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Synthesis and characterization of novel triazol compounds containing a thiophen ring as potential antifungal agents and the structure of 2-(2-hydroxy-2-p-tolyethyl)-5-(thiophen-2-ylmethyl)-4-(4H-1,2,4-triazol-4-yl)-2H-1,2,4-triazol-3(4H)-one

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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-ylmethyl)-4,5-dihydro-1,2,4-triazol-1-yl)acetates (**6a-c**) were obtained by reaction of compounds **3** and bromoacetophenon derivatives and bromo ethylacetate, 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, ^{1}H-NMR, and ^{13}C-NMR spectral data. The structure of compound **5d** was inferred through IR, ^{1}H-, ^{13}C-NMR, elemental analyses, and X-ray spectral techniques. 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

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Introduction

The 1,2,4-triazol 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 azole 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- triazol 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 D2 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 triazol derivatives containing thiophen (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 12 standard organisms including bacterial and fungal strains. In addition, the crystal data for compound **5d,** Orthorhombic, space group *Pbca*, a = 11.2122 (5), b = 15.2298 (6), c = 22.4200 (9) (Å), V=3828.4(3) (Å^3), Z = 8, are given in Experimental section.

Experimental

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-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. Elemental analyses were performed on a Hewlett-Packard 185 CHN analyzer; their values agreed with the calculated ones. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). 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-triazol **(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, thiophen H), 12.20 (s, NH); ^{13}C-NMR (DMSOd_6) δ 25.76 (thiophen-CH_2), thiophen C:[125.76 (CH), 127.65 (CH), 127.71 (CH), 135.63 (C)], 143.76 (C=N triazol-3-one), 144.02 (C=N triazol), 150.92 (C=O triazol-3-one); Analysis (%Calculated/found) for $C_{11}H_{12}N_6SO$ 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); ^{13}C-NMR (200 MHz, DMSOd_6)δ 26.64 (thiophen-CH_2), thiophen C:[126.55 (CH), 127.70 (CH), 127.71 (CH), 135.40 (C)], 138.28 (C=N triazol-3-one), 144.12 (C=N triazol), 154.86 (C=O triazol-3-one); Analysis (%Calculated/found) for $C_{13}H_{16}N_6SO$ 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 bromoacetophenon 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 (acetophenon 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, NCH_2), 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, triazol H); ^{13}C-NMR (DMSO-d_6) δ (ppm) 26.49 (thiophen-CH_2), 52.29 (NCH_2), thiophen C:[126.33 (CH), 127.27 (CH), 127.74 (CH), 133.91 (C)], arom. C: [128.06 (CH), 129.07 (CH), 133.90 (CH), 134.47 (C)], 143.30 (C=N triazol-3-one), 144.65 (C=N triazol) 167.60 (C=O triazol-3-one), 191.07 (C=O acetophenon); 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. Mp 115-116 °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 (acetophenon 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, CH_3), 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); ^{13}C-NMR (DMSO-d_6) δ (ppm) 13.70 (CH_2), 15.80 (CH_3), 25.90 (thiophen-CH_2), 60.10 (NCH_2), thiophen C:[123.40 (CH), 125.00 (CH), 126.50 (CH), 139.40 (C)], ar C: [128.45 (CH), 128.65 (CH), 132.90

(CH),137.40 (C)], 155.20 (C=N triazol-3-one), 160.45 (C=N triazol), 162.65 (C=O triazol-3-one), 192.07 (C=O acetophenon); 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.

