

# Synthesis and Antimicrobial Evaluation of Novel Di-triazoles and 4-Arylidene Amino 4,5 Dihydro-1H-[1,2,4] triazole-5-one Derivatives

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A series of novel di-[3(thiophen-2-yl-methyl)-4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-alkanes (**2a-h**) were obtained by the reaction of N'-1-ethoxy-2-thiophen-2-yl-ethyldene hydrazino carboxylic acid ethyl ester (**1**) and diamines. Compound **3** was reacted with aldehydes and 4-(arylidene-amino)-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-ones (**4**, **5**, and **8**) with Schiff base character were synthesized. (4-(arylidene-amino)-5-oxo-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-1-yl)-acetic acid ethyl esters (**6**, **7**, and **9**) were obtained by the reaction of 4-(arylidene-amino)-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-ones (**4**, **5**, and **8**) and ethyl bromoacetate. The structures of the new compounds were inferred through IR, <sup>1</sup>H/<sup>13</sup>C NMR, elemental analyses, and mass spectral data. Compound **8i** was characterized by IR, <sup>1</sup>H/<sup>13</sup>C NMR, elemental analyses, mass, and X-ray spectral techniques. Geometry optimization of compounds **2a**, **2c**, **2f**, **4**, and **5** was achieved by computer using the AM1 method.

Compounds **2f**, **4**, **5**, **6**, **7**, **8i**, and **9k** showed good antifungal activity only against yeast fungi, while compound **2d** showed antimicrobial activity against the bacteria *Pseudomonas aeruginosa* ATCC10145, *Enterococcus faecalis* ATCC29212 and the yeast fungi *Candida albicans* ATCC 60193 and *Candida tropicalis* ATCC 13803.

**Key Words:** Triazole-5-one, Schiff base, 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- triazole residues are fluconazole,<sup>1</sup> the powerful azole antifungal agent, as well as the potent antiviral N-nucleoside ribavirin.<sup>2</sup> Furthermore, various 1,2,4-triazole derivatives have been reported as having fungicidal,<sup>3</sup> insecticidal,<sup>4</sup> and antimicrobial activity,<sup>5</sup> and

some showed antitumor activity,<sup>6a-b</sup> as well as being anticonvulsants,<sup>7</sup> antidepressants,<sup>8</sup> and plant growth regulator anticoagulants.<sup>9</sup> Other laboratories reported the biological activity of the triazole family.<sup>10-12</sup>

Moreover, some biheterocyclic compounds incorporating [1,3,4] thiadiazole and [1,2,4] triazole rings have been produced as antimicrobial agents.<sup>13-18</sup> It was reported that bis (4-aryl-1,2,4-triazoline-3-thione-5-yl) pentane, their sulfides and sulfones, as well as bis (2-aryl-amino-1,3,4-thiadiazol-5-yl) pentanes were synthesized to study their antimicrobial activities.<sup>19</sup> Compounds having triazole moieties such as vorozole, anastrozole, and letrozole appear to be very effective aromatase inhibitors very useful for preventing breast cancer.<sup>20-22</sup> The 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazole-5-one compounds, studied in detail by Ikizler,<sup>23</sup> were reported to be good nucleophiles in most reactions. For example, 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazole-5-ones were obtained via nucleophilic attack of amino nitrogen at position 4 on the 1,2,4-triazole-5-one ring to carbonyl carbon of various aldehydes. It has also been reported that the conversion of the amino group in the 4 position in the 1,2,4-triazole ring into an arylidene amino group causes antitumor activity.<sup>24</sup>

Here we report the synthesis of a series of di [(3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl)n-alkanes, 4-(arylidene-amino)-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-ones, and 4-(arylidene-amino)-5-oxo-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-1-yl-acetic acid ethyl esters and their antimicrobial activities against the bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC10145, *Yersinia pseudotuberculosis* ATCC 911, *Klebsiella pneumonia* ATCC 13883, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 roma, the yeast fungi *Candida albicans* ATCC 60193, and *Candida tropicalis* ATCC 13803. In addition, the crystal data for compound **8i** [Monoclinic, space group P 2<sub>1</sub>/n with cell dimensions of a = 9.4654(4) Å, b = 10.2344(3) Å, c = 13.6653(5) Å, β = 100.864(3), and V = 1300.07(8)] is given in the experimental section and geometry optimization of compounds **2a**, **2c**, **2f**, **4**, and **5** was performed using the molecular mechanics MM+ module and AM1 semiempirical calculations in the HyperChem 6.03 molecular modeling program package.<sup>25</sup>

## Experimental

### Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. <sup>1</sup>H-NMR and <sup>13</sup>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. The MS spectra were measured with a Micromass Quattro LC/ULTIMA LC-MS/MS spectrometer with ethanol as solvent. All experiments were performed in the positive ion mode. 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**, **3**, and **4** were synthesized by published methods<sup>26-28</sup> respectively.

**General method for the synthesis of Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4] triazole-5-one-4yl]n-alkanes (2):** To a solution of corresponding compound **1** (0.02 mol) in 50 mL of water was added diamine (0.01 mol). Having refluxed this mixture for 4 h the precipitate formed was filtered off. The solid obtained was washed with H<sub>2</sub>O and crystallized from appropriate solvent to afford the desired compound.

**1,3-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-propane (2a):**

Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:2) (yield 69.65%) to afford the desired compound. mp 102-103 °C. Analysis (Calc/found %): for C<sub>17</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 50.73/50.75, H: 4.51/4.52, N: 20.88/20.87; IR (KBr) cm<sup>-1</sup>: 3179 ( $\nu_{NH}$ ), 1700 ( $\nu_{C=O}$ ), 1579 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.52 (bs, CH<sub>2</sub>, 2H), 3.45 (bs, NCH<sub>2</sub>, 4H), 4.09 (s, thiophen-CH<sub>2</sub>, 4H), 6.94-7.43 (m, ar-H, 6H), 11.58 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)  $\delta$  26.06 (CH<sub>2</sub>), 27.83 (2thiophen-CH<sub>2</sub>), 40.58 (2NCH<sub>2</sub>), thiophen-C: [125.52 (2CH), 126.46 (2CH), 126.97 (2CH), 137.34 (2C)], 145.46 (2C=N), 154.79 (2C=O). MS: m/z 403.82 (M+1)<sup>+</sup>.

**1,5-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-pentane (2b):**

Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:2) (yield 68.76%) to afford the desired compound. mp 167-168 °C. Analysis (Calc/found %): for C<sub>19</sub>H<sub>22</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 53.00/53.01, H: 5.15/5.14, N: 19.52/19.53; IR (KBr) cm<sup>-1</sup>: 3165 ( $\nu_{NH}$ ), 1694 ( $\nu_{C=O}$ ), 1581 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.05 (bs, CH<sub>2</sub>, 6H), 3.42 (bs, NCH<sub>2</sub>, 4H), 4.15 (s, thiophen-CH<sub>2</sub>, 4H), 6.94-7.43 (m, ar-H, 6H), 11.56 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)  $\delta$  22.86(CH<sub>2</sub>), 26.13 (2CH<sub>2</sub>), 27.66 (2thiophen-CH<sub>2</sub>), 40.59 (2NCH<sub>2</sub>), thiophen-C: [125.46 (2CH), 126.49 (2CH), 126.92 (2CH), 137.65 (2C)], 145.64 (2C=N), 154.88 (2C=O). MS: m/z 431.10 (M+1)<sup>+</sup>.

