whereas compound **7i** was not tested because it did not show any antibacterial activity against this bacterium in the agar well diffusion test. Compound **7c** showed an intermediate antibacterial activity with an MIC value of 1 µg/mL against *B. cereus*. The MIC value of **7g** against *B. cereus* was also high (2 µg/mL) compared with the values found with the other compounds. Compound **7c** and **7g** were not tested against *S. aureus* nor tested against *P. aeruginosa* because they did not show any antibacterial activity against these two bacteria using the agar well diffusion method (Table 2).

When comparing compound **6g** and **7i** (the most active ones of the 4 tested compounds against this panel of bacterial stains) with penicillin, we found that both drugs were having similar MIC values as those found with penicillin (0.5 µg/mL) against *B. cereus*, penicillin was not active against *P. aeruginosa* but active against *S. aureus* (MIC value 0.5 µg/mL). These results suggest that both compounds **6g** and **7i** could be selected as potential antibacterial agents with an activity similar or stronger than penicillin against some bacterial stains.

4. Conclusion

We report the synthesis and *in vitro* antibacterial evaluation of new 1,2,4-triazole derivatives. The four tested compounds, **6g** and **7i** were the most active ones against P. aeruginosa (one of the most resistant bacteria against many drugs) and *B. cereus* ATCC11778 (MIC 0.5 µg/mL), followed by compound **7c** against *B. cereus* ATCC11778 (MIC 1 µg/mL). These results suggest that compound **6g** and **7i** could be good candidates as antibacterial agents; further studies could be done on other bacterial strains as well as the study of the interactions caused by these compounds with the bacterial DNA.

Disclosure statement ⒟Ⓢ

Conflict of interests: The authors declare that they have no conflict of interest.
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