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. Mp 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); ^{13}C-NMR (DMSO-d_6)δ (ppm) 21.17 (CH_3), 25.08 (thiophen-CH_2), 52.15 (NCH_2), thiophen C:[126.00 (CH), 127.00 (CH), 128.23 (CH), 134.22 (C)], ar C: [129.37 (CH), 131.44 (CH), 136.92 (CH), 142.81 (C)], 143.35 (C=N triazol-3-one), 144.70 (C=N triazol), 150.14 (C=O triazol-3-one), 191.79 (C=O acetophenon); 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) (ν, cm^{-1}) 1711 (acetophenon 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); ^{13}C-NMR (DMSO-d_6)δ (ppm) 25.93 (thiophen-CH_2), 52.73 (NCH_2), thiophen C:[125.83 (CH), 127.06 (CH), 127.25 (CH), 134.04 (C)], ar C: [119.36 (CH), 123.93 (CH), 129.53 (CH), 138.56 (C)], 142.44 (C=N triazol-3-one), 143.76 (C=N triazol), 150.59 (C=O triazol-3-one), 190.80 (C=O acetophenon); 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 recrystallized from 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) (ν, 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, NCH_2), 4.17 (s, 2H, thiophen-CH_2), 4.95 (bs, 1H, C<u>H</u>-OH), 5.79 (d, 1H, OH, J= 5 Hz), 6.80-6.98 (m, 8H, thiophen+arom.H), 8.79 (s, 2H, triazol H); ^{13}C-NMR (DMSO-d_6)δ 25.13 (thiophen-CH_2), 52.90 (N-CH_2), 69.88 (CH-OH); thiophen-C:[125.97 (CH), 127.00 (CH), 127.43 (CH), 134.42 (C)], ar-C: [127.32 (CH), 128.15 (CH), 129.00 (CH), 131.00 (C)], 142.08 (C=N triazol-3-one), 142.70 (triazol C=N), 149.47 (C=O triazol-3-one); 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-yl-methyl)-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) (ν, 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-CH_2), 4.95 (bs, 1H, C<u>H</u>-OH), 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); ^{13}C-NMR (DMSO-d_6)δ (ppm) 8.21 (CH_3), 8.45(CH_3), 25.08 (thiophen-CH_2), 52.17 (N-CH_2), 70.04 (CH-OH); thiophen-C:[126.14 (CH), 127.28 (CH), 127.42 (CH), 134.74 (C)], ar-C: [126.46 (CH), 128.15 (CH), 128.35 (CH), 131.15(C)], 141.94 (C=N triazol-3-one), 142.55 (triazol C=N), 150.19 (C=O triazol-3-one); 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. Mp 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, C<u>H</u>-OH), 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). ^{13}C-NMR (DMSO-d_6)δ 13.68 (CH_2), 15.82 (CH_3), 25.10 (thiophen-CH_2), 52.15 (N-CH_2), 70.10 (CH-OH); thiophen-C: [126.17 (CH), 127.31 (CH), 127.46 (CH), 134.79 (C)], ar-C: [126.49 (CH), 128.21 (CH), 128.39 (CH), 131.17 (C)], 141.98 (C=N triazol-3-one), 142.58 (triazol C=N), 150.21 (C=O triazol-3-one); 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, C<u>H</u>-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); ^{13}C-NMR (DMSO-d_6)δ 20.62 (CH_3), 25.08 (thiophen-CH_2), 52.08 (N-CH_2), 69.76 (CH-OH); thiophen-C:[125.92 (CH), 126.91 (CH), 127.00 (CH), 134.40 (C)], ar-C: [128.71 (CH), 136.50 (CH), 138.79 (CH), 139.79(C)], 142.59 (C=N triazol-3-one), 142.69 (triazol C=N), 149.67 (C=O); 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. Mp 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, C<u>H</u>-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); ^{13}C-NMR (DMSO-d_6) δ 25.00 (thiophen-CH_2), 52.50 (N-CH_2), 70.00 (CH-OH); thiophen-C:[125.37 (CH), 126.97 (CH), 127.39 (CH), 134.31 (C)], ar-C: [123.34 (CH), 129.63 (CH), 132.82 (CH), 133.79 (C)], 142.73 (C=N triazol-3-one), 146.82 (triazol C=N), 149.82 (C=O); 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 compound 3 (0.01 mol) was refluxed with an equivalent amount of natrium in absolute ethanol for 1 h. Then ethyl

