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Synthesis and Study of Biological Activities of Water-Soluble Derivatives of Metronidazole Based on Carbohydrate

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Conceptually, the present work will lead to preparation of new compounds with interesting characteristics for pharmaceutics due to increase in water solubility. Synthesis and study as potential antibacterial activity inhibitors of metronidazole–1,2,3-triazole ring based on carbohydrate are reported. All the prepared compounds of metronidazole were confirmed by NMR, FTIR elemental analysis, and mass spectroscopies. The compounds established potent to strong antibacterial activity against Gram-negative and Gram-positive bacteria. The tested compounds exhibited better antibacterial activities than the reference compound.

Key words: Metronidazole; antibacterial activities; solubility; 1,2,3-Triazole; click chemistry

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Nitroimidazole derivatives have been enticing increasing attention because of their large potentiality in pharmaceuticals and medicinal chemistry **[1–6]**. Nitroimidazole-based materials showed several bioactivities, such as, antibacterial **[7,8]**, anti-tubercular **[9]**, antiparasitic **[10]**, and anticancer **[11,12]**. Mainly as antimicrobial agents, various nitroimidazoles like benznidazole, ornidazole, metronidazole, secnidazole, tinidazole, and nimorazole have been in common medical use to treat diseases caused by bacteria (anaerobic bacteria) **[13,14]**. Notably, in spite of the long-term medical usage of nitroimidazoles, the occurrence of resistance in anaerobic bacteria is still actual low. This encourages the continuation of researches with the attention on the development of nitroimidazoles with potential pharmaceutical and medicinal applications.

Predominantly, metronidazole as an active artificial drug, presented in 1960, has good inhibitory efficacies against Gram-negative anaerobic bacteria, such as *Helicobacter pylori*, and protozoa, such as *Giardia*, *Lamblia, and Entomoeba histolytic* **[15]**. Metronidazole, l-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, is known to be a strong antibacterial and antiprotozoal compound **[16]**. Nonetheless, there are some difficulties concerning poor absorption, toxicity, and low aqueous solubility. Ester derivatives have been prepared and studied as prodrugs to adjust such problems. The parent compound can be liberated non-enzymatically or enzymatically. Several esters and hemi-esters of metronidazole have been synthesized and studied; e.g., hemimaleate, hemiglutarate and hemisuccinate **[17]**. (Larsen *et al.*, 1988; Vermeersch *et al.*, 1990). However, amino acid esters and phosphates of metronidazole have been studied to increase aqueous solubility of metronidazole compounds **[18-20]**. In 2009, Mital **[21]** reported a brief explanation of numerous biological activities shown by artificial nitroimidazole materials, in addition to their structure–mutagenicity associations. Recently, Mubarak *et al*. and Al-Soudet *et al*. **[22–25]** reported the preparation of new metronidazole derivatives with estimation of their biological activities (antifungal and antibacterial activities).

Based on these pharmacological activities, and in continuance of the work on nitroimidazole derivatives **[26–30]**, this report presents the synthesis of water-soluble derivatives for metronidazole based on glucopyranoside and the antibacterial activity evaluation.

EXPERIMENTAL SECTION

General experimental information

All solvents and chemical materials were obtained from commercial sources, such as, BDH, Merck, Sigma Aldrich, and Fluke. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR), Mashhad University, Iran. Infrared spectra were obtained by using Bruker ALPHA FT-IR, Faculty of Science, University of Kufa. Absorbance recorded by Appel spectrometer, Japan. Melting points were measured with Electro Thermal Melting Point Apparatus, UK. Glass TLC 1020GS with silica 60, thickness 0.25, size 10x20cm. Mass spectra were recorded on

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LC/MS/MS system, model CBM-20A, SHIMADZU, Japan, Mashhad University, Iran.

Synthesis of 1-(2-chloroethyl) -5-methyl-2-nitro-1H-imidazole (1) [31]

Thionyl chloride (1.18 g, 0.01 mole) was added slowly to a cold solution of metronidazole (1.71 g, 0.01 mole) in dry toluene (20 mL). The reaction solution was left at room temperature and refluxed for 7 hrs. The solvent was evaporated and the end product was obtained and purified by recrystallization in a mixture of water and ethanol. Yield of reaction: 90%; m.p.: 85-87°C; IR (ν, cm^{-1}): 2981,2831 (C-H aliph.), 1614 (C=N), 1539, 1464 (NO_2), 761 (C-Cl); 1H-NMR (300 MHz, *" DMSO-d6"* δ ppm: 8.51 (s, 1H, imidazole), 3.74 (t, *J* = 5.1 Hz, CH_2-Cl), 2.98 (t, *J* = 5.2 Hz, N-CH_2), 1.76 (s, CH_3); ^{13}C-NMR (75 MHz, *" DMSO-d6"* δ ppm: 154.4 (C-2 imidazole), 139.1 (C-5 imidazole), 130.3 (C-4 imidazole), 53.9 (CH_2-Cl), 44.8 (N-imidazole-CH_2), 15.6(C2- CH_3 imidazole). Anal. % Calc. for $C_6H_8N_3O_2Cl$: C, 38.01, H, 4.25, N, 22.16; found: C, 36.78; H, 4.12; N, 21.24.

