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Synthesis, Characterization AND Antimicrobial Evaluation of some Triazole derivative containing Thiophen Ring

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Abstract : A series of new 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones (**3a-c**) were obtained by reaction N'-1-ethoxy-2-thiophen-2-yl-ethylydene hydrazino carboxylic acid ethyl ester (**1**) and 4-amino-4H-1,2,4-triazoles (**2**). 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-2-(2-oxo-2-arylethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones (4a-e) and ethyl 2-(4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-5-oxo-3-(thiophen-2-lmethyl)-4,5-dihydro-1,2,4-triazol-1-yl)acetates (**6a-c**) were obtained by reaction of compounds **3** and bromoacetophenone derivatives and bromoethylacetate, respectively. Compounds **5a-e** were synthesized from the reaction of corresponding compounds **4a-e** with $NaBH_4$. Compounds **7a-c** were obtained by the reaction compounds **6** and $LiAlH_4$. Seventeen new compounds were synthesized and characterized by elemental analyses, IR and ^{1}H-NMR. In addition, the newly synthesized chemicals were screened for their antibacterial and antifungal properties. Among the chemicals tested, **6a** and **6b** exhibited the highest degree of antifungal activity.

Key Words: Synthesis, 1, 2, 4-triazole-3-one, $NaBH_4$, $LiAlH_4$, antimicrobial activity, X-ray.

Introduction

The 1,2,4-triazole compounds possess important pharmacology activities such as antifungal and antiviral activities. Examples of such compounds bearing the 1,2,4-triazol residues are fluconazole,1 the powerful azoles antifungal agent, as well as the potent antiviral N- nucleoside ribavirin. [2] Furthermore, various 1,2,4-triazole derivatives have been reported to have fungicidal,[3] insecticidal,[4] and antimicrobial activity,[5] and some showed antitumor activity[6] as well as anticonvulsant,[7] antidepressant,[8] and plant growth regulator anticoagulant activities.[9] It was reported that compounds having triazole moieties such as vorozole, anastrozole, and letrozole appear to be very effective aromatase inhibitors very useful for preventing breast cancer.[10–12]

It is known that 1,2,4-triazole moieties interact strongly with heme iron, and aromatic substituents on the triazoles are very effective for interacting with the active site aromatase.[13] In recent years, there has been an increasing interest in the chemistry of thiophenes because of their biological significance. Many of them have been widely investigated for therapeutic uses, especially as antifungal, antibacterial, antiinflammatory, anticonvulsant, antiasthmatic, and analgesic agents. They are also known to show anti-HIV, antiproliferative, germicidal, and D_2 dopaminergic activities.[14] Antimicrobial agents having different structures are frequently used in the treatment of microbial infections.

However, there is an increasing resistance to these drugs. Moreover, some azole derivatives used as common antibiotics such as Amphotericin B have a

toxic effect on humans as well as their antimicrobial effects.[15] To overcome the development of drug resistance, it is crucial to synthesize a new class of antimicrobials possessing different chemical properties from those of used commonly. In view of these facts, the aim of this present study was to obtain triazole derivatives containing thiophene (Scheme) to be used as antimicrobial agents. For years, several articles have been devoted to the synthesis and pharmacological investigation of certain chemicals.[16–20]We investigated the possible antimicrobial activity of some **3a, 3b, 3c, 4a, 4b, 6a, 6b**, and **6c** to organisms including bacterial and fungal strains

Experimental

Chemistry:

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. Compounds **1**,**3a**,and **4b** were synthesized by published methods,[21–23] respectively.

General method for the synthesis of 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones (3a-c): Compound **1** (0.01 mol) and 3,5-disubstitue-4-amino-4H-1,2,4-triazole **(2)** (0.01 mol) were heated at 165-170˚C in an oil bath for 2 h. After cooling to room temperature, a solid appeared. It was recrystallized from an appropriate solvent to afford the desired compound.

Synthesis of 4-(3,5-dimethyl-4H-1,2,4-triazol-4-yl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol- 3(4H)-one (3b): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 64%) to afford the desired compound. Mp 220-221˚C. IR (KBr) (ν, cm−1) 3152(NH), 1738 (C=O), 1605 (C=N); ^{1} H-NMR ($DMSOd_6$) δ 3.84 (s,2H, CH_2), 6.59-6.61 (m, 3H, thiophene H),12.20 (s, NH); Analysis (%Calculated/found) for $C_{11}H_{12}N_6$ SO C:47.81/47.83, H:4.38/4.39, N:30.41/30.43.