**1,6-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-hexane (2c):**

Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:2) (yield 75.22%) to afford the desired compound. mp 198-199 °C. Analysis (Calc/found %): for C<sub>20</sub>H<sub>24</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 54.03/54.03, H: 5.44/5.43, N: 18.90/18.91; IR (KBr) cm<sup>-1</sup>: 3168( $\nu_{NH}$ ), 1698 ( $\nu_{C=O}$ ), 1580 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.03 (bs, CH<sub>2</sub>, 8H), 3.41 (bs, NCH<sub>2</sub>, 4H), 4.15 (s, thiophen-CH<sub>2</sub>, 4H), 6.94-7.43 (m, ar-H, 6H), 11.57 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)  $\delta$  25.47 (2CH<sub>2</sub>), 26.33 (2CH<sub>2</sub>), 28.06 (2thiophen-CH<sub>2</sub>), 40.58 (2NCH<sub>2</sub>), thiophen-C: [125.59 (2CH), 126.67 (2CH), 127.14 (2CH), 137.77 (2C)], 145.95 (2C=N), 155.12 (2C=O). MS: m/z 445.15 (M+1)<sup>+</sup>.

**1,7-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-heptane (2d):**

Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 69.45%) to afford the desired compound. mp 105-106 °C. Analysis (Calc/found %): for C<sub>21</sub>H<sub>26</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 55.00/55.01, H: 5.71/5.72, N: 18.33/18.31; IR (KBr) cm<sup>-1</sup>: 3168 ( $\nu_{NH}$ ), 1698 ( $\nu_{C=O}$ ), 1580 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.03 (bs, CH<sub>2</sub>, 10H), 3.41 (bs, NCH<sub>2</sub>, 4H), 4.15 (s, thiophen-CH<sub>2</sub>, 4H), 6.94-7.42 (m, ar-H, 6H), 11.57 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) $\delta$  25.64 (CH<sub>2</sub>), 26.20 (2CH<sub>2</sub>), 27.95 (2CH<sub>2</sub>), 28.06 (2thiophen-CH<sub>2</sub>), 40.58 (2NCH<sub>2</sub>), thiophen-C: [125.59 (2CH), 126.67 (2CH), 127.14 (2CH), 137.77 (2C)], 145.95 (2C=N), 155.12 (2C=O). MS: m/z 459.19 (M+1)<sup>+</sup>.

**1,8-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-octane (2e):**

Following the general procedure above, a white solid was obtained. It was recrystallized from DMSO/water (1:1) (yield 70.76%) to afford the desired compound. mp 159-160 °C. Analysis (Calc/found %): for C<sub>22</sub>H<sub>28</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 55.91/55.90, H: 5.97/5.98, N: 17.78/17.77; IR (KBr) cm<sup>-1</sup>: 3159 ( $\nu_{NH}$ ), 1699 ( $\nu_{C=O}$ ), 1578 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.07 (bs, CH<sub>2</sub>, 12H), 3.41 (bs, NCH<sub>2</sub>, 4H), 4.16 (s, thiophen-CH<sub>2</sub>, 4H), 6.95-7.44 (m, ar-H, 6H), 11.56 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) $\delta$  25.70 (2CH<sub>2</sub>), 26.19 (2CH<sub>2</sub>), 28.03 (2CH<sub>2</sub>), 28.22 (2thiophen-CH<sub>2</sub>), 41.46 (2NCH<sub>2</sub>), thiophen-C: [125.29 (2CH), 126.45 (2CH), 127.24 (2CH), 136.54 (2C)], 146.08 (2C=N), 156.00 (2C=O). MS: m/z 473.15 (M+1)<sup>+</sup>.

**1,9-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-nonane (2f):**

Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water

(1:1) (yield 68.31%) to afford the desired compound. mp 78-79 °C. Analysis (Calc/found %): for C<sub>23</sub>H<sub>30</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 56.76/56.77, H: 6.21/6.20, N: 17.27/17.27; IR (KBr) cm<sup>-1</sup>: 3183 ( $\nu_{NH}$ ), 1701 ( $\nu_{C=O}$ ), 1587 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.05 (bs, CH<sub>2</sub>, 14H), 3.40 (bs, NCH<sub>2</sub>, 4H), 4.16 (s, thiophen-CH<sub>2</sub>, 4H), 6.95-7.44 (m, ar-H, 6H), 11.56 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) $\delta$  25.76 (CH<sub>2</sub>), 26.19 (2CH<sub>2</sub>), 28.06 (2CH<sub>2</sub>), 28.29 (2CH<sub>2</sub>), 28.48 (2thiophen-CH<sub>2</sub>), 40.58 (2NCH<sub>2</sub>), thiophen-C: [125.43 (2CH), 126.44 (2CH), 126.93 (2CH), 137.73 (2C)], 145.70 (2C=N), 154.92 (2C=O). MS: m/z 487.16 (M+1)<sup>+</sup>.

**1,10-Di-[3(thiophen-2-yl-methyl)4,5-dihydro-1H-[1,2,4]triazole-5-one-4yl]n-decane (2g):**

Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:1) (yield 72.00%) to afford the desired compound. mp 171-172 °C. Analysis (Calc/found %): for C<sub>24</sub>H<sub>32</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 56.57/56.55, H: 6.44/6.45, N: 16.79/16.78; IR (KBr) cm<sup>-1</sup>: 3160 ( $\nu_{NH}$ ), 1698 ( $\nu_{C=O}$ ), 1576 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.12 (bs, CH<sub>2</sub>, 16H), 3.45 (bs, NCH<sub>2</sub>, 4H), 4.16 (s, thiophen-CH<sub>2</sub>, 4H), 6.95-7.44 (m, ar-H, 6H), 11.56 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) $\delta$  25.81 (2CH<sub>2</sub>), 26.21 (2CH<sub>2</sub>), 28.10 (2CH<sub>2</sub>), 28.39 (2CH<sub>2</sub>), 28.61 (2thiophen-CH<sub>2</sub>), 40.61 (2NCH<sub>2</sub>), thiophen-C: [125.44 (2CH), 126.45 (2CH), 126.92 (2CH), 137.75 (2C)], 145.70 (2C=N), 154.93 (2C=O). MS: m/z 501.12 (M+1)<sup>+</sup>.

**1,12-Di-[3(thiophen-2-yl-methyl) 4,5-dihydro-1H-[1,2,4] triazole-5-one-4yl] n-dodecane (2h):** Following the general procedure above, a white solid was obtained. It was recrystallized from DMSO/water (1:1) (yield 70.45%) to afford the desired compound. mp 149-150 °C. Analysis (Calc/found %): for C<sub>26</sub>H<sub>34</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> C: 59.06/59.05, H: 6.86/6.86, N: 15.89/15.88; IR (KBr) cm<sup>-1</sup>: 3181 ( $\nu_{NH}$ ), 1701 ( $\nu_{C=O}$ ), 1584 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  1.16 (bs, CH<sub>2</sub>, 20H), 3.44 (bs, NCH<sub>2</sub>, 4H), 4.16 (s, thiophen-CH<sub>2</sub>, 4H), 6.95-7.44 (m, ar-H, 6H), 11.54 (s, NH, 2H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) $\delta$  25.82 (2CH<sub>2</sub>), 26.19 (2CH<sub>2</sub>), 28.09 (2CH<sub>2</sub>), 28.43 (2CH<sub>2</sub>), 28.71 (2CH<sub>2</sub>), 28.77 (2thiophen-CH<sub>2</sub>), 43.00 (2NCH<sub>2</sub>), thiophen-C: [125.42 (2CH), 126.44 (2CH), 126.89 (2CH), 137.72 (2C)], 145.69 (2C=N), 154.91 (2C=O). MS: m/z 529.27 (M+1)<sup>+</sup>.

