**In vitro** biological activity of some new 1,2,4-triazole derivatives with their potentiometric titrations

H Yüksekd, Ö Aktaş-Yokuşd, Ö Gürsoy-Kol* and Ş Alpay-Karaoğlu

*Department of Chemistry, Kafkas University, 36100 Kars, Turkey
**Department of Chemistry, Kafkas University, 36100 Kars, Turkey
*Department of Biology, Recep Tayyip Erdogan University, 53100 Rize, Turkey

E-mail: ozlemgursoy@gmail.com

Received 17 March 2016; accepted (revised) 21 December 2016

In this study, 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives are reported to possess a broad spectrum of biological activities such as anti-inflammatory, antibacterial, antioxidant, antifungal, anticancer, analgesic, anticonvulsant, antiparasitic, antiviral, anti-HIV, antihypertensive and diuretic properties. In addition, several articles reporting the synthesis of some 4,5-dihydro-1H-1,2,4-triazol-5-one rings have weak acidic properties, so that some 1,2,4-triazole and 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives have been published so far.

On the other hand, it is known that 1,2,4-triazole and 4,5-dihydro-1H-1,2,4-triazol-5-one rings have weak acidic properties, so that some 1,2,4-triazole and 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were titrated potentiometrically with TBAH in non-aqueous solvents, and the $pK_a$ values of the compounds were determined.

In order to investigate the antimicrobial and antioxidant activity of some 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives, nine new 3-alkyl(aryl)-4-[4-methoxy-3-(p-toluene-sulfonyloxy)benzylidnamino]-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were synthesized. For that purpose, 4-methoxy-3-(p-toluene-sulfonyloxy)benzaldehyde was used by the reactions of 3-hydroxy-4-methoxybenzaldehyde with p-toluene sulfonfyl chloride by using trimethylamine. The 3-alkyl(aryl)-4-[4-methoxy-3-(p-toluene-sulfonyloxy)benzylidnamino]-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were obtained from the reactions of compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives with 4-methoxy-3-(p-toluene-sulfonyloxy)benzaldehyde. Then, the reactions of compounds 4a-e and 4g with acetic anhydride were investigated, and compounds 5a-e and 5g were prepared (Scheme 1). On the other hand, the newly synthesized 4a-i compounds were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents and the $pK_a$ values of the compounds were determined.

Results and Discussion

In this study, the structures of nine new 3-alkyl(aryl)-4-[4-methoxy-3-(p-toluene-sulfonyloxy)benzylidnamino]-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were synthesized. For that purpose, 4-methoxy-3-(p-toluene-sulfonyloxy)benzaldehyde was used by the reactions of 3-hydroxy-4-methoxybenzaldehyde with p-toluene sulfonfyl chloride by using trimethylamine. The 3-alkyl(aryl)-4-[4-methoxy-3-(p-toluene-sulfonyloxy)benzylidnamino]-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were obtained from the reactions of compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives with 4-methoxy-3-(p-toluene-sulfonyloxy)benzaldehyde. Then, the reactions of compounds 4a-e and 4g with acetic anhydride were investigated, and compounds 5a-e and 5g were prepared (Scheme 1). On the other hand, the newly synthesized 4a-i compounds were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents and the $pK_a$ values of the compounds were determined.

Antioxidant Activity

The antioxidant activities of fifteen new compounds 4a-i, 5a-e and 5g were determined. Several methods have been used to determine...
antioxidant activities and the methods used in the study are given below:

**Total reductive capability using the potassium ferricyanide reduction method**

The reductive capabilities of compounds were assessed by the extent of conversion of the Fe\(^{3+}\) / ferricyanide complex to the Fe\(^{2+}\) / ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and \(\alpha\)-tocopherol. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity\(^{19}\). The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging\(^{20}\). In the study, examined compounds did not show the reductive activities. In other words, all the amount of the compounds showed lower absorbance than standard antioxidants such as BHA, BHT and \(\alpha\)-tocopherol. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction.

**DPPH radical scavenging activity**

The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability\(^{21}\). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule\(^{22}\). The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by reaction between antioxidant molecules and radical, progresses, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants\(^{23}\).
Antiradical activities of compounds and standard antioxidants such as BHA and α-tocopherol were determined by using DPPH method. Scavenging effect values of compounds 4 and 5 with BHA and α-tocopherol at different concentrations are respectively given in Figure 1 and Figure 2. The newly synthesized compounds showed no activity as radical scavengers.