Synthesis of 4-(3, 5-ethyl-4H-1,2,4-triazol-4-yl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-one (3c): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 671%) to afford the desired compound. Mp 231-232˚C. IR (KBr) (ν, cm−1)3095 (NH), 1744 (C=O), 1598 (C=N); ^{1} H-NMR ($DMSOd_6$)δ 3.87 (s, 2H, CH_2), 6.81-6.85 (m, 3H, thiophen H), 11.68 (s, NH); Analysis (%Calculated/found) for $C_{13}H_{16}N_6$ SO C:51.30/51.32, H:5.30/5.31, N:27.61/27.63.

General method for the synthesis of 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-2-(2-oxo-2-arylethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones (4a-e): The corresponding 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones **(3a-c)** (0.01 mol) were refluxed with an equivalent amount of natrium in absolute ethanol for 1 h. Then bromoacetophenone derivatives (0.01 mol) were added and refluxed for an additional 5 h. The precipitate was filtered off, washed with H_2O, and recrystallized from an appropriate solvent to afford the desired compound.

2-(2-oxo-2-phenylethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (4a): Following the general procedure above, a white solid was obtained. It was recrystallized from benzene/petroleum ether (1:1) (yield 70%) to afford the desired compound. Mp 109-110˚C. Analysis(%Calculated/found) for $C_{17}H_{14}N_6SO_2$ C:55.73/55.74, H:3.85/3.87, N:22.94/22.96; IR (KBr) (ν , cm−1) 1658(acetophenone C=O), 1746 (triazol-3-one C=O), 1597 (C=N); 1 H-NMR ($DMSO-d_6$)δ 4.02 (s, 2H, thiophen-CH_2), 5.27 (s, 2H, NCH2), 6.62-6.91 (m, 3H, thiophen+arom.H),7.46-7.50(m,2H,thiophen+arom.H),7.57-7.68 (m, 2H, thiophen+arom.H), 8.05(s,2H,triazoleH);Analysis(%Calculated/found)for $C_{17}H_{14}N_6SO_2$C:55.73/55.74,H:3.85/3.87,N:22.94/22.96 .

4-(3,5-diethyl-4H-1,2,4-triazol-4-yl)-2-(2-oxo-2-phenylethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-one (4c): Following the general procedure above, a white solid was obtained. It was recrystallized from benzene/petroleum ether (1:1) (yield 70%) to afford the desired compound. M.p115-116˚C. Analysis(%Calculated/found)for $C_{21}H_{22}N_6SO_2$ C:59.70/59.71, H:5.25/5.27, N:19.89/19.90; IR (KBr) (ν ,cm−1) 1659 (acetophenone C=O), 1748 (triazol-3-one C=O), 1600 (C=N); ^{1}H-NMR ($DMSO-d_6$)δ 1.24 (t, 6H,J=3.4 Hz, CH3), 2.5 (q, 4H, J=3.4 Hz, CH_2), 4.05 (s, 2H, thiophen-CH_2), 5.35 (s, 2H, NCH_2), 6.66-6.98 (m,3H, thiophen+arom.H), 7.45-7.50 (m, 2H, thiophen+arom.H), 7.59-7.78 (m, 2H, thiophen+arom.H), 8.01 (s, 2H,triazolH); Analysis(%Calculated/found) for $C_{21}H_{22}N_6$ SO_2C:59.70/59.71, H:5.25/5.27, N:19.89/ 19.90.

2-(2-oxo-2-p-tolylethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (4d): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 70%) to afford the desired compound. M.p 210-211˚ C. IR (KBr) (ν,

cm−1)1691(acetophenon-C=O), 1715(triazol-3-one C=O), 1597 (C=N); ^{1}H-NMR (DMSO-d_6)δ 2.41 (s, 6H, CH_3),4.18 (s, 2H, thiophen-CH_2), 5.48 (s, 2H, NCH_2), 6.73-6.95 (m, 2H, thiophen+arom.H), 7.39-7.45 (m, 4H,thiophen+arom.H), 7.94-7.98 (m, 2H, triazol H); Analysis (%Calculated/found) for $C_{18}H_{16}N_6SO_2$ C:56.83/56.84, H:4.24/4.25, N:22.09/22.00.