**4-[(3-Hydroxy-4-methoxy-benzylidene)-amino]-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-one (5):** 4-amino-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-one (**3**) (0.01 mol) and vanillin (0.01 mol) were heated at 160 °C in an oil bath for 2 h. After cooling to room temperature, a solid appeared and it was crystallized from methanol (yield 80.00%) to afford the desired compound. mp 231-232 °C. Analysis (Calc/found %): for C<sub>15</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>S C: 54.53/54.52, H: 4.27/4.28, N: 16.96/16.95; IR (KBr) cm<sup>-1</sup>: 3175 ( $\nu_{NH}$ ), 1705 ( $\nu_{C=O}$ ), 1620 ( $\nu_{C=N}$ ); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) $\delta$  3.84 (s, OCH<sub>3</sub>, 3H), 4.24 (s, thiophen-CH<sub>2</sub>, 2H), 6.93-7.24 (m, ar-H, 4H), 7.35-7.41 (m, ar-H, 2H), 9.42 (s, N=CH, 1H), 9.52 (s, OH, 1H), 11.98 (s, NH, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) $\delta$  25.61 (thiophen-CH<sub>2</sub>), 55.50 (OCH<sub>3</sub>), thiophen-C: [125.99(CH), 126.53 (CH), 126.79 (CH) 137.34 (C)], ar-C: [111.65 (CH), 112.32 (CH), 121.71 (CH), 126.79 (C), 146.79 (C), 151.10 (C)], 145.39 (C=N), 150.86 (N=CH), 153.96 (C=O). MS: m/z 331.67 (M+1)<sup>+</sup>.

**General method for the synthesis of 4-(arylidene-amino)-5-oxo-3-(thiophen-2-yl methyl)-4,5-dihydro-1H-[1,2,4]triazole-1-yl)-acetate (6,7):** 3-thiophen-2-yl-methyl-4-arylidene amino-4,5-dihydro-1H-[1,2,4] triazole-5-one (**3**) (0.01 mol) was refluxed with an equivalent amount of sodium in absolute ethanol for 1 h. Then ethyl bromoacetate (0.02 mol) was added and refluxed for an additional 5 h. The precipitate was filtered off, washed with H<sub>2</sub>O, and recrystallized from appropriate solvent to afford the desired compound.

**Ethyl 2-(4-(2-(2-ethoxy-2-oxoethoxy)benzylidene-amino)-5-oxo-3-(thiophen-2-yl-methyl)-4,5-dihydro-1H-[1,2,4]triazole-1-yl)acetate (6):** Following the general procedure above, a white solid was obtained. It was recrystallized from DMSO/water (1:2) (yield 80.93%) to afford the desired compound. mp 114-115 °C. Analysis (Calc/found %): for C<sub>22</sub>H<sub>24</sub>N<sub>4</sub>O<sub>6</sub>S C: 55.92/55.91, H: 5.12/5.13, N: 11.86/11.87; IR

(KBr)  $\text{cm}^{-1}$ : 1734, 1744 ( $\nu_{\text{ester-C=O}}$ ), 1708 ( $\nu_{\text{triazole-C=O}}$ ), 1599 ( $\nu_{\text{C=N}}$ ), 1216 ( $\nu_{\text{C-O}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  1.12 (t,  $\text{CH}_3$ , 6H,  $J = 7.0$  Hz), 4.16 (q,  $\text{OCH}_2\text{CH}_3$ , 4H,  $J = 7.0$  Hz), 4.94 (s,  $\text{OCH}_2\text{CO}$ , 2H), 4.33 (s, thiophen- $\text{CH}_2$ , 2H), 4.63 (s,  $\text{NCH}_2$ , 2H), 6.94-7.12 (m, ar-H, 4H), 7.38-7.89 (m, ar-H, 3H), 10.04 (s,  $\text{N=CH}$ , 1H);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ )  $\delta$  (ppm) 13.99 ( $\text{OCH}_2\text{CH}_3$ ), 25.51 (thiophen- $\text{CH}_2$ ), 46.45 ( $\text{NCH}_2$ ), 60.89, 61.39, 65.11 ( $\text{OCH}_2$ ), thiophen-C: [125.88 (CH), 126.83 (CH), 127.00 (CH), 136.98 (C)], ar-C: [113.19 (CH), 121.62 (CH), 121.69 (CH), 133.32 (C), 149.51 (C)], 145.08 (C=N), 149.68 (N=CH), 157.17 (C=O), 167.67, 168.48 (ester-C=O). MS:  $m/z$  473.16 (M+1) $^+$ .

**Ethyl 2(4-(3-(2-ethoxy-2-oxoethoxy)-4-methoxybenzylidene-amino)-5-oxo-3-(thiophen-2-yl-methyl)-4,5-dihydro-1H-[1,2,4]triazole-1-yl)-acetate (7):** Following the general procedure above, a white solid was obtained. It was recrystallized from ethanol/water (1:2) (yield 76.32%) to afford the desired compound. mp 146-147 °C. IR (KBr)  $\text{cm}^{-1}$ : 1747, 1749 ( $\nu_{\text{ester-C=O}}$ ), 1699 ( $\nu_{\text{triazole-C=O}}$ ), 1605 ( $\nu_{\text{C=N}}$ ), 1213 ( $\nu_{\text{C-O}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  1.14-1.23 (m,  $\text{CH}_3$ , 6H), 3.66 (s,  $\text{OCH}_3$ , 3H), 4.15 (q,  $\text{OCH}_2\text{CH}_3$ , 4H,  $J = 7.0$  Hz, 4H), 4.86 (s,  $\text{OCH}_2\text{CO}$ , 2H), 4.31 (s, thiophen- $\text{CH}_2$ , 2H), 4.63 (s,  $\text{NCH}_2$ , 2H), 6.93-7.12 (m, ar-H, 3H), 7.37-7.47 (m, ar-H, 3H), 9.52 (s,  $\text{N=CH}$ , 1H);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  (ppm) 13.88 ( $\text{OCH}_2\text{CH}_3$ ), 25.35 (thiophen- $\text{CH}_2$ ), 46.33 ( $\text{NCH}_2$ ), 55.64 ( $\text{OCH}_3$ ), 60.59, 61.16, 64.93 ( $\text{OCH}_2$ ), thiophen-C: [125.42 (CH), 126.68 (CH), 126.80 (CH), 136.88 (C)], ar-C: [110.35 (CH), 111.92 (CH), 123.81 (CH), 127.05 (C), 147.18 (C), 149.57 (C)], 144.78 (C=N), 151.93 (N=CH), 153.92 (C=O), 167.50, 168.41 (ester-C=O). MS:  $m/z$  503.16 (M+1) $^+$ .

**General method for the synthesis 4-[arylidene-amino]-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-ones (8):** The corresponding 4-amino-3-thiophen-2-yl-methyl 4,5-dihydro-1H-[1,2,4] triazole-5-one (**3**) (0.01 mol) and aldehydes (0.01 mol) were heated at 160 °C in an oil bath for 2 h. After cooling to room temperature, a solid appeared and it was crystallized from appropriate solvent to afford the desired compound.