Synthesis of 1-(2-chloroethyl)-2-nitro-1H-imidazol -5-carboxylic acid (2) [31]

To a stirred solution of compound **1** (0.01 mole, 1.89 g) was added a solution of Na_2CO_3 (0.01 mole, 1.06 g) and potassium permanganate ($KMnO_4$) (0.01 mole,1.58 g) in water (20 mL), and the mixture was stirred for 15 hrs. The reaction solution was left to cool and acidified by adding conc. HCl, and the end product was obtained and recrystallized from ethanol. Yield of reaction: 64%; m.p.: 181-183°C; FTIR (ν, cm^{-1}): 3378 cm^{-1} (OH acid), 2967,2842 cm^{-1} (C-H aliph.), 1724 cm^{-1} (C=O acid), 1607 cm^{-1} (C=N), 1541, 1361 cm^{-1} (NO_2); ^{1}HNMR (300 MHz, $(CD_3)_2SO$) δ ppm: 12.27 (s, acid OH), 8.78 (s, 1H, imidazole); 3.57 (t, *J*=5.1 Hz, CH_2-Cl), 3.31 (t *J*=5.1 Hz, N-CH_2), ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 176.1 (COOH), 155.7 (C-2 imidazole), 141.4 (C-5 imidazole), 133.4 (C-4 imidazole), 52.4 (CH_2-Cl), 46.4 (N-imidazole-CH_2). Anal. % Calc. for $C_6H_6ClN_3O_4$: C, 32.82; H, 2.75; N, 19.14 found: C, 31.74; H, 2.09; N, 18.71.

Synthesis of 1-(2-azidoethyl)-2-nitro-1H-imidazol-5-carboxylic acid (3,4)

Sodium azide (0.01 mole) was added rapidly to a solution of compound **1 or 2** (0.01 mole) in DMF (20 mL), then the reaction solution was stirred for 6-7 hrs. After evaporation, the product was collected and recrystallized from ethanol-water.

1-(2-azidoethyl)-2-methyl-5-nitro-1H-imidazole (3): Yield: 84%; m.p.: 121-123°C; FTIR (ν, cm^{-1}): 2947, 2868 cm^{-1} (C-H aliph.), 2125 cm^{-1} due to azide group (N_3), 1621 cm^{-1} (C=N), 1561,1347 cm^{-1} (NO_2); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ ppm: 8.71 (s, 1H, imidazole); 3.67 (t, *J*=5.2Hz, CH_2-N_3), 3.21 (t, *J*=5.3Hz, N-CH_2), 1.85(s, CH_3); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 153.2 (C-2 imidazole), 140.1 (C-5 imidazole), 131.8 (C-4 imidazole), 56.7 (CH_2-N_3), 46.9 (N-imidazole-CH_2), 14.7 (C2-CH_3 imidazole). Anal. % Calc. for $C_6H_8N_6O_2$: C, 36.74; H, 4.11; N, 42.84 found: C, 36.04; H, 3.89; N, 42.02.

1-(2-azidoethyl)-5-nitro-1H-imidazole-2-carboxy licacid (4): Yield: 80%; m.p.: 104-106°C; FTIR (ν, cm^{-1}): 3298 (OH acid), 2978, 2857 (C-H aliph.), 2132 cm^{-1} due to azide group (N_3), 1608(C=N), 1567,1337 (NO_2); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ ppm: 12.31 (s, acid OH), 8.9 (s, 1H, imidazole); 3.73 (t, *J*=5.2Hz, CH_2-N_3), 3.34 (t *J*=5.3Hz, N-CH_2); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 175.4 (COOH), 152.4 (C-2 imidazole), 141.5 (C-5 imidazole), 132.4 (C-4 imidazole), 58.4 (CH_2-N_3), 47.7 (N-imidazole-CH_2). Anal. % Calc. for $C_6H_6N_6O_4$: C, 31.87; H, 2.67; N, 37.16 found: C, 30.24; H, 2.47; N, 36.37.