bromoacetate (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); ^{1}H-NMR (DMSO-d_6) δ 1.65 (t, 6H, J= 7.0 Hz, $OCH_2C\underline{H}_3$,), 4.36 (s, 2H, thiophen-CH_2), 4.62 (q, 4H, J= 7.0 Hz, $OC\underline{H}_2CH_3$,), 4.92 (s, 2H, NCH_2), 7.25 (s, 2H, thiophen H), 7.38 (s, 1H, thiophen H), 8.38 (s, 2H, triazol H); ^{13}C-NMR (DMSO-d_6) δ (ppm) 14.10 (-$OCH_2\underline{C}H_3$), 26.42 (thiophen-CH_2), 47.16 (-$O\underline{C}H_2CH_3$), 62.28 (NCH_2), thiophen C:[126.35 (CH), 127.31 (CH), 127.73 (CH), 133.68 (C)], 143.29 (C=N triazol), 150.07 (C=N triazol-3-one), 157.07 (C=O triazol-3-one), 167.01 (C=O ester); 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 (C-O); ^{1}H-NMR (DMSO-d_6) δ 1.31 (t, 3H, J= 7.2 Hz, $OCH_2C\underline{H}_3$), 2.08 (s, 6H, triazol-CH_3), 3.98 (s, 2H, thiophen-CH_2), 4.26 (q, 2H, J= 7.2 Hz, $OC\underline{H}_2CH_3$), 4.63 (s, 2H, NCH_2), 6.57 (s, 2H, thiophenH), 6.91 (s, 1H, thiophenH); ^{13}C-NMR (DMSO-d_6)δ 9.07 (CH_3 triazole), 14.12 ($OCH_2\underline{C}H_3$), 26.57 (thiophen-CH_2), 47.43($O\underline{C}H_2CH_3$), 62.27 (NCH_2), thiophen-C:[126.56 (CH),127.56 (CH), 127.74 (CH), 133.40(C)], 143.63 (C=N triazol), 150.63 (C=N triazol-3-one), 150.64 (C=O triazol-3-one), 166.84 (C=O ester); 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-d_6)δ 1.56-1.59 (m, 9H, -$OCH_2C\underline{H}_{3+}$ triazol-$CH_2C\underline{H}_3$), 2.56-2.60 (m, 4H, triazol-CH_2), 4.27 (s, 2H, thiophen-CH_2), 4.58 (q, 4H, J= 7.0 Hz, $OC\underline{H}_2CH_3$), 4.94 (s, 2H, NCH_2), 6.88 (s, 2H, thiophen H), 7.56 (s, 1H, thiophen H); ^{13}C-NMR (DMSO-d_6)δ 10.56, 14.05 ($OCH_2\underline{C}H_3$+triazolCH_3), 15.22 (triazol-CH_2), 26.47 (thiophen-CH_2), 47.32 ($O\underline{C}H_2CH_3$), 62.18 (N-CH_2), thiophen-C:[126.91 (CH), 126.60 (CH), 127.53 (CH), 133.32(C)], 143.56 (C=N triazol), 150.25 (C=N triazol-3-one), 154.77 (C=O triazol-3-one), 167.79 (C=O ester); 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 ethyl acetate/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-d_6)δ 3.35-3.42 (m, 2H, NCH_2), 4.44 (s, 2H, thiophen-CH_2), 3.79-3.85 (m, 2H, CH_2-OH), 4.78 (bs, 1H, OH), 6.60-6.72 (m, 2H, thiophen), 6.91 (s, 1H, thiophen), 8.30 (s, 2H, triazol H); ^{13}C-NMR (DMSO-d_6)δ 25.90 (thiophen-CH_2), 42.75 (N-CH_2), 56.80 (CH_2-OH); thiophen-C:[126.87 (CH), 126.65 (CH), 127.55 (CH), 133.38 (C)], 144.47 (triazol C=N), 155.08 (C=N triazol-3-one), 162.18 (C=O triazol-3-one); 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.