Ferrous ion chelating activity
The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe$^{3+}$. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator$^{24}$. Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe$^{3+}$) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe$^{2+}$, depending on condition, particularly pH$^{25}$ and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of

![Figure 1 — Scavenging effect of compounds 4a-i, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)](image1)

![Figure 2 — Scavenging effect of compounds 5a-e, 5g, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)](image2)
these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may not activate metal ions and potentially inhibit the metal-dependent processes\textsuperscript{26}. Also, the production of highly active ROS such as $O_2^-$, $H_2O_2$ and $OH^-$ is also catalyzed by free iron though Haber-Weiss reactions:

$$O_2 + H_2O_2 \rightarrow O_2^+ + OH^- + OH^-$$

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down the hydrogen and lipid peroxides to reactive free radicals via the Fenton reactions:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$$

Fe$^{3+}$ ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe$^{2+}$ ion, which is the most powerful pro-oxidant among the various types of metal ions\textsuperscript{27}. Ferrous ion chelating activities of the compounds 4, 5, BHT and BHA are respectively shown in Figure 3 and Figure 4.

![Figure 3](image1.png)

**Figure 3** — Metal chelating effect of different amount of the compounds 4a-i, BHT and BHA on ferrous ions

![Figure 4](image2.png)

**Figure 4** — Metal chelating effect of different amount of the compounds 5a-e, 5g, BHT and BHA on ferrous ions
In this study, metal chelating capacity was significant since it reduced the concentrations of the catalyzing transition metal. It was reported that chelating agents that form σ-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion. The data obtained from Figure 3 and Figure 4 reveal that the compounds, especially 4b, 4d and 5d demonstrate a marked capacity for iron binding, except 4i, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. On the other hand, free iron is known to have low solubility and a chelated iron complex has greater solubility in solution, which can be contributed solely by the ligand. Furthermore, the compound-iron complex may also be active, since it can participate in iron-catalyzed reactions.

**Potentiometric titrations**

In order to determine the $pK_a$ values of the compounds 4a-i, they were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl alcohol, tert-butyl alcohol, acetone and DMF. The mV values read in each titration were plotted against 0.05 M TBAH volumes (mL) added, and potentiometric titration curves were obtained for all the cases. From the titration curves, the HNP values were measured, and the corresponding $pK_a$ values were calculated. The data obtained from the potentiometric titrations was interpreted, and the effect of the C-3 substituent in 4,5-dihydro-1H-1,2,4-triazol-5-one ring as well as solvent effects were studied.

As an example for the potentiometric titration curves for 0.001 M solutions of compounds 4b titrated with 0.05 M TBAH in isopropyl alcohol, tert-butyl alcohol, DMF and acetone are shown in Figure 5.

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: DMF ($\varepsilon=36.7$) > acetone ($\varepsilon=36$) > isopropyl alcohol ($\varepsilon=19.4$) > tert-butyl alcohol ($\varepsilon=12$).

![Figure 5 — Potentiometric titration curves of 0.001 M solutions of compound 4b titrated with 0.05 M TBAH in isopropyl alcohol, tert-butyl alcohol, DMF and acetone at 25°C](image-url)
As seen in Table I, the acidity order for compound 4a is: acetone > DMF > tert-butyl alcohol, for compound 4b it is: acetone > tert-butyl alcohol > DMF, for compounds 4c and 4d it is: acetone > DMF, for compound 4e it is: acetone > isopropyl alcohol > DMF > tert-butyl alcohol, for compound 4f it is: acetone > isopropyl alcohol > DMF, while the order for compound 4g is: acetone > DMF > tert-butyl alcohol > isopropyl alcohol and for compound 4h it is: DMF > acetone and for compound 4i it is: tert-butyl alcohol > acetone > DMF > isopropyl alcohol. Moreover, as seen in Table I, for compounds 4a, 4b and 4c in isopropyl alcohol, for compounds 4c, 4d, 4f and 4h in tert-butyl alcohol the HNP values and the corresponding $pK_a$ values were not obtained.

As it is well known, the acidity of a compound depends on some factors. The two most important factors are the solvent effect and molecular structure$^{4,5,14-18,29}$. Table I and Figure 5 show that the HNP values and corresponding $pK_a$ values obtained from the potentiometric titrations depend on the non-aqueous solvents used and the substituents at C-3, in 4,5-dihydro-1$H$-1,2,4-triazol-5-one ring.