**4-[(Pyridin-3-ylmethylene)-amino]-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-one (8i):** Recrystallized from ethanol (yield: 84.42%). mp 190-192 °C. Analysis (Calc/found %): for  $\text{C}_{13}\text{H}_{11}\text{N}_5\text{OS}$  C: 54.72/54.73, H: 3.89/3.88, N: 24.55/24.53; IR (KBr)  $\text{cm}^{-1}$ : 3179 ( $\nu_{\text{NH}}$ ), 1723 ( $\nu_{\text{triazole-C=O}}$ ), 1613 ( $\nu_{\text{C=N}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  (ppm) 4.32 (s, thiophen- $\text{CH}_2$  2H), 6.95-7.06 (m, ar-H, 2H), 7.39-7.41 (m, ar-H, 1H), 7.51-7.58 (m, ar-H, 1H), 8.27 (d, ar-H,  $J = 7.6$  Hz, 2H), 8.60 (d, ar-H,  $J = 7.6$  Hz, 1H), 9.02 (s, ar-H, 1H), 9.83 (s,  $\text{N=CH}$ , 1H), 12.12 (s, NH, 1H);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ )  $\delta$  (ppm) 25.56 (thiophen- $\text{CH}_2$ ), thiophen-C: [125.28 (CH), 126.62 (CH), 126.85 (CH), 137.26 (C)], ar-C: [124.00 (CH), 129.40 (CH), 134.12 (CH), 150.51 (CH), 150.95 (C)], 145.51 (C=N), 149.32 (N=CH), 151.85 (C=O). MS:  $m/z$  286.01 (M+1) $^+$ .

**4-[(4-Flouro-benzylidene)-amino]-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-one (8j):** Recrystallized from DMSO/water (1:3) (yield: 80.79%). mp 160-161 °C. Analysis (Calc/found %): for  $\text{C}_{14}\text{H}_{11}\text{FN}_4\text{OS}$  C: 55.62/55.61, H: 3.67/3.67, N: 18.53/18.52; IR (KBr)  $\text{cm}^{-1}$ : 3172 ( $\nu_{\text{NH}}$ ), 1706 ( $\nu_{\text{C=O}}$ ), 1607 ( $\nu_{\text{C=N}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  (ppm) 4.28 (s, thiophen- $\text{CH}_2$ , 2H), 6.93-7.04 (m, ar-H, 2H), 7.32-7.41 (m, ar-H, 3H), 7.90-7.98 (m, ar-H, 2H), 9.72 (s,  $\text{N=CH}$ , 1H), 12.06 (s, NH, 1H);  $^{13}\text{C-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  (ppm) 25.54 (thiophen- $\text{CH}_2$ ), thiophen-C: [125.05 (CH), 126.55 (CH), 126.80 (CH), 137.20 (C)], ar-C: [115.83 (CH), 116.27 (CH), 129.98 (CH), 130.16 (C)], 145.47 (C=N), 150.95 (N=CH), 152.20 (C=O). MS:  $m/z$  302.98 (M+1) $^+$ .

**4-[(4-Nitro-benzylidene)-amino]-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4] triazole-5-one (8k):** Recrystallized from ethanol/water (1:2) (yield: 82.37%). mp 255-256 °C. Analysis (Calc/found %): for  $\text{C}_{14}\text{H}_{11}\text{N}_5\text{O}_3\text{S}$  C: 51.06/51.07, H: 3.37/3.38, N: 21.27/21.29; IR (KBr)  $\text{cm}^{-1}$ : 3187 ( $\nu_{\text{NH}}$ ), 1712 ( $\nu_{\text{C=O}}$ ), 1615 ( $\nu_{\text{C=N}}$ );  $^1\text{H-NMR}$  (DMSO- $\text{d}_6$ ) $\delta$  (ppm) 4.32 (s, thiophen- $\text{CH}_2$ , 2H), 6.96-7.06 (m, ar-H, 2H),

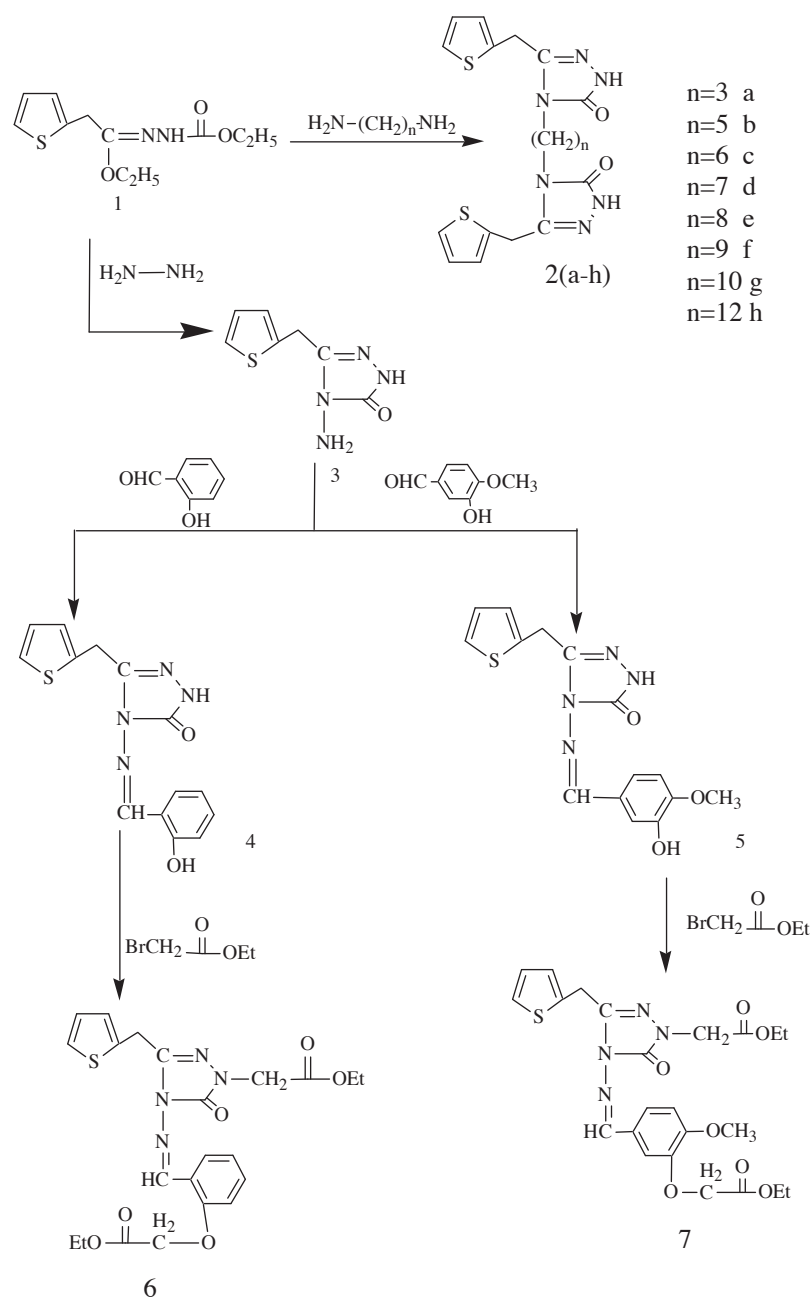
7.37-7.41 (m, ar-H, 1H), 8.13 (d, ar-H, J= 8 Hz, 2H), 8.34 (d, ar-H, J= 8 Hz, 2H), 9.87 (s, N=CH, 1H), 12.15 (s, NH, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)δ (ppm) 25.50 (thiophen-CH<sub>2</sub>), thiophen-C: [125.32 (CH), 126.67 (CH), 126.86 (CH), 137.09(C)], ar-C: [123.97 (CH), 128.72 (CH), 139.43 (CH), 148.64 (C)], 145.51 (C=N), 150.44 (N=CH), 150.75 (C=O). MS: m/z 330.03 (M+1)<sup>+</sup>.

**General method for the synthesis (4-(arylidene-amino)-5-oxo-3-thiophen-2-yl- methyl-4,5-dihydro-1H-[1,2,4]triazole-1-yl)-acetic acid ethyl ester (9):** The corresponding 3-thiophen-2-yl-methyl-4-arylidene-amino-4,5-dihydro-1H-[1,2,4]-triazole-5-one (**8**) (0.01 mol) was refluxed with an equivalent amount of sodium 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<sub>2</sub>O, and recrystallized from appropriate solvent to afford the desired compound.