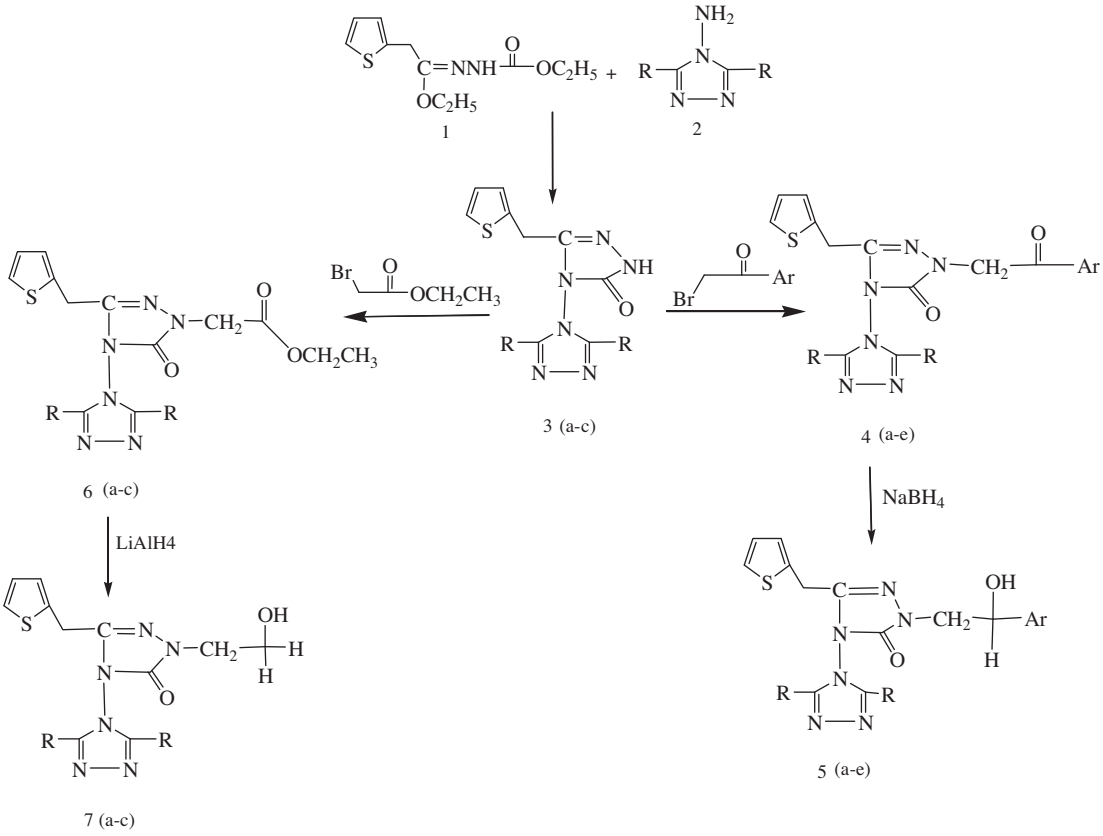
4-(3,5-dimethyl-4H-1,2,4-triazol-4-yl)-2-(2-ethoxy-2-hydroxyethyl)-5-(thiophen-2-yl-methyl)-2H-1,2,4-triazol-3(4H)-one (7b): Following the general procedure above, a white solid was obtained. It was recrystallized from ethyl acetate/petroleum ether (1:2) (yield 63.06%) to afford the desired compound. Mp 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)δ 1.55-1.64 (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); ^{13}C-NMR (DMSO-d_6)δ 25.87 (thiophen-CH_2), 42.75 (N-CH_2), 56.88 (CH_2-OH); thiophen-C: [126.58 (CH),127.66 (CH), 127.78 (CH), 133.40(C)], 144.49 (triazol C=N), 155.07 (C=N triazol-3-one), 162.21 (C=O triazol-3-one); 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. Mp 128-129 °C. IR (KBr) (ν, cm^{-1}) 3345 (OH), 1737 (triazol-3-one C=O), 1595 (C=N); ^{1}H-NMR (DMSO-d_6)δ 2.10 (s, 6H, triazol-CH_3), 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); ^{13}C-NMR (DMSO-d_6) δ 25.86 (thiophen-CH_2), 42.74 (N-CH_2), 56.86 (CH_2-OH); thiophen-C: [126.95 (CH), 126.67 (CH), 127.55 (CH), 133.36 (C)], 144.48 (triazol C=N), 155.07 (C=N triazol-3-one), 162.17 (C=O triazol-3-one); 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.