Synthesis of 2-propynyl(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside) (g) [32]

A solution of the starting compound (1,2,3,4,6-penta-*O*-acetyl-**α**-D-glucopyranose, 1.5 g, 0.004 mole) in dry dichloromethane (40 mL) was cooled to 0°C. Stannic chloride ($SnCl_4$, 5 mL, 0.005 mol) was added slowly and the mixture was stirred for (20 min) at 0°C. Then, propargyl alcohol (5.05 mmol) was added dropwise, and the reaction solution was allowed to warm to room temperature and the stirring was continued for 3 hours. After which time, the reaction solution was quenched and diluted with 75 mL of DCM, then poured onto crushed ice (75 mL). The organic solvent was separated and washed many times with a solution of $NaHCO_3$ (aq., sat., 3X50 mL) and then with water, dried with Na_2SO_4, and concentrated by evaporated solvent.

It was synthesized as a white solid. Chemical formula: $C_{17}H_{22}O_{10}$, reaction yield: 74%, m.p.: 114-116°C; FTIR, *ν* (cm^{-1}) 3288 (C-H) alkyne, 2955, 2860 (C-H alipha.), 2232(C≡C) alkyne, 1744(C=O), 1448, 1233; ^{1}H-NMR (300 MHz, Chloroform-*d*): δ ppm 5.21 (t, *J* = 9.4 Hz, 1H,H3), 5.11 (t, *J* = 9.9 Hz, 1H,H4), 4.96 (dd, *J* = 9.4, 7.9 Hz, 1H,H2), 4.64 (d, *J* = 7.9 Hz, 1H,H1), 4.39 (d, *J* = 2.4 Hz, 2H), 4.24 (dd, *J* = 12.4, 4.5 Hz, 1H, H6-b), 4.18 (s, 2H,CH_2), 4.07 (dd, *J* = 12.4, 2.4 Hz,1H,H6-a), 3.69 (ddd, *J* = 9.8, 4.8, 2.4 Hz,1H,H5), 2.48(s,1H,C≡CH), 2.07, 2.04, 200, 1.99 (s,12H, $4CH_{3acetate}$).

Synthesis of 1,2,3-triazole derivative (a1) [33]

1,2,3-triazole derivatives were prepared according to click chemistry conditions. 0.001 mole of α-D-glucopyranoside alkyne derivative was dissolved in 15 mL of DMF. CuCl (0.002 mole) and sodium ascorbate (0.004 mole) were added to the solution. Then, 0.001 mol of azidometronidazole (2,3) was added and stirred continuously at 60-70°C until the reaction was complete. The progress of reaction was checked by TLC. The final products were separated

with diethyl ether and D.W. three times. The organic phase was dried over $MgSO_4$. The solvent was evaporated to produce 1,2,3-triazole derivatives.

2-methyl-5-nitro-1-(2-((5-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-imidazole (1a): Yield: 84%; m.p.: 174-176ºC; FTIR (ν, cm^{-1}): 3088 (C-triazol), 2968, 2874 (C-H aliph.), 1725 (C=O acetate), 1608 (C=N), 1541, 1358 (NO_2); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ ppm:8.64 (s, 1H, imidazole), 7.58 (s, 1H, triazol), 6.45(d, *J*= 4.8 Hz,1H,H-1), 5.54 (t, *J* =8.9 Hz, 1H, H-4), 5.42(t, *J* =8.9 Hz, 1H, H-3),5.21 (t, *J* =8.7 Hz, 1H, H-2),4.52 (s, O-CH_2- triazol, 2H), 4.31 (ddd, *J* =7.8 Hz, *J* =4.1 Hz, *J* =2.1 Hz, 1H, H-5), 3.91-3.58 (m, 2H, H-6a, H-6b),3.67 (t,*J* = 5.2 Hz, 2H, CH_2-triazol), 3.08 (t, *J* = 5.2 Hz, 2H, N-CH_2), 2.06,2.04,2.02, 2.01 (s, 12H, CH_3 acetate), 1.79 (s, CH_3, 3H); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 170.9, 170.3, 169.5, 169.4 (4C, C=O acetate), 152.7 (C-2 imidazole), 146.2(1C-C4 triazol), 136.4 (C-5 imidazole), 131.7 (C-4 imidazole), 122.1 (1C-C-5 triazol),87.1(1C-C1), 74.2 (1C-C4), 72.1 (1C-C3), 70.2 (1C-C2), 66.9 (1CC6), 62.6(1C-C5), 59.2 (Ntriazole-CH_2), 47.4 (N-imidazole-CH_2),20.6, 20.4 (4C-CH_3 acetate), 14.8 (C2- CH_3 imidazole); m/z, Calc. for ($C_{23}H_{30}N_6O_{12}$)[M+]: 582.1; found: 581.2, 583.4.