**{5-Oxo-4-[(pyridin-3-yl-methylene)-amino]-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-1-yl}-acetic acid ethyl ester (9i):** Recrystallized from ethanol/water (1:2) (yield: 82.86%). mp 123-124 °C. Analysis (Calc/found %): for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>S C: 54.97/54.98, H: 4.61/4.60, N: 18.86/18.85; IR (KBr) cm<sup>-1</sup>: 1756 (ν<sub>esterC=O</sub>), 1704 (ν<sub>triazoleC=O</sub>), 1613 (ν<sub>C=N</sub>), 1209 (ν<sub>C-O</sub>); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)δ (ppm) 1.20 (t, CH<sub>3</sub>, J= 7.4 Hz, 3H), 4.16 (q, OCH<sub>2</sub>, J= 7.4 Hz, 2H), 4.36 (s, thiophen-CH<sub>2</sub>, 2H), 4.66 (s, NCH<sub>2</sub>, 2H), 6.94-7.59 (m, ar-H, 4H), 8.26-8.71 (m, ar-H, 2H), 9.03 (s, ar-H, 1H), 9.76 (s, N=CH, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)δ (ppm) 13.89 (OCH<sub>2</sub>CH<sub>3</sub>), 25.28 (thiophen-CH<sub>2</sub>), 46.33 (NCH<sub>2</sub>), 61.23 (OCH<sub>2</sub>CH<sub>3</sub>), thiophen-C:[125.71 (CH), 126.83 (C), 126.88 (CH), 136.17 (C)], ar-C: [120.43 (CH), 125.49 (CH), 136.64 (CH), 149.44 (CH), 152.02 (C)], 144.86 (C=N), 146.86 (N=CH), 153.33 (C=O), 167.46 (ester-C=O). MS: m/z 372.12 (M+1)<sup>+</sup>.

**{4-[(4-Flouro-benzylidene)-amino]-5-oxo-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-1-yl}-acetic acid ethyl ester (9j):** Recrystallized from ethanol (yield: 79.38%). mp 114-115 °C. Analysis (Calc/found %): for C<sub>18</sub>H<sub>17</sub>N<sub>4</sub>O<sub>3</sub>S C: 55.66/55.67, H: 4.41/4.40, N: 14.42/14.40; IR (KBr) cm<sup>-1</sup>: 1754 (ν<sub>esterC=O</sub>), 1711 (ν<sub>triazoleC=O</sub>), 1597 (ν<sub>C=N</sub>), 1217 (ν<sub>C-O</sub>); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)δ (ppm) 1.20 (t, CH<sub>3</sub>, J= 7.0 Hz, 3H), 4.15 (q, OCH<sub>2</sub>, J= 7.0 Hz, 2H), 4.34 (s, thiophen-CH<sub>2</sub>, 2H), 4.64 (s, NCH<sub>2</sub>, 2H), 6.95-7.02 (m, ar-H, 2H), 7.33-7.42 (m, ar-H, 3H), 7.93-7.97 (m, ar-H, 2H), 9.67 (s, N=CH, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) δ (ppm) 13.89 (OCH<sub>2</sub>CH<sub>3</sub>), 25.35 (thiophen-CH<sub>2</sub>), 46.35 (NCH<sub>2</sub>), 61.19 (OCH<sub>2</sub>CH<sub>3</sub>), thiophen-C: [125.44 (CH), 126.72 (C), 126.84 (CH), 136.79 (C)], ar-C: [115.89 (CH), 116.33 (CH), 130.22 (CH), 130.40 (C)], 144.84 (C=N), 149.48 (N=CH), 153.00 (C=O), 167.49 (ester-C=O). MS: m/z 389.17 (M+1)<sup>+</sup>.

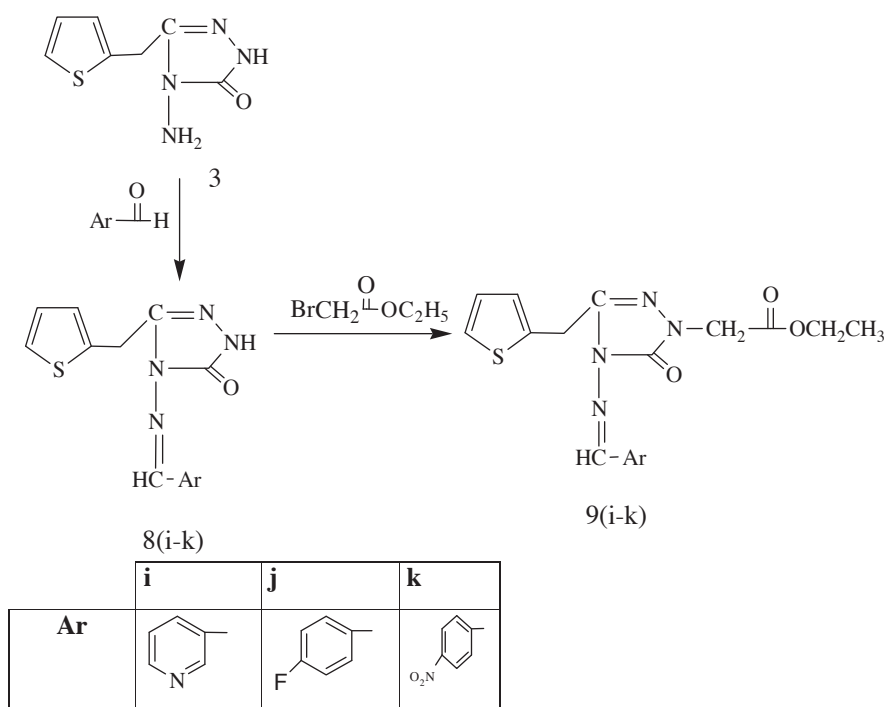
**{4-[(4-Nitro-benzylidene)-amino]-5-oxo-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-1-yl}-acetic acid ethyl ester (9k):** Recrystallized from ethanol (yield: 79.38%). mp 164-165 °C. Analysis (Calc/found %): for C<sub>18</sub>H<sub>17</sub>N<sub>5</sub>O<sub>5</sub>S C: 52.04/52.03, H: 4.12/4.13, N: 16.86/16.87; IR (KBr) cm<sup>-1</sup>: 1745 (ν<sub>esterC=O</sub>), 1702 (ν<sub>triazoleC=O</sub>), 1600 (ν<sub>C=N</sub>), 1212 (ν<sub>C-O</sub>); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)δ (ppm) 1.20 (t, CH<sub>3</sub>, J= 7.2 Hz, 3H), 4.16 (q, OCH<sub>2</sub>, J= 7.2 Hz, 2H), 4.38 (s, thiophen-CH<sub>2</sub>, 2H), 4.67 (s, NCH<sub>2</sub>, 2H), 6.96-7.05 (m, ar-H, 2H), 7.39-7.41 (m, ar-H, 1H), 8.11 (d, ar-H, J=10 Hz, 2H), 8.35 (s, ar-H, J= 8 Hz, 2H), 9.83 (s, N=CH, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)δ (ppm) 13.89 (OCH<sub>2</sub>CH<sub>3</sub>), 25.32 (thiophen-CH<sub>2</sub>), 46.37 (NCH<sub>2</sub>), 61.24 (OCH<sub>2</sub>CH<sub>3</sub>), thiophen-C: [125.52 (CH), 126.84 (C), 126.91 (CH), 136.62 (C)], ar-C: [124.02 (CH), 128.96 (CH), 136.62 (CH), 148.84 (C)], 144.91 (C=N), 149.28 (N=CH), 151.36 (C=O) 167.44 (ester-C=O). MS: m/z 416.14 (M+1)<sup>+</sup>.