**Antimicrobial Activity**

The microbiological results are summarized in Table II. Microbiology results are not promising; only compounds 4a, 5b and 5d showed low antimicrobial activity (10μg/100μL) against *Mycobacterium smegmatis* and *Bacillus cereus*.

**Experimental Section**

Chemical reagents used in this study were purchased from Merck AG, Aldrich and Fluka. The

### Table I — The HNP and the corresponding $pK_a$ values of compounds 4a-i in isopropyl alcohol, tert-butyl alcohol, DMF and acetone

<table>
<thead>
<tr>
<th>Compd</th>
<th>DMF HNP (mV) $pK_a$</th>
<th>Acetone HNP (mV) $pK_a$</th>
<th>tert-Butyl alcohol HNP (mV) $pK_a$</th>
<th>Isopropyl alcohol HNP (mV) $pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>−364</td>
<td>15.32</td>
<td>−256</td>
<td>13.08</td>
</tr>
<tr>
<td>4b</td>
<td>−340</td>
<td>15.04</td>
<td>−172</td>
<td>10.58</td>
</tr>
<tr>
<td>4c</td>
<td>−381</td>
<td>16.13</td>
<td>−184</td>
<td>11.08</td>
</tr>
<tr>
<td>4d</td>
<td>−334</td>
<td>14.9</td>
<td>−243</td>
<td>10.06</td>
</tr>
<tr>
<td>4e</td>
<td>−343</td>
<td>15.45</td>
<td>−105</td>
<td>8.82</td>
</tr>
<tr>
<td>4f</td>
<td>−351</td>
<td>14.92</td>
<td>−201</td>
<td>10.02</td>
</tr>
<tr>
<td>4g</td>
<td>−301</td>
<td>13.67</td>
<td>−266</td>
<td>13.21</td>
</tr>
<tr>
<td>4h</td>
<td>−316</td>
<td>14.50</td>
<td>−451</td>
<td>17.70</td>
</tr>
<tr>
<td>4i</td>
<td>−327</td>
<td>14.75</td>
<td>−221</td>
<td>11.84</td>
</tr>
</tbody>
</table>

### Table II — Antimicrobial and antifungal activity of the compounds 4 and 5

<table>
<thead>
<tr>
<th>Compd</th>
<th>Stock solution (µg/mL)</th>
<th>Microorganisms and zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ec</td>
</tr>
<tr>
<td>4a</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4b</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4c</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4d</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4e</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4f</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4g</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4h</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>4i</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>5a</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>5b</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>5c</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>5d</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>5e</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>5f</td>
<td>10.000</td>
<td>−</td>
</tr>
<tr>
<td>Amp.</td>
<td>10.000</td>
<td>10</td>
</tr>
<tr>
<td>Strep.Fl.</td>
<td>10.000</td>
<td>25</td>
</tr>
</tbody>
</table>

Strep.Fl.
starting materials 3a-i were prepared from the reactions of the corresponding ester ethoxycarbonyl-hydrazones 2a-i with an aqueous solution of hydrazine hydrate as described in the literature.³⁰,³¹ Melting points were determined in open glass capillaries by using a Stuart SMP-30 melting point apparatus and are uncorrected. The IR spectra were obtained by an ALPHA-P BRUKER FT-IR spectrometer. ¹H and ¹³C NMR spectra were recorded in DMSO-ᵈ with TMS as internal standard using a Bruker 400 NMR spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C NMR with TMS as internal standard. UV-Vis absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV-Vis spectrometer. Extinction coefficients (ε) are expressed in L.mol⁻¹.cm⁻¹. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a TSQ Quantum Access Max Triple Stage Quadrupole Mass Spectrometer.

**General procedure for the synthesis of compounds 4**

3-Hydroxy-4-methoxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (20 mL) was treated with p-toluenesulfonyl chloride (0.01 mol) and to this solution was slowly added triethylamine (0.01 mol) with stirring at 0-5°C. The process of stirring continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated in vacuo, and the crude product was washed with water and recrystallized from acetic acid-water to afford compound 1 (Ref 32), m.p. 144-46°C. IR: 2843 (CH=O), 1692 (C=O), 1590 (C=N), 1363, 1179 (SO₂sulfonyloxy)-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones 3 as colorless crystals.