5-nitro-1-(2-((5-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-imidazole-2-carboxylic acid (2a): Yield: 81%; m.p.: 148-150ºC; FTIR (ν, cm^{-1}): 3358 (OH acid), 3104 (C-triazol), 2972, 2841 (C-H aliph.), 1716 (C=O acetate), 1617 (C=N), 1551, 1338(NO_2); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ ppm: 12.24 (s, 1H, acid OH),8.76 (s, imidazole), 7.61 (s, 1H, triazol), 6.52 (d, *J*= 4.4Hz, 1H, H-1), 5.51 (t, *J* =8.8Hz, 1H, H-4), 5.39 (t, *J* =8.9 Hz, 1H, H-3), 5.26 (t, *J* =8.9Hz, 1H, H-2), 4.49 (s, O-CH_2- triazol, 2H), 4.28 (ddd, *J* =7.8 Hz, *J* =4.2 Hz, *J* =2.2 Hz, 1H, H-5), 3.87-3.61 (m, 2H, H-6a, H-6b), 3.74 (t, *J* = 5.1 Hz, 2H, CH_2-triazol), 3.11 (t, *J* = 5.2 Hz, 2H, N-CH_2), 2.05, 2.03, 2.02, 2.01 (s, 12H, CH_3 acetate); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 174.8 (1C-COOH), 171.7, 171.1, 169.9, 169.1 (4C, C=O acetate), 154.1 (C-2 imidazole), 144.8 (C-4triazol), 142.9 (C-4 imidazole), 138.6 (C-5 imidazole), 120.7 (1C-C5triazol), 89.2(1C-C1), 72.8 (1C-C4), 72.7 (1C-C3), 70.5 (1C-C2), 67.8 (1CC6), 62.1(1C-C5), 59.5 (Ntriazole-CH_2), 49.8 (N-imidazole-CH_2),20.7, 20.05, 20.3 (4C-CH_3 acetate); m/z, Calc. for ($C_{23}H_{28}N_6O_{14}$)[M+]: 612.5; found: 612.1, 613.5.

Removal of protective groups and synthesis of 1b and 2b [34]

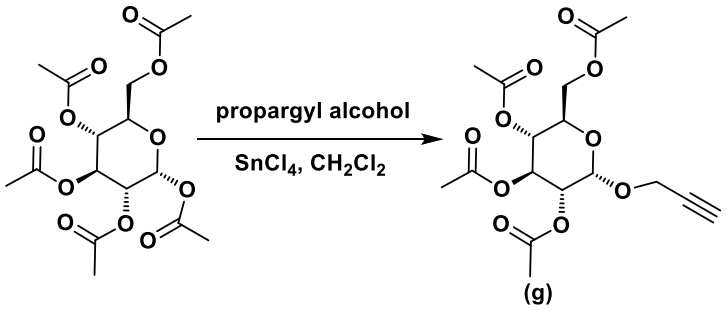
Compounds (0.3 mmol) were dissolved in methanol (5 mL), and potassium carbonate (60 mmol) was added to the solution. The reaction mixture was stirred (6 hours) at room temperature until of the starting material disappears and the mixture becomes concentrated. The mixture was separated using 35 mL of diethyl ether several times, and then the organic layer was treated with 100 mL of water three times. Subsequently, the diethyl ether phase was dried by adding Na_2SO_4 and filtered. The organic solvent (diethyl ether) was evaporated and then the end product was dried.