**Scheme 1.** Synthetic pathway for the preparation of target compounds (2, 5, 6, and 7).

## Antimicrobial Activity

All test microorganisms were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey) and are as follows; Ec: *Escherichia coli* ATCC 25922, Pa: *Pseudomonas aeruginosa* ATCC 10145, Yp: *Yersinia pseudotuberculosis* ATCC 911, Kp: *Klebsiella pneumonia* ATCC 13883, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus* 709 roma, Ca: *Candida albicans* ATCC 60193, and Ct: *Candida tropicalis* ATCC 13803. Some of the newly compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare extract stock solution.



**Scheme 2.** Synthetic pathway for the preparation of target compounds (**8**, **9**).

The results were interpreted in terms of the diameter of the inhibition zone (5 mm: no antimicrobial activity; >5 mm: positive antimicrobial activity). Ec: *Escherichia coli* ATCC 25922, Pa: *Pseudomonas aeruginosa* ATCC 10145, Yp: *Yersinia pseudotuberculosis* ATCC 911, Kp: *Klebsiella pneumonia* ATCC 13883, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus* 709 ROMA, Ca: *Candida albicans* ATCC 60193, Ct: *Candida tropicalis* ATCC 13803.

### Agar well diffusion method

A simple susceptibility screening test using agar-well diffusion<sup>29</sup> as adapted earlier<sup>30</sup> was used. Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI, USA) broth and diluted to 10<sup>6</sup> colony forming units (cfu) per milliliter. They were ‘flood-inoculated’ onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA) (Difco) and then dried. For *C. albicans*, *C. tropicalis*, *Penicillium* spp., and *Aspergillus* spp., SDA was used. Five millimeter diameter wells were cut from the agar using a sterile cork-borer, and 250-5000 μg/50 μμL of the chemical substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ceftazidime (Fortum) (10 μg) and Triflucan (5 μg) were the standard drugs. DMSO served as the solved control. The results are shown in Table 1.

### Crystallographic structure determination of compound 8i

The crystal structure of compound **8i**, C<sub>13</sub>H<sub>11</sub>N<sub>5</sub>OS, was determined by single crystal X-ray diffraction (Figure 1). Compound **8i** crystallizes in the monoclinic space group *P*2<sub>1</sub>/*c* with the following unit-cell parameters: a = 9.4654(4) Å, b = 10.2344(3) Å, c = 13.6653(5) Å, β = 100.864(3), and V = 1300.07(8) Å<sup>3</sup>; crystallographic data shown in Table 2.



**Table 1.** Antibacterial and antifungal activities of the synthesized compounds (10 mg/mL).

Compound no.	Microorganism and inhibition zone (mm)								
	Ec	Pa	Yp	Kp	Ef	Sa	Bc	Ca	Ct
2a	5	<b>7</b>	5	5	5	5	5	5	5
2b	5	5	5	5	5	5	5	5	5
2c	5	5	5	5	5	5	5	5	5
2d	5	<b>7</b>	5	5	<b>10</b>	5	5	<b>10</b>	<b>13</b>
2e	5	5	5	5	5	5	5	5	5
2f	5	5	5	5	5	5	5	<b>12</b>	<b>13</b>
2g	5	5	5	5	5	5	5	5	5
2h	5	<b>10</b>	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	<b>15</b>	<b>10</b>
5	5	5	5	5	5	5	5	<b>8</b>	<b>13</b>
6	5	5	5	5	5	5	5	<b>9</b>	<b>10</b>
7	5	5	5	5	5	5	5	<b>15</b>	<b>15</b>
8i	5	5	5	5	5	5	5	5	<b>10</b>
8j	5	5	5	5	5	5	5	5	5
8k	5	5	5	5	5	5	5	5	5
9i	5	5	5	5	5	5	5	5	5
9j	5	5	5	5	5	5	5	5	5
9k	5	5	5	5	5	5	5	<b>10</b>	<b>11</b>
DMSO	5	5	5	5	5	5	5	5	5
Ampicillin	<b>8</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>11</b>	<b>15</b>	<b>14</b>		
Fortum	<b>45</b>	<b>45</b>	<b>45</b>	<b>20</b>	<b>30</b>	<b>30</b>	<b>35</b>		
Triflucan								<b>25</b>	<b>25</b>

The molecular data were collected on a Stoe IPDS II<sup>31</sup> diffractometer using  $MoK_{\alpha}$  radiation at room temperature. For compound **8i** data collection: X-AREA;<sup>32</sup>; cell refinement: X-AREA; data reduction: X-RED32;<sup>31</sup>; program used to solve structure: SHELXS97;<sup>32</sup>; program used to refine structure: SHELXL-97;<sup>32</sup>; molecular figures: ORTEP III;<sup>33</sup>; publication software: WinGX<sup>34</sup> and PARST.<sup>35</sup> The structure was solved by direct methods with SHELXS-97 and refined by full-matrix least-squares procedures on  $F^2$ , using the program SHELXL-97 in the WinGX software package. All non-hydrogen atoms were refined anisotropically. All hydrogen atom positions were refined using a riding model.

In the molecular structure of compound **8i**, the whole molecule is non-planar. The thiophene system makes a dihedral angle of 72.76(8) ° with the triazole ring and 74.39(7) ° with the pyridine ring. It means that these rings are almost perpendicular to each other, while triazole and pyridine rings are almost planar with the angle of 3.02(7) °. The bond lengths and angles in the 5-membered thiophene ring in the title molecules are in agreement with expected values.<sup>36,37</sup>

The structure of compound **8i** contains C-H...O and N-H...N type contacts, namely intra-molecular C5-H5...O1 and inter-molecular C9-H9...O1 (symmetry code:  $x, y+1, z$ ) where O1 atom added to the triazole ring accepts H bonds from C-H donors [graph set  $R_2^2(8)$ ] [7], N2-H2...N5 (symmetry code:  $x, y-1, z$ ) (Figure 2). In addition, it exhibits weak C-H... $\pi$  interactions [C11-H11...Cg1, Cg1 is the centroid of the

S1-C19 ring with the symmetry code: 1-x,1/2+y,1/2-z and C14-H14...Cg1 with symmetry code: -1-x,y,z]. The details of the H bonds are shown in Table 3.

**Table 2.** Crystal and experimental data.

Formula: C <sub>13</sub> H <sub>11</sub> N <sub>5</sub> OS
Formula weight: 285.28
Crystal system: monoclinic
Space group: P2 <sub>1</sub> /c
Weight = 285.33 Z = 4
a = 9.4654(4) Å
b = 10.2344(3) Å
c = 13.6653(5) Å
β = 100.864(3) °, and V = 1300.07(8) Å <sup>3</sup> ,
No. of reflections used = 13,145
2θ <sub>max</sub> = 60° MoK <sub>α</sub>
R = 0.058
(Δ/σ) <sub>max</sub> = 0.000
(Δρ) <sub>max</sub> = 0.291 eÅ <sup>-3</sup>
(Δρ) <sub>min</sub> = -0.396 eÅ <sup>-3</sup>
Measurement: STOE IPDS II
Program system: STOE X-RED
Structure determination: Direct methods
Refinement: Full matrix