Microbiology: All test microorganisms except for Penicillum spp. and Aspergillus spp. were obtained from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 10145, Yersinia pseudotuberculosis ATCC 911, Klebsiella pneumoniae ATCC 13883, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633, Candida albicans ATCC 60193, Candida tropicalis ATCC 13803, and Candida glabrata ATCC 66032. Penicillum spp. and Aspergillus spp. were isolated from soil. The chemicals were dissolved in ethanol to prepare chemical stock solutions of 1 mg/mL.

Agar well diffusion method: A simple susceptibility screening test using the agar-well diffusion method[24] as adapted earlier[25] was used. Microorganisms were suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI, USA) broth and diluted approximately 10^6 colony forming unit (cfu) per mL. They were "flood-inoculated" onto the surface of BHI agar and Sabouraud Dextrose agar (SDA) (Difco) and then dried. For *C. albicans*, *C. tropicalis*, *C. glabrata*, *Penicillum* spp., and *Aspergillus* spp., SDA was used. Five-millimeter diameter wells were cut from the agar by using a sterile cork-borer, and filled with 500 μg/50 μL of the chemical substances. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the inhibition zones against the test organisms. The tests were carried out in duplicate. Ceftazidime (10 μg)

and fluconazole (5 μg) were the standard drugs. Ethanol was used as the solved control. The data are not shown.



3,4,5,6,7	R	Ar
a	-H	
b	$-CH_3$	
c	$-C_2H_5$	
d	-H	$-CH_3$
e	-H	$-NO_2$

Scheme. Synthetic pathway for the preparation of target compounds (**3, 4, 5, 6,** and **7).**

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 °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

Crystallographic structure determination of compound 5d

The crystal structure of compound **5d** ($C_{18}H_{18}N_6O_2$ S) with dimensions of 0.29 mm × 0.16 mm × 0.12 mm was determined by single crystal X-ray diffraction. X-ray data for compound 5d were collected with a Bruker Smart Area-CCD diffractometer by w-scan mode using Cu-Kα radiation($\lambda = 1.54178$ Å). For compound 5d cell refinement: Bruker *SMART*[29] ; data reduction: Bruker *SMART*[29]; program used to solve structure: *SHELXS-97*[30]; program used to refine structure: *SHELXL-97*[30]; molecular graphics: *ORTEP-3 for Windows*[31]; software used to prepare material for publication: *WinGX*[32]. The geometrical parameters are given in Table 2.

Crystal data for $C_{18}H_{18}N_6O_2$ S: $Mr = 382.44$, Orthorhombic, space group *Pbca,* T =293(2)K, μ(a= 11.2122 (5), b = 15.2298 (6), c = 22.4200 (9) (Å), V=3828.4(3) (Å^3), Z = 8. 17,746 measured reflections, 3424 were unique ($R_{int} = 0.066$), R_1 , GOF = 1.05, largest peak and hole in final Fourier difference map were 0.38 and –0.29 e. Å^{-3}.