2-methyl-5-nitro-1-(2-((5-(α-D-glucopyranosyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-imidazole (1b): Yield: 78%, semi sold; FTIR (ν, cm^{-1}): 3461,3327 (OH), 3074 (C-triazol), 2958, 2871 (C-H aliph.), 1621 (C=N), 1552, 1351 (NO_2); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ ppm: 8.71 (s, 1H, imidazole), 7.62 (s, 1H, triazol), 6.53 (d, *J*= 4.6 Hz,1H,H-1), 5.39 (t, *J* =8.9 Hz, 1H, H-4), 5.24(t, *J* =8.8 Hz, 1H, H-3),5.17 (t, *J* =8.7 Hz, 1H, H-2), 4.46 (s, O-CH_2- triazol, 2H), 4.26 (ddd, *J* =7.8 Hz, *J* =4.2 Hz, *J* =2.1 Hz, 1H, H-5), 3.97-3.42 (m, 2H, H-6a, H-6b),3.63 (t,*J* = 5.2 Hz, 2H, CH_2-triazol), 3.10 (t, *J* = 5.2 Hz, 2H, N-CH_2), 1.76 (s, CH_3, 3H); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 154.1 (C-2 imidazole), 144.8 (1C-C4 triazol), 137.6 (C-5 imidazole), 133.2 (C-4 imidazole), 120.8(1C-C5 triazol), 84.8(1C-C1), 72.3 (1C-C4), 70.4 (1C-C3), 68.5(1C-C2), 65.7 (1C-C6), 62.1 (1C-C5), 57.4 (Ntriazole-CH_2), 45.7 (N-imidazole-CH_2),13.7 (C2- CH_3 imidazole); m/z, Calc. for ($C_{15}H_{22}N_6O_8$)[M+]: 414.3; found: 414.1,415.2.

5-nitro-1-(2-((5-(α-D-glucopyranosyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1H-imidazole -2-carboxylic acid (2b): Yield: 80%; m.p.:133-135ºC; FTIR (ν, cm^{-1}): 3428, 3315 (OH acid), 3084 (C-triazol), 2982, 2839 (C-H aliph.), 16124 (C=N), 1565, 1347 (NO_2); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ ppm: 12.37 (s, 1H, acid OH), 8.2 (s, imidazole), 7.52 (s, 1H, triazol), 6.61(d, *J* = 4.4Hz, 1H, H-1), 5.47 (t, *J* = 8.7Hz, 1H, H-4), 5.4 (t, *J* = 8.9 Hz, 1H, H-3), 5.27 (t, *J* = 8.9Hz, 1H, H-2), 4.43 (s, O-CH_2- triazol, 2H), 4.31 (ddd, *J* = 7.7 Hz, *J* = 4.1 Hz, *J* = 2.2 Hz, 1H, H-5), 3.38-3.54 (m, 2H, H-6a, H-6b), 3.82 (t, *J* = 5.2 Hz, 2H, CH_2-triazol), 3.14 (t, *J* = 5.2 Hz, 2H, N-CH_2); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$) δ ppm: 176.7 (1C-COOH), 156.4 (C-2 imidazole), 143.4 (C-4 triazol), 144.2 (C-4 imidazole), 137.8 (C-5 imidazole), 122.1 (1C-C5 triazol), 85.5 (1C-C1), 73.7 (1C-C4), 70.9 (1C-C3), 69.5 (1C-C2), 66.9 (1CC6), 61.8 (1C-C5), 59.7 (N triazole-CH2), 51.2 (N-imidazole-CH_2); m/z, Calc. for ($C_{15}H_{20}N_6O_{10}$)[M+]: 444.36; found: 443.1, 444.5.

Aqueous solubility [35]

The water solubility of metronidazole derivatives (**1a, 2a, 1b** and **2b**) and parent metronidazole was evaluated at 25°C. An additional quantity of the respective substances was added to 2 mL of water in a test tube. The suspension was rotated and controlled on a thermostatic mechanical shaker set at 30 strokes min^{-1} for 24 h to attain equilibrium. The mixture was altered and a portion was diluted with an appropriate quantity of water and spectrophotometrically analyzed for metronidazole derivatives content at 320 nm.



Scheme I: Synthesis of compound (g)