2-(2-(4-nitrophenyl)-2-oxoethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (4e): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 65%) to afford the desired compound. Mp 185-186 °C. IR (KBr)(*v*, cm−1) 1711 (acetophenone C=O), 1737 (triazol-3-one C=O), 1602 (-C=N) ; ^{1} H-NMR (DMSO-d_6)δ 4.14(s, 2H, thiophen-CH_2), 5.51 (s, 2H, NCH_2), 6.70-6.94 (m, 2H, thiophen + arom.H), 7.30-7.83 (m, 2H, thiophen+arom.H), 8.26-8.41 (m, 3H, thiophen+ arom. H); Analysis (%Calculated/found) for $C_{17}H_{13}N_7SO_4$ C:49.63/49.65, H:3.19/3.20,N:23.83/23.84.

General method for the synthesis of 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-2-(2-hydroxy-2-arylethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones (5a-e): A mixture of corresponding compound **4** (0.01 mol) and $NaBH_4$ (0.04 mol) in absolute ethanol (50 mL) was refluxed for 4 h. After cooling to room temperature ice water was added to it with vigorous stirring. The solid obtained was filtered off and recrystallized from an appropriate solvent to afford the desired compound.

2-(2-hydroxy-2-phenylethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (5a): Following the general procedure above, a white solid was obtained. It was recrystallizedfrom ethanol/water (1:1) (yield 60%) to afford the desired compound. Mp 186-187 ◦ C. Analysis (%Calculated/found) for $C_{17}H_{16}N_6SO_2$ C:55.42/55.48, H:4.38/4.42, N:22.81/22.82; IR (KBr) (*v* , cm−1) 3434 (OH),1746 (triazol-3-one C=O), 1635 (C=N); ^{1}H-NMR (DMSO-d_6)δ 3.41-3.78 (m, 2H, NCH2), 4.17 (s, 2H, thiophen-CH2), 4.95 (bs, 1H, CH-OH), 5.79 (d, 1H, OH, J= 5 Hz), 6.80-6.98 (m, 8H, thiophen+arom.H), 8.79 (s, 2H,triazol H); Analysis (%Calculated/found) for $C_{17}H_{16}N_6SO_2$ C:55.42/55.48, H:4.38/4.42, N:22.81/22.82.

4-(3,5-dimethyl-4H-1,2,4-triazol-4-yl)-2-(2-hydroxy-2-phenylethyl)-5-(thiophen-2-ylmethyl)-2H-1,2,4-triazol-3(4H)-one (5b): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 60%) to afford the desired compound. Mp 195-196° C. IR (KBr) (*v*, cm−1) 3412 (OH), 1746 (triazol-3-one C=O), 1637 (C=N); ^{1} H-NMR (DMSO-d_6)δ 1.65(s, 3H, CH_3), 1.91 (s, 3H, CH_3), 3.81-3.95 (m, 2H, NCH_2), 4.19 (s, 2H, thiophen-CH2), 4.95 (bs, 1H, CHOH),5.80 (d, 1H, OH, J= 5 Hz), 6.98-7.06 (m, 2H, thiophen+arom.H), 7.27-7.47 (m, 6H, thiophen+arom.H); Analysis (Calculated/found) for $C_{19}H_{20}N_6SO_2$ C:57.56/57.58, H:5.08/5.10, N:21.20/21.24.

4-(3,5-diethyl-4H-1,2,4-triazol-4-yl)-2-(2-hydroxy-2-phenylethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-one (5c): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 58%) to afford the desired compound. M.p 200-201°C. IR (KBr)(ν , cm−1) 3445 (OH), 1747 (triazol-3-one C=O), 1687 (-C=N); ^{1}H-NMR (DMSO-d_6)δ 1.26 (q, 6H, J=3.6 Hz,CH_3), 2.59 (t, 4H, J=3.6 Hz, CH_2), 3.78-3.90 (m, 2H, NCH_2), 4.14 (s, 2H, thiophen-CH_2), 4.96 (bs, 1H, CHOH),5.83 (d, 1H, OH, J= 5 Hz), 6.95-6.97 (m, 2H, thiophen+arom.H), 7.35-7.44 (m, 6H, thiophen+arom.H). Analysis (%Calculated/found) for $C_{21}H_{24}N_6SO_2$ C:59.41/59.43, H:5.70/5.72, N:19.80/19.83.