**Table 3.** Hydrogen-bond geometry (Å, °).

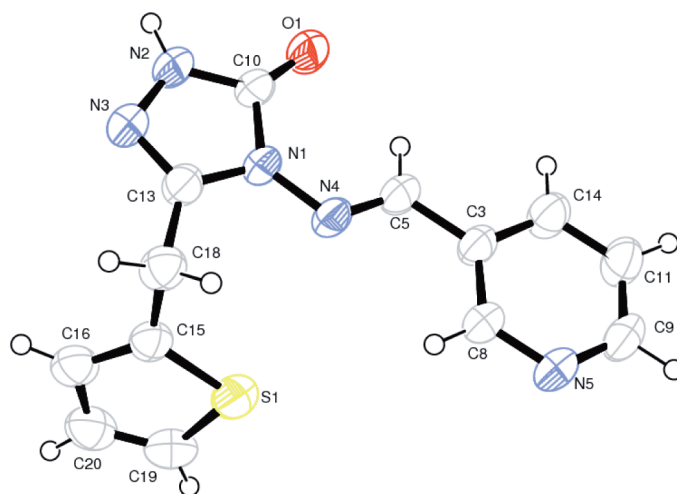
D-H...A	D-H	H...A	D...A	D-H...A
C9-H9...O1 <sup>i</sup>	0.93	2.56	3.400(3)	150.8
N2-H2...N5 <sup>ii</sup>	0.86	2.03	2.879(3)	168.3
C5-H5...O1	0.93	2.17	2.879(3)	132.3

(i) x,y+1,z (ii) x,y-1,z

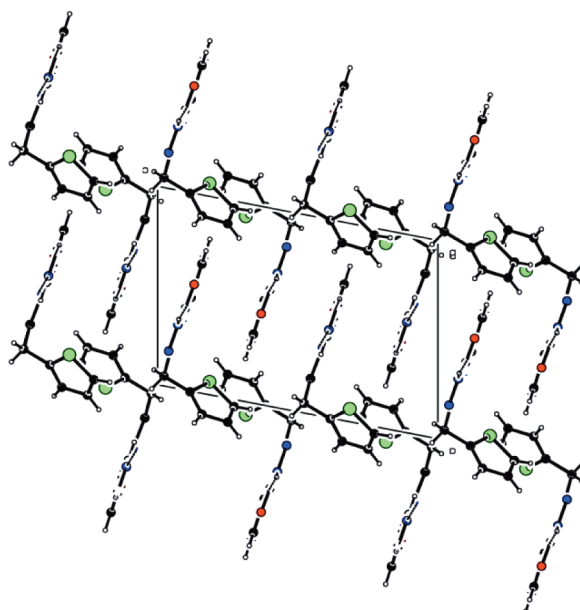
## Results and Discussion

The treatment of various ester ethoxy carbonyl hydrazones with some amines and hydrazines was reported.<sup>26</sup> The present study describes the reaction of N'-1-ethoxy-2-thiophen-2-yl-ethyldene hydrazino carboxylic acid ethyl ester with several diamines. The synthesis of di [(3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-one-4-yl] alkanes (**2a-h**) was carried out by the reaction of N'-1-ethoxy-2-thiophen-2-yl-ethyldene hydrazino carboxylic acid ethyl ester with diamines (Scheme 1) and their structures were confirmed using IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, elemental analyses, and mass spectral data. The signals observed at 11.54-11.58 ppm in the <sup>1</sup>H-NMR spectra of compounds **2a-h** were attributed to the -NH proton (exchangeable with D<sub>2</sub>O). The <sup>13</sup>C signals of azomethyn function and carbonyl function of the triazole ring of compounds **2a-h** appeared at 145.46-145.69 and 154.79-156.00 ppm, respectively. The geometrical optimization of compounds **2a**, **2c**, and

**2f** was achieved by computer using the AM1 method and the most stable conformations were determined (Figure 3).



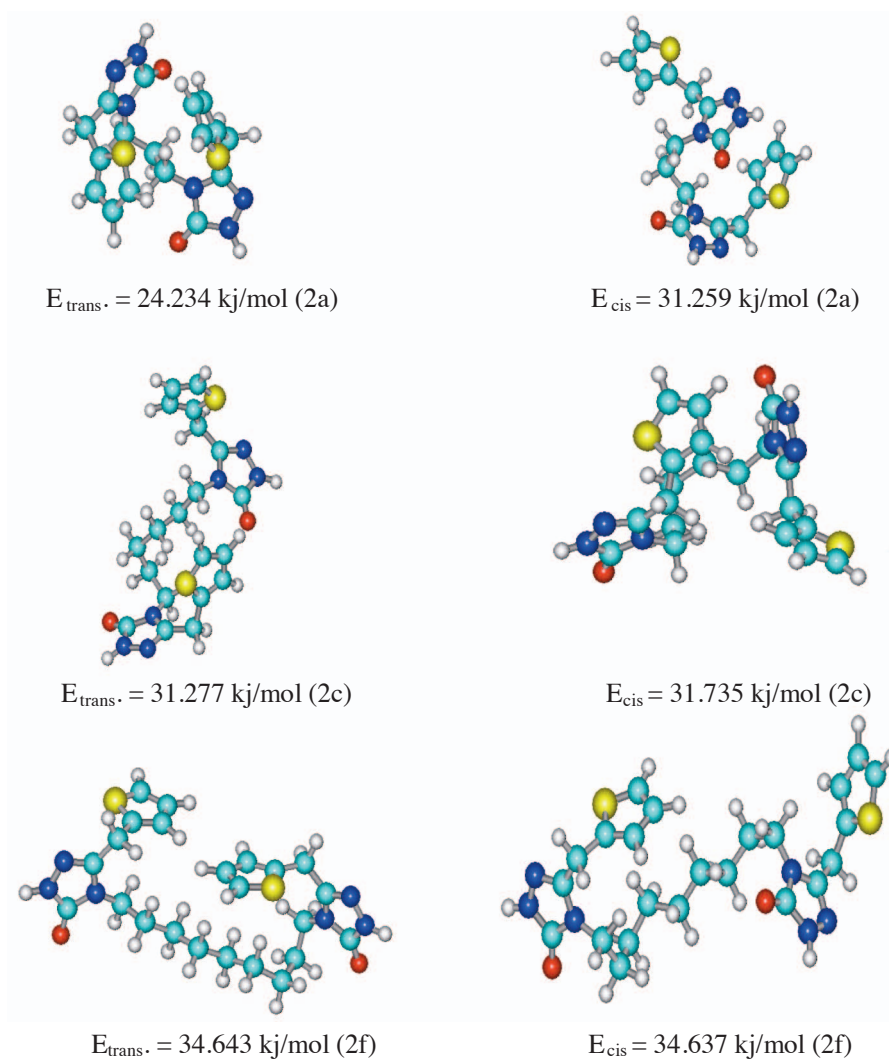
**Figure 1.** Ortep III diagram of compound **8i**.