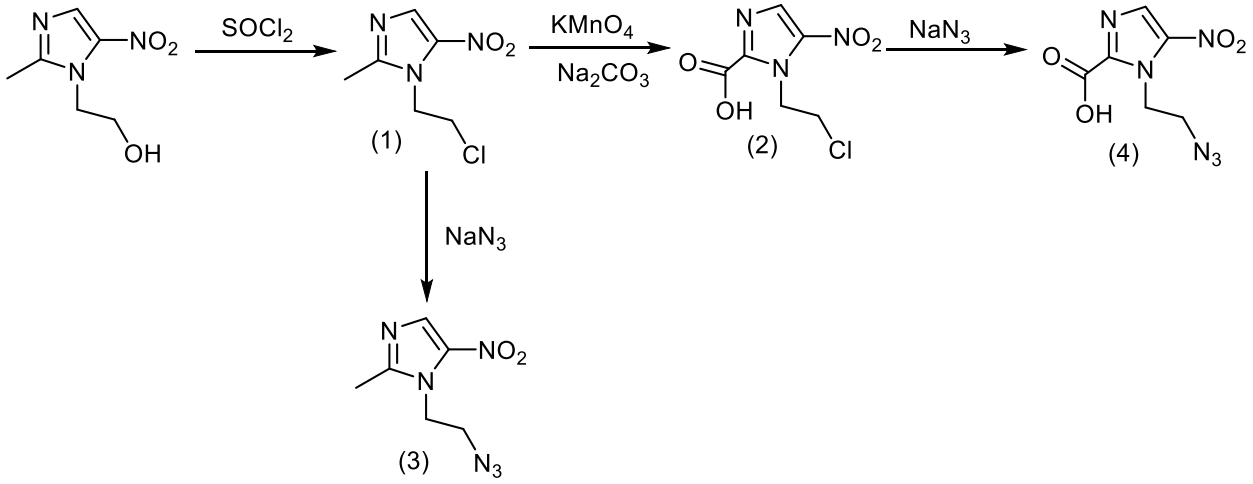
RESULTS AND DISCUSSION

Chemistry

The main goal of this study was to prepare a series of novel metronidazole-glucosyl derivatives (**1a**, **2a**, **1b** and **2b**), and study their solubility behavior in aqueous solution. Conceptually, the present work will lead to new compounds with interesting properties for pharmaceutics due to increase in water solubility. The first step of this study was the synthesis of 2-propynyl (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside) (g), as shown in Scheme I, by glycosidation of propargyl alcohol with glucose pentaacetate, in the presence of stannic chloride ($SnCl_4$) dissolved in dry DCM to afford the glycoside in high yields **[32,34]**. Stannic chloride and acetate group in position 2 played a good role in activation of the anomeric acetate. FTIR of **(g)** showed the following important bands: 3273 cm^{-1} due to alkyne hydrogen (C-H alkyne), 2118 cm^{-1} due to triple bond stretching (C≡C), and 1758 cm^{-1} and 1732 cm^{-1} assigned to carbonyl group of acetate stretching (C=O). The ^{1}H-NMR data showed singlet signals at 2.07, 2.04, 200, and 1.99 ppm for the protons of acetate, and other signals at 5.21 (t, *J* = 9.4 Hz,H-3), 5.11 (t, *J* = 9.9 Hz, H-4), 4.96 (dd, *J* = 9.4,7.9 Hz, H-2), 4.64 (d, *J* = 7.9 Hz, H-1), 4.24 (dd, *J* = 12.4, 4.5 Hz, Hb-6), 4.18 (s, 2H-methyl propargyl proton), 4.07 (dd, *J* = 12.4, 2.4 Hz,Ha-6), 3.69 (ddd, *J*= 9.8, 4.8, 2.4 Hz,H-5), and 2.48 (s, H-propargyl proton).

The metronidazole compounds were prepared according to Scheme **II**. Reaction of metronidazole with thionyl chloride at room temperature afforded 1-(2-chloroethyl)-5-methyl-2-nitro-1*H*-imidazole (**1**) **[31]**. The FTIR spectrum of the compound collected and purified from ethanol showed the disappearance of absorption peaks assigned to hydroxyl group (OH) and the appearance of a new peak due to of a halide (C-Cl) at 768 cm^{-1}. The ^{1}H-NMR spectrum of this compound showed the following signals: 8.51 ppm (s, 1H, imidazole), 3.74 ppm (t, *J* = 5.1 Hz, CH_2-Cl), 2.98 ppm (t, *J* = 5.2 Hz, N-CH_2), and 1.76 ppm (s, CH_3); whereas the ^{13}C-NMR spectrum showed the signals: 154.4 ppm (C-2 imidazole), 139.1 ppm (C-5 imidazole), 130.3 ppm (C-4 imidazole), 53.9 ppm (CH_2-Cl), 44.8 ppm (N-imidazole-CH_2), and 15.6 ppm (C2-CH_3 imidazole). 1-(2-Chloroethyl)-2-nitro-1*H*-imidazol-5-carboxylic acid **(2) [31,35]** was readily synthesized by the oxidation process of the methyl group of metronidazole in potassium permanganate. The structure of derivative **(2)** was determined by FTIR and NMR spectral and elemental analyses. In the FTIR spectrum of compound **(2)**, the presence of a hydroxyl group absorption (OH) at 3378 cm^{-1} was shown, in addition to a carbonyl absorption at 1724 cm^{-1}. The ^{1}H-NMR spectrum showed a new singlet signal at 12.27 ppm assigned to a carboxyl group proton. In addition, the ^{13}C- NMR data of the same compound indicated an additional peak at 176.1 ppm due to carbons of the carboxyl group. Finally, metronidazole azides **3** and **4** were prepared via displacement reaction of the chloro groups at 1 and 2 via S_N2 mechanism by a treatment with sodium azide. The structures of compounds **3** and **4** characterized by the FTIR data showed new bands at 2125 and 2132 cm^{-1} due to azide groups of compounds **3** and **4**, respectively. The NMR data of these compounds showed shifting in values of (CH_2-N_3) and other signals.