2-(2-hydroxy-2-p-tolylethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (5d): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 78%) to afford the desired compound. Mp 197-198 ◦ C. IR (KBr) (ν ,cm−1) 3360 (OH), 1748 (triazol-3-one C=O), 1595 (C=N); ^{1} H-NMR (DMSO-d_6)δ 2.29 (s, 3H, CH_3), 3.66-3.94(m, 2H, NCH_2), 4.15 (s, 2H, thiophen-CH_2), 4.90 (bs, 1H, CH-OH), 5.60 (d, 1H, OH, J= 5.6 Hz), 6.65-6.83 (m,3H, thiophen+arom.H), 7.14-7.29 (m, 2H, thiophen+arom.H), 7.42-7.44 (m, 2H, thiophen+arom.H), 8.75 (s,2H, triazolH); Analysis (%Calculated/found) for $C_{18}H_{18}N_6SO_2$ C:56.53/56.55, H:4.74/4.73, N:21.97/21.98.

2-(2-hydroxy-2-(4-nitrophenyl)ethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (5e): Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 78%) to afford the desired compound. M p 188-189°C. IR (KBr)(ν, cm−1) 3350 (OH), 1738 (triazol-3-one C=O), 1600 (C=N); ^{1} H-NMR (DMSO-d_6)δ 3.85-3.94 (m, 2H,NCH_2), 4.01 (s, 2H, thiophen-CH_2), 5.05 (bs, 1H, CH-OH), 6.05 (d, 1H, OH, J= 5.4 Hz), 6.66-6.89 (s, 2H,thiophen+arom.H), 7.41-7.64 (m, 3H, thiophen+arom.H), 8.20 (s, 2H, triazol H); Analysis (%Calculated/found) for $C_{17}H_{15}N_7SO_4$ C:49.39/49.41, H:3.66/3.68,N:23.72/23.73.

General method for the synthesis of ethyl 2-(4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-5-oxo-3-(thiophen-2-yl-methyl)-4,5-dihydro-1,2,4-triazol-1-yl) acetates (6a-c): The corresponding compound3 (0.01 mol) was refluxed with an equivalent amount of natrium in absolute ethanol for 1 h. Then ethylbromoacetate (0.01 mol) was added and refluxed for an additional 5 h. The precipitate was filtered off, washed with H_2O, and recrystallized from appropriate solvent to afford the desired compound.

Ethyl 2-(5-oxo-3-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-4,5-dihydro-1,2,4-triazol-1-yl) acetate (6a): Following the general procedure above, a white solid was obtained. It was recrystallized from ethyl acetate/petroleum ether (1:2) (yield 67.78%) to afford the desired compound. Mp 123-124° C. IR (KBr) (ν, cm−1) 1763 (ester C=O), 1732 (triazol-3-one C=O), 1595 (C=N), 1220 (C-O); 1H-NMR (DMSO-d_6)δ 1.65 (t, 6H,J= 7.0 Hz, OCH_2CH_3 ,), 4.36 (s, 2H, thiophen-CH_2), 4.62 (q, 4H, J= 7.0 Hz, OCH_2CH_3 ,),4.92 (s, 2H, NCH_2), 7.25 (s, 2H, thiophen H), 7.38 (s, 1H, thiophen H), 8.38 (s, 2H, triazol H); Analysis (%Calculated/found) for $C_{13}H_{14}N_6SO_3$ C:46.70/46.71, H:4.22/4.23, N:25.14/25.15.

Ethyl 2-(4-(3,5-dimethyl-4H-1,2,4-triazol-4-yl)-5-oxo-3-(thiophen-2-yl-methyl)-4,5-dihydro-1,2,4-triazol-1-yl) acetate (6b): Following the general procedure above, a white solid was obtained. It was recrystallized from ethyl acetate/petroleum ether (1:2) (yield 66.78%) to afford the desired compound. Mp 155-156°C IR (KBr) (ν, cm−1) 1759 (ester C=O), 1735 (triazol-3-one C=O), 1597 (C=N), 1240 (CO);[1]H-NMR (DMSO-d_6) δ 1.31 (t, 3H, J= 7.2 Hz, OCH_2CH_3), 2.08 (s, 6H, triazol-CH_3), 3.98 (s, 2H,thiophen-CH_2), 4.26 (q, 2H, J= 7.2 Hz, OCH_2CH_3), 4.63 (s, 2H, NCH_2), 6.57 (s, 2H, thiophenH), 6.91(s, 1H, thiophenH); Analysis (%Calculated/found) for $C_{15}H_{18}N_6SO_3$ C:49.71/49.72, H:5.01/5.04, N:23.19/23.20.