**Figure 2.** Packing diagram of compound **8i** along the b axes.

We obtained the Schiff bases of 1,2,4-triazole-5-one derivatives. Schiff base **5** was prepared by the condensation of 4-amino-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-one with vanillin. The IR spectra of Schiff base **5** showed a characteristic absorption band at  $1620\text{ cm}^{-1}$  (C=N). In the  $^1\text{H-NMR}$  spectra of compound **5** the proton signal due to -N=CH was recorded at 9.42 ppm. The peak belonging to the same group was observed at 150.86 ppm in the  $^{13}\text{C-NMR}$  spectra. In the  $^1\text{H-NMR}$  spectra of compound **5**, proton signals of -OH and triazole -NH were observed at 9.52 and 11.98 ppm integrating for one proton (exchangeable with  $\text{D}_2\text{O}$ ). In addition, the proton signal due to the -OCH<sub>3</sub> group was recorded at 3.84 ppm. The peak belonging to the same group was observed at 55.50 ppm in the  $^{13}\text{C-NMR}$  spectra. The geometrical

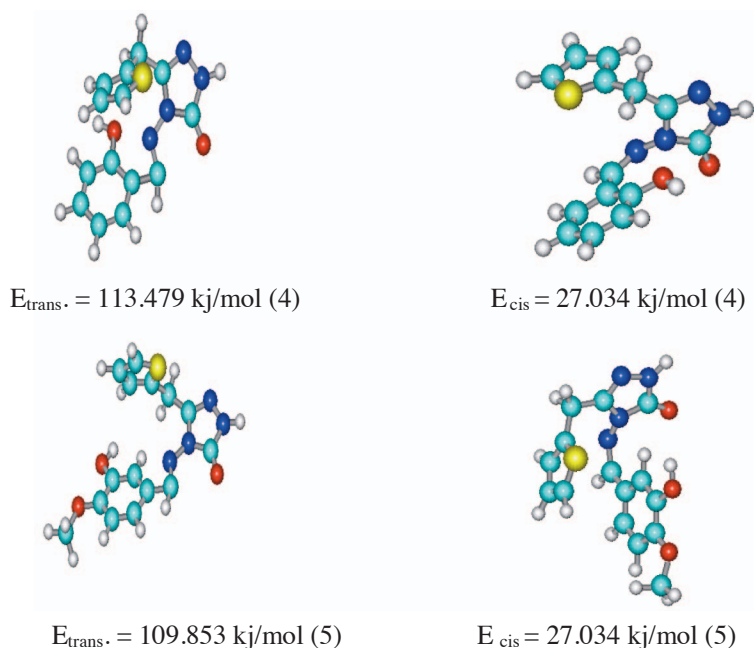
optimization of compounds **4** and **5** was achieved by computer using the AM1 method and the most stable conformations were determined (Figure 4).



**Figure 3.** Geometric optimization of compounds **2a**, **2c**, and **2f**.

New compounds **6** and **7** were obtained from the reaction of compounds **4** and **5** with ethyl bromoacetate in reasonably good yields (Scheme 1). The IR spectra of compounds **6** and **7** showed 2 sharp absorption bands, one of which, appearing at 1699-1708  $\text{cm}^{-1}$ , was attributed to carbonyl function of the 1,2,4-triazole-5-one ring, and the other, observed at 1744-1747  $\text{cm}^{-1}$ , was assigned to  $-\text{C}=\text{O}$  stretching frequency corresponding to ester carbonyl. The  $-\text{NH}$  signal disappeared in the  $^1\text{H-NMR}$  and IR spectra of compounds **6** and **7**. In the  $^1\text{H-NMR}$  spectra of these compounds, the proton signals due to the ester group were recorded at 1.21-1.23 ppm ( $-\text{OCH}_2\text{CH}_3$ ) integrating for 6 protons and 4.16-4.15 ppm ( $-\text{OCH}_2\text{CH}_3$ ) integrating for 4 protons. In these compounds (**6**, **7**), proton signals of  $-\text{OCH}_2\text{CO}$  and  $-\text{NCH}_2\text{CO}$  were observed at 4.94-4.86 and 4.63-4.63 ppm, respectively. The  $^{13}\text{C}$  signals of  $-\text{OCH}_2$  of compound **6** appeared at 60.89, 61.39, and 65.11 ppm. The  $^{13}\text{C}$  signal of  $-\text{NCH}_2$  of compound **7** appeared at 46.45 ppm. In compound **7**, the  $^{13}\text{C}$  signals of  $-\text{OCH}_2$  were observed at 60.59, 61.16, and 64.93 ppm. The  $^{13}\text{C}$  signal of  $-\text{NCH}_2$  appeared at around 46.33 ppm. The peaks belonging to carbonyl groups of 2 different esters linked to different atoms

were seen at 167.67 and 168.48 ppm in the  $^{13}\text{C}$ -NMR spectra for compound **6**. In the  $^{13}\text{C}$ -NMR spectra for compound **7**, the same peaks were observed at 167.50 and 168.41 ppm.



**Figure 4.** Geometric optimization of compounds **4** and **5**.

Schiff bases **8i-k** were prepared by the condensation of 4-amino-3-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-one with certain aldehydes (Scheme 2). The IR spectra of Schiff bases **8i-k** showed characteristic absorption bands between 1607 and 1615  $\text{cm}^{-1}$  ( $-\text{C}=\text{N}$ ). The  $^1\text{H}$ -NMR characteristic signals of compounds **8i-k** were observed at 9.72-9.87 ppm ( $-\text{N}=\text{CH}$ ). The  $^{13}\text{C}$ -NMR signals for the  $-\text{N}=\text{CH}$  group of compounds **8i-k** were recorded at 149.32-150.95 ppm.

The  $-\text{NH}$  proton at position 1 of 4,5-dihydro-1H-1,2,4-triazole-5-one ring is adequately acidic for further reactions. In the new study, some new (4-(arylidene-amino)-5-oxo-3-thiophen-2-yl-methyl-4,5-dihydro-1H[1,2,4]triazole-1-yl)-acetic acid ethyl ester compounds (**9i-k**) were obtained from the reaction of (arylidene-amino)-5-thiophen-2-yl-methyl-4,5-dihydro-1H-[1,2,4]triazole-5-ones (**8i-k**) with bromo ethyl acetate in reasonably good yields (Scheme 2). The IR spectra of compounds **9i-k** showed 2 sharp absorption bands, one of which, appearing at 1702-1711  $\text{cm}^{-1}$ , was attributed to carbonyl function of the 1,2,4-triazole-5-one ring, and the other, observed at 1745-1756  $\text{cm}^{-1}$ , was assigned to  $-\text{C}=\text{O}$  stretching frequency corresponding to ester carbonyl. The  $-\text{NH}$  signal disappeared in the  $^1\text{H}$ -NMR and IR spectra of compounds **9i-k**. In the  $^1\text{H}$ -NMR spectra of these compounds, the proton signals due to the ester group were recorded at 1.20 ppm ( $-\text{OCH}_2\text{CH}_3$ ) integrating for 3 protons and 4.15-4.16 ppm ( $-\text{OCH}_2\text{CH}_3$ ) integrating for 2 protons. The  $^{13}\text{C}$  signals of  $-\text{OCH}_2\text{CH}_3$  and  $-\text{OCH}_2\text{CH}_3$  were observed at 13.89 ppm and 61.19-61.24 ppm. The  $^{13}\text{C}$  signals of carbonyl function of ester were seen at 167.44 and 167.49 ppm.

Geometry optimization of compounds **2a**, **2c**, **2f**, **4**, and **5** was performed using the molecular mechanics MM+ module and AM1 semiempirical calculations in the HyperChem 6.03 molecular modeling program package.<sup>25</sup> Molecular mechanics used the MM+ as a classical Newtonian calculation method, which, in the energy minimization procedure, includes bond lengths, bond angles, torsion angles, and noncovalent interactions. Energy minimization used the Smart Minimizer, which is a combination of methods, starting

with the Steepest Descent Method, followed by the Fletcher-Reeves and Block-diagonal Newton-Raphson methods, and ending with the accurate Polak-Ribiere method.<sup>25</sup>

Compounds **2f**, **4**, **5**, **6**, **8i**, and **9k** showed good antifungal activity only against yeast-like fungi, while compound **2d** showed antimicrobial activity against bacteria and yeast-like fungi. Compounds **2a** and **2h** were only effective against *Pseudomonas aeruginosa* ATCC 10145. The best activity was observed against *Candida albicans* ATCC 60193 and *Candida tropicalis* ATCC 13803 by compound **7**.

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