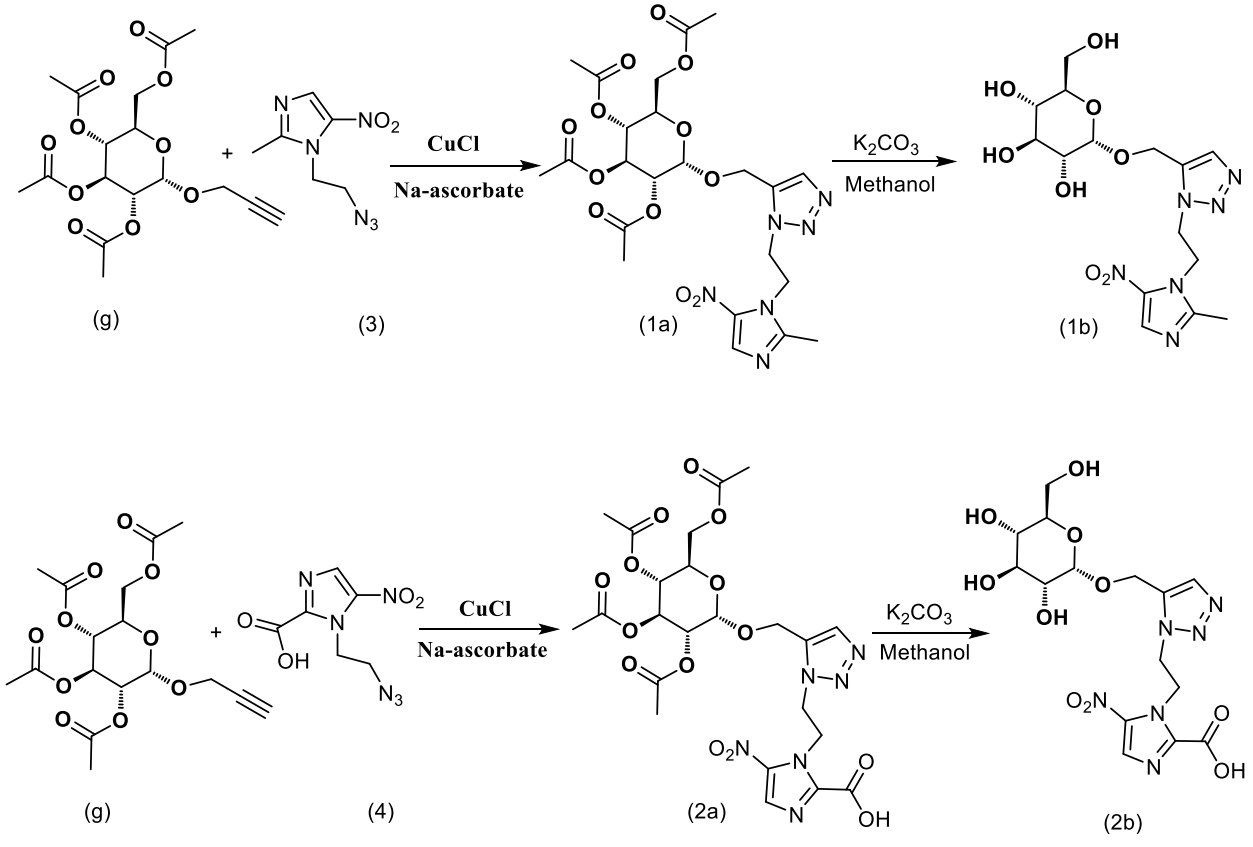
In pyranoses sugars, the β-anomer resonance is upfield compared to the α-anomer. The value of the coupling constant between H-2 and H-1, $^3J_{1,2}$ is confirmed for the stereochemistry relationship of these protons and can be utilized to differentiate α- and β-anomers for glucosides. Normally, β-anomers exhibit a value of 8 Hz for the axial-axial interactions, while 3-4 Hz is observed for the axial-equatorial interactions of α-anomers **[36]**. The target glucose -based 1,2,3-triazole compounds of metronidazole (**1a** and **2a**) were synthesized via 1,3-dipolar Huisgen cycloaddition (click chemistry) catalyzed by copper chloride and sodium ascorbate (Scheme **III**). The FTIR spectra of the triazole compounds showed the absence of bands at 2118 and 2132 cm^{-1} assigned to azide groups in compounds **3** and **4** and an absorption band at 2118 cm^{-1} assigned to the triple bond of glycosyle alkyne. The ^{1}H-NMR spectra of the metronidazole glucosyles showed 7.58 and 7.61 ppm (s, 1H, triazol) for compounds **1a** and **2a**, respectively, and singlet signals at 2.06, 2.04, 2.02, and 2.01 ppm due to methyl groups of acetate, but the ^{13}C-NMR spectra showed new signals at 146.2 and 144.8 ppm (1C-C4 triazol), and 122.1 and