Ethyl 2-(4-(3,5-diethyl-4H-1,2,4-triazol-4-yl)-5-oxo-3-(thiophen-2-yl-methyl)-4,5- dihydro-1,2,4-triazol-1-yl) acetate (6c): Following the general procedure above, a white solid was obtained. It was recrystallized from ethyl acetate/petroleum ether (1:2) (yield 72.78%) to afford the desired compound. Mp 143-144°C. IR (KBr) (ν , cm−1) 1754 (ester C=O), 1734 (triazol-3-one C=O), 1595 (C=N), 1240 (C-O); [1] H-NMR(DMSO-d6)δ 1.56-1.59 (m, 9H, -OCH_2CH_3+ triazol-CH_2CH_3), 2.56-2.60 (m, 4H, triazol-CH_2), 4.27 (s, 2H,thiophen-CH2), 4.58 (q, 4H, J= 7.0 Hz, OCH_2CH_3), 4.94 (s, 2H, NCH_2), 6.88 (s, 2H, thiophen H), 7.56 (s,1H, thiophen H); Analysis (%Calculated/found) for $C_{17}H_{22}N_6SO_3$ C:52.29/52.30, H:5.68/5.67, N:21.52/21.53.

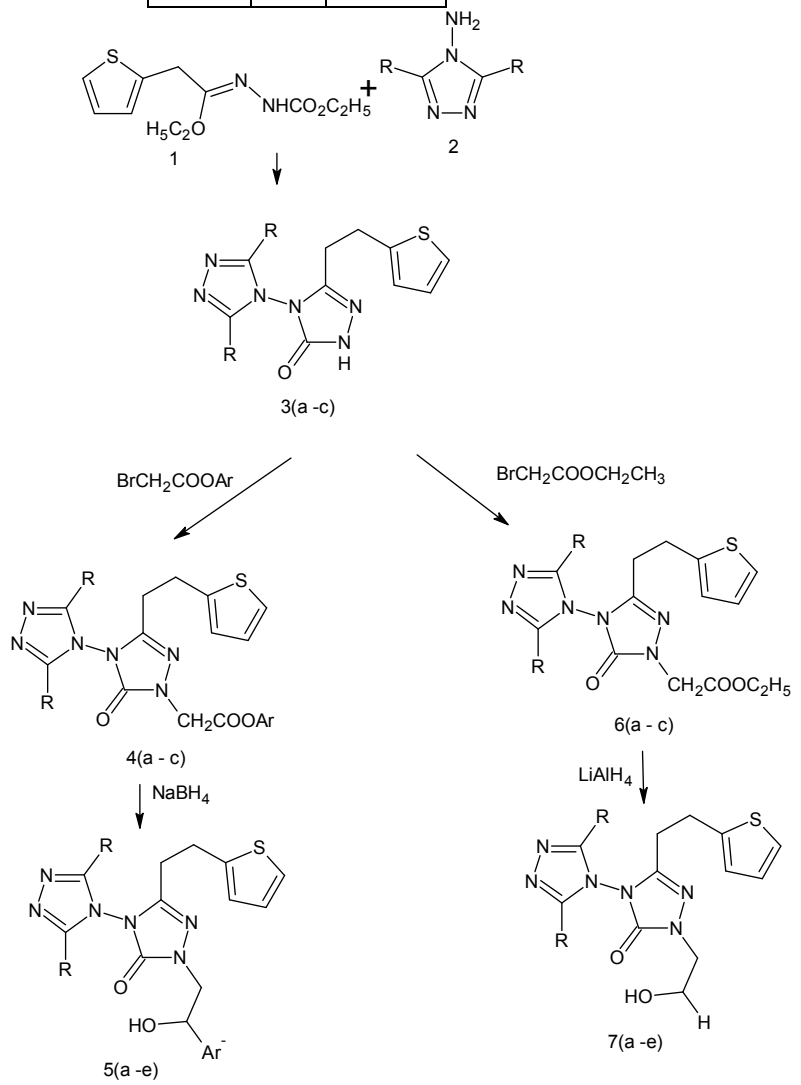
General method for the synthesis of 4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-2-(2-hydroxyethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones7(a-c): A mixture of corresponding compound **6** (0.01 mol) and $LiAlH_4$ (0.04 mol) in absolute ethanol (50 mL) was refluxed for 4 h. After cooling to room temperature ice water was added to it with vigorous stirring. The solid obtained was filtered off and recrystallized from an appropriate solvent to afford the desired compound.