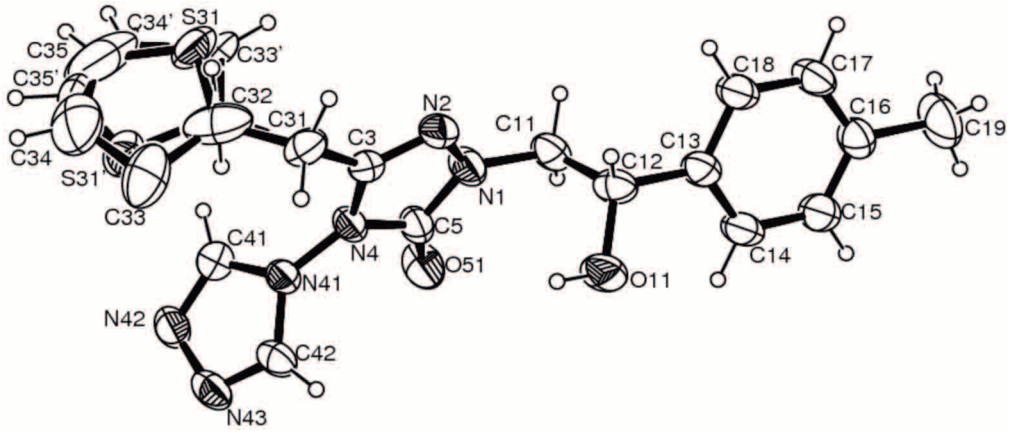
Supplementary material

The compound **5d** consists of two triazole, one benzene and one thiophene ring. The thiophene ring of compound **5d** is disordered over 2 positions.The length of N1-N2 and the length of N42-N43 bonds in the triazole rings are [1.405 (4) Å] and [1.371 (4) Å], respectively. Because of the substituents connected to N1 atom, the N1-N2 bond is longer than N42-N43 bond and these lengths are compatible to the values in the literature [1.404 (4) Å

Table 2. Crystallographic data for compound **5d.**

	ev10552s
Crystal data	
Chemical formula	$C_{18}H_{18}N_6O_2S$
M_r	382.44
Cell setting, space group	Orthorhombic, *Pbca*
Temperature (K)	293 (2)
a, b, c (Å)	11.2122 (5), 15.2298 (6), 22.4200 (9)
V (Å^3)	3828.4 (3)
Z	8
D_x (Mg m^{-3})	1.327
Radiation type	Cu K
Crystal form, colour	Prism, colourless
Crystal size (mm)	0.29 × 0.16 × 0.12
Data collection	
Diffractometer	Bruker CCD 6000 area detector
Data collection method	phi and scans
Absorption correction	None
No. of measured, independent and observed reflections	17,746, 3424, 2004
Criterion for observed reflections	$I > 2(I)$
R_{int}	0.066
Refinement	
Refinement on	F^2
No. of relections	3424 reflections
No. of parameters	301
H-atom treatment	Constrained to parent site
Weighting scheme	Calculated $w = 1/[(F_o^2) + (0.1756P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$

in $C_{18}H_{14}Cl_2N_4O_2$; 1.378 (3) Å in $C_{18}H_{12}ClN_5$]. The C5= O51 [1.202 (4) Å][33−34] bond length has nearly the same value with the C= O [1.209 (2) Å in $C_{12}H_{11}ClN_4O_2$][35] double bond length in the triazole rings. The dihedral angle between the triazole rings is 87,88 (12)^o and this points, that the triazole rings are nearly perpendicular to each other.



The crystal structure is stabilized by C-H. . .N, O-H. . .N and C-H. . .O intra- and intermolecular hydrogen bonds.

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. ^{13}C-NMR spectra data were also in agreement with the proposed structures.

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 acetophenon 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 acetophenon was recorded at 4.95-5.51 ppm integrating for one proton. The signals belonging to carbonyl carbon of ketone group were seen at 190.80-191.75 ppm in the ^{13}C-NMR spectra of compounds **4**.

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. The signals of reduced (-CH-OH) carbon atom of compounds **5** were observed at 69.88-70.04 ppm in the ^{13}C-NMR spectra respectively. New compounds **6** were obtained synthesized via the nucleophilic attack of N-1 on 5-oxo-[1,2,4] triazol ring to bromine-bearing C atom of ethyl bromoacetate (Scheme).

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 ($OCH_2C\underline{H}_3$) and 4.26-4.62 ppm ($OC\underline{H}_2CH_3$) integrating for 2 protons and 3 protons, respectively. In the ^{13}C-NMR spectra of these compounds, the signals belonging to the same groups were recorded at 14.05-14.12 and 47.16-47.43 ppm, respectively.

The synthesis of compounds **7a-c** was performed by the reaction of compounds **6** with $LiAlH_4$ 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 signals of reduced (-CH_2-OH) carbon atom of compounds **7** were observed at 56.80-56.88 ppm in the ^{13}C-NMR spectra.

The chemical compositions of synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analyses. All spectral data were presented in experimental section. In addition, only the **5d** crystal was suitable for single-crystal X-ray structure determination and a summary of crystallographic data and details of the structure refinement are listed in Table 2. The X-ray crystal structure data of compound **5d** verified the structure of the compound besides IR, ^{1}H-NMR, ^{13}C-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.

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