Scheme II: Synthesis of compounds 1-4

120.7 ppm (1C-C5 triazol) due to carbons of triazole compounds **1a** and **2a** (see Experimental section).

Finally, deprotection of heterocyclic compounds **1b** and **2b** was carried out by using potassium carbonate in methanol, as shown in Scheme **III**. The structures of the prepared compounds confirmed by using the FTIR data indicated new absorption bands above 3300 cm^{-1} due to hydroxyl groups in the glucose moiety and absence of bands above 1700 cm^{-1} related to carbonyl groups of acetate. The ^{1}H-NMR data showed absence of singlet signals at 2.06, 2.04, 2.02, and 2.01 ppm due to methyl groups of acetate, and showed singlet signals at 7.62 and 7.52 ppm (s, 1H, triazol) of compounds **1b** and **2b**, respectively, whereas the ^{13}C-NMR data of these compounds showed signals at 144.8 and 143.4 ppm (1C-C4 triazole), and 120.8 and 122.1 ppm (1C-C5 triazol) due to carbons of triazole of compounds **1a** and **2a**, and absence of peaks at 171.7, 171.1, 169.9, and 169.1 and 170.9, 170.3, 169.5, and 169.4 ppm due to carbonyl group carbons of acetate for compounds **1b** and **2b**, respectively. (see Experimental section).



Scheme III: Synthesis of compounds 1a, 2a, 1b, 2b

Table 1. Antibacterial Activities of New Compounds

Compound	*Sa*	*Ec*	*Pa*	*St*
1a	**6**	**8**	**11**	**10**
2a	**8**	**8**	**10**	**9**
1b	**11**	**10**	**12**	**14**
2b	**12**	**9**	**15**	**13**
DMSO	**5**	**5**	**5**	**5**
Metronidazole	**8**	**5**	**15**	**15**

Diameter of growth inhibition zone was measured in mm: (5 mm: no antimicrobial activity; >5 mm: positive antimicrobial activity). *Ec*: *Escherichia coli* ATCC 25922; *Pa*: *Pseudomonas aeruginosa* ATCC 10145; *Sa*: *Staphylococcus aureus* ATCC 25923; *St*: *Streptococcus pneumoniae;* Conc. 10^{-3} M

Table 2. Solubility data of metronidazole and its derivatives at 25°C

Compound	Solubility (mg mL^{-1})
1a	**587.8**
2a	**808.9**
1b	**1247.3**
2b	**1914.8**
Metronidazole	**11.2**

***Average of three experiments.**

Antibacterial activity

All evaluated bacteria (*Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*) were obtained from the microbiology lab of Alfurat al-Awsat Hospital (Najaf, Iraq) **[37]**. The chemical compounds were weighed and then dissolved in an organic solvent (DMSO) to produce stock solutions of 10^{-3} M. The procedure of the antibacterial activity evaluation using the agar well diffusion method was adapted from previous studies and literature **[30,31]**. The results are summarized in Table 1.

Solubility Properties

The aqueous solubilities of metronidazole **(1) [17]** and its derivatives (**1a**, **2a**, **1b** and **2b**) are listed in Table 2. Aqueous solubility properties of the metronidazole glucosyles were evaluated at room temperature. They were observed as a common design that the prepared compounds have higher water solubility compared with the parent compound metronidazole. The end products with free hydroxyl groups (**1b**, **2b**) showed higher water solubility that exceeded the water solubility of metronidazole. Also, compound **(2b)** had higher solubility than other prepared compounds because it has free hydroxyl groups, in addition to carboxylic acid groups. On the other hand, derivative **(1a)** possessed lower aqueous solubility than other prepared compounds.

CONCLUSION

In summary, a series of new sugar-based metronidazole derivatives have been described by applying a dipolar cycloaddition reaction (click chemistry). All the end compounds were obtained with very good yields, as well as they showed good antibacterial activities and aqueous solubilities in comparison with metronidazole.

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