2-(2-hydroxyethyl)-5-(thiophen-2-yl-methyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one (7a): Following the general procedure above, a white solid was obtained. It was recrystallized from ethylacetate/petroleum ether (1:2) (yield 65.80%) to afford the desired compound. Mp 111-112 ◦ C. IR (KBr) (ν , cm−1) 3348 (OH), 1735 (triazol-3-one C=O), 1594 (C=N); [1]H-NMR (DMSO-d6)δ 3.35-3.42 (m, 2H, NCH2),4.44 (s, 2H, thiophen-CH2), 3.79-3.85 (m, 2H, CH2 -OH), 4.78 (bs, 1H, OH), 6.60-6.72 (m, 2H, thiophen),6.91 (s, 1H, thiophen), 8.30 (s, 2H, triazol H); Analysis (%Calculated/found) for $C_{11}H_{12}N_6SO_2$C:45.20/45.23, H:4.14/4.12, N:28.75/ 28.75.

4-(3,5-dimethyl-4H-1,2,4-triazol-4-yl)-2-(2-ethoxy-2-hydroxyethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-one (7b): Following the general procedure above, a white solid was obtained. It was recrystallized from ethyl acetate/petroleum ether (1:2) (yield 63.06%) to afford the desired compound. M.p 135-136°C. IR (KBr) (ν ,cm−1) 3346 (OH), 1738 (triazol-3-one C=O), 1595 (C=N); [1] H-NMR (DMSO-d_6)δ1.55-1.64 (m, 6H, triazol-CH_2CH_3), 2.57-2.63 (m, 4H, triazol-CH_2), 3.35-3.42 (m, 2H, NCH_2), 4.43 (s, 2H,thiophen-CH_2), 3.79-3.88 (m, 2H, CH_2 -OH), 4.78 (bs, OH), 6.60-6.76 (m, 2H, thiophen), 6.96 (s, 1H, thiophen);
Analysis (%Calculated/found) for $C_{13}H_{16}N_6SO_2$ C:48.74/48.74, H:5.03/5.05, N:26.23/26.24.

4-(3,5-diethyl-4H-1,2,4-triazol-4-yl)-2-(2-ethoxy-2-hydroxyethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-one (7c): Following the general procedure above, a white solid was obtained. It was recrystallized from ethyl acetate/petroleum ether (1:2) (yield 62.58%) to afford the desired compound. M.p128-129°C. IR (KBr) (ν, cm−1) 3345 (OH), 1737 (triazol-3-one C=O), 1595 (C=N); [1] H-NMR (DMSO-d_6)δ2.10 (s, 6H, triazol-CH3), 3.35-3.42 (m, NCH_2), 4.43 (s, thiophen-CH_2), 3.79-3.85 (m, 2H,CH_2 -OH), 4.81(bs,OH), 6.60-6.72 (m, 2H, thiophen), 6.91(s,1H,thiophen); Analysis (%Calculated/found) for$C_{15}H_{20}N_6SO_2$ C:51.71/51.70, H:5.79/5.78, N:24.12/24.14.

3,4,5,6,7	R	Ar
a	H	C_6H_5-
b	CH_3	C_6H_5-
c	C_2H_5	C_6H_5-
d	H	$CH_3C_6H_4$-
e	H	$NO_2C_6H_4$-



SCHEME

Antimicribial Activity

Agar well diffusion method: A simple susceptibility screening test using the agar-well diffusion method[24] as adapted earlier[25] was used . Representative organisms selected for evaluation of antifungal activity were *Penicillum* spp., and *Aspergillus* spp., SDA was used.

Antimicrobial activity was evaluated by measuring the inhibition zones against the test organisms. fluconazole (5 μg) was the standard drugs. Ethanol was used as the solved control.

Mycelial growth inhibition test: Mycelial growth inhibition was tested by the agar diffusion method.[26–28] Five-millimeter diameter mycelial agar disks were placed on PDA plates containing test chemicals. The final concentration in the medium was adjusted to 1 mg/mL. The plates were incubated at 25^{o}C for 3 days, and diameters of the mycelium colonies were then measured to examine the effects of the chemicals on fungal growth. The tests were carried out in triplicate. The results are shown in Table 1.

Table 1

Chemicals No	Microorganisms (Colony diameters, mm)	
	***Penicillum* spp.**	***Aspergillus* spp.**
3a	20	15
3b	20	**13**
3c	22	**11**
4a	18	**12**
4b	18	**12**
6a	**8**	**8**
6b	20	**8**
6c	12	**8**
Fluconazole	17	9
With ethanol	20	17
Untreated control	20	17

Results and discussion

In the first part of this study, the synthesis of compounds **3a-c** was performed from condensation of compound **1** with compound **2** in reasonably good yields (Scheme).

Analytical and spectroscopic data of compounds **3a-c** confirmed the success of the cyclization reaction. The IR data indicated the formation of compounds **3a-c** by the disappearance of $COCH_2$ (esteric) band of compound **1** at 1247 cm−1 , and the new band at 1738- 1744 cm−1 belonging to triazole C=O. In the ^{1}H-NMR spectra of compounds **3a-c**, the existence of **3** was revealed by the disappearance the chemical shifts belonging to esteric methylenes (4.18-4.24 ppm) in the precursor **1** after the cyclization and the appearance of a new peak at 11.68-12.20 ppm integrating for one proton (exchangeable with D_2O) belonging to N-H.

4-(3,5-disubstitue-4H-1,2,4-triazol-4-yl)-2-(2-oxo-2-arylethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-ones **4a-e** were obtained from the reaction compounds **3a-c** with bromo acetophenone derivatives in reasonable good yields (Scheme).

The IR spectra of compounds **4a-e** showed 2 sharp absorption bands, one of which, appearing at 1715-1748 cm−1 , was attributed to the carbonyl function of 1,2,4-triazol-3-one ring and the other, observed 1658-1691 cm−1 , at was assigned to C=O stretching frequency corresponding to ketone carbonyl. The NH signal disappeared in the 1 H-NMR and IR spectra of compounds **4a-e**. In the ^{1}H NMR spectra of compounds **4a-e**, a new additional signal belonging to methylene protons of acetophenone was recorded at 4.95-5.51 ppm integrating for one proton. The synthesis of compounds **5a-e** was performed by the reaction of compounds **4** with $NaBH_4$ at the reflux temperature in the presence of absolute ethanol (Scheme). In the IR spectra of compounds **5**, only one carbonyl function (C=O) belonging to 1,2,4-triazol-3-one ring was observed at 1738-1748 cm−1 . While a C=O carbon signal belonging to acetophenon derivatives of compounds **4** was recorded, these signals disappeared and new signals at 4.95-5.05 and 5.60-6.05 ppm belonging to -CH and -OH protons of -CH-OH group of compounds **5** were seen In the 1 H-NMR spectra of compounds **6a-c** additional signals derived from the ester group were observed at 1.31-1.56 ppm (OCH_2CH_3) and 4.26-4.62 ppm (OCH_2CH_3) integrating for 2 protons and 3 protons, respectively. The synthesis of compounds **7a-c** was performed by the reaction of compounds **6** with LiAlH4 at the reflux temperature in the presence of absolute ethanol (Scheme). In the IR spectra of compounds **7**, only one carbonyl function (C=O) belonging to 1,2,4-triazol-3-one ring was observed at 1735-1738 cm−1 . While a C=O carbon signal belonging to the ester group of compounds **6** was recorded, these signals disappeared and new signals at 3.79-3.85 and 4.78-4.81 ppm belonging to CH_2 and OH protons of the CH_2 -OH group of compounds **7** were seen. The chemical compositions of synthesized compounds were confirmed by IR, ^{1}H-NMR, and elemental analyses. The chemicals were found to be inactive against gram-positive and gram-negative bacteria and the yeast like fungi. However, the test chemicals showed antifungal activity against *Penicillum* spp. and *Aspergillus* spp. Chemicals **4** and **6** showed very potent in vitro antifungal activity against *Penicillum* spp. (Table 1). Chemicals **4**, **5**, and **6** also showed antifungal activity against *Aspergillus* spp.

Conclusion

In conclusion, a group of 1, 2, 4-triazole derivatives were synthesized and characterized. All these compounds containing 1, 2, 4-triazole moiety is more active and plays a prominent role in biological activity. The structure of all the compounds were confirmed by IR, PMR and elemental analysis.

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