**3-Methyl-4-(4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one, 4a:** Yield 3.90 g (97%). m.p.196-98°C. IR: 3148 (NH), 1689 (C=O), 7,19 (d, 1H, ArH), 7,27 (d, 2H, ArH), 7,47 (m, 1H, ArH), 7,72 (d, 2H, ArH), 7,89 (d, 1H, ArH), 9,86 (s, 1H, CHO); ¹³C NMR (DMSO-ᵈ): δ 11.44 (CH₃), 21.61 (PhCH₃), 56.45 (OCH₃), 114.02, 121.35, 126.73, 128.75 (2C), 129.87, 130.38 (2C), 132.51, 138.50, 144.59, 154.33 (arom-C), 146.18 (triazole C₅), 151.65 (N=CH), 152.63 (triazole C₆); UV-Vis λ⁰max (ε): 270 (20427), 228 (24688), 212 (20323) nm; MS (70 eV): m/z (%) 426 (M+1+23), 425 (M+2, 100), 404 (M+2), 403 (M+1, 60), 398, 361, 330, 329, 307, 282, 277, 261, 243, 234, 186, 155, 139, 104.

**3-Ethyl-4-(4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one, 4b:** Yield 4.10 g (99%). m.p.200-201°C. IR: 3183 (NH), 1694 (C=O), 1591 (C=N), 1365, 1180 (SO₂), 820 cm⁻¹ (1,4-disubstituted benzenoid ring); ¹H NMR (DMSO-ᵈ): δ 1.18 (t, 3H, CH₂CH₃), 2.42 (s, 3H, PhCH₃), 2.62 (q, 2H, CH₂CH₂), 3.62 (s, 3H, OCH₃), 7.19 (d, 1H, ArH), 7.47, 7.50 (m, 3H, ArH), 7.70, 7.75 (m, 3H, ArH), 9.60 (s, 1H, NH); ¹³C NMR (DMSO-ᵈ): δ 10.43 (CH₃CH₂), 19.00 (CH₂CH₂), 21.60 (PhCH₃), 56.45 (OCH₃), 114.04, 121.24, 126.76, 128.72 (2C), 129.87, 130.37 (2C), 132.54, 138.54, 146.16, 154.36 (arom-C), 148.35 (triazole C₅), 151.81 (N=CH), 152.64 (triazole C₆); UV-Vis λ⁰max (ε): 308 (19656), 226 (22854), 222 (22646) nm; MS (70 eV): m/z (%) 440 (M+1+23), 439 (M+2, 100), 434, 418 (M+2), 417 (M+1, 45), 361, 329, 307, 289, 284, 276, 243.

**3-n-Propyl-4-(4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one, 4c:** Yield 4.20 g (98%). m.p.207-208°C. IR: 3177 (NH), 1692 (C=O), 1590 (C=N), 1363, 1179 (SO₂), 813 cm⁻¹ (1,4-disubstituted benzenoid ring); ¹H NMR (DMSO-ᵈ): δ 0.93, 0.96 (m, 3H, CH₃CH₂CH₂), 1.63, 1.68 (m, 2H, CH₂CH₂CH₂), 2.43 (s, 3H, PhCH₃), 2.57, 2.60 (m, 2H, CH₂CH₂CH₃), 3.60 (s, 3H, OCH₃), 7.18, 7.20 (m, 1H, ArH), 7.47, 7.53 (m, 3H, ArH), 7.72, 7.75 (m, 3H, ArH), 9.60 (s, 1H, NH); ¹³C NMR (DMSO-ᵈ): δ 13.91 (CH₂CH₂CH₃), 19.37 (CH₃CH₂CH₂), 21.61 (PhCH₃), 27.09 (CH₂CH₂CH₂), 56.46 (OCH₃), 114.09, 121.41, 126.78, 128.73 (2C), 129.79, 130.37 (2C), 132.50, 138.52, 146.17, 154.33 (arom-C), 147.25 (triazole C₅), 151.74 (N=CH), 152.76 (triazole C₆); UV-Vis λ⁰max (ε): 308 (18000), 226 (21065), 220 (20183) nm; MS (70 eV): m/z (%) 454 (M+1+23), 453 (M+23, 100), 432 (M+2), 431 (M+1, 65), 428, 397, 361, 330, 329, 324, 296, 291, 275, 245, 211, 183, 155, 128.
3-Benzyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 4d: Yield 4.60 g (96%). m.p.204-205°C. IR: 3173 (NH), 1691 (C=O), 1586 (C=N), 1365, 1179 (SO₂), 838 (1,4-disubstituted benzenoid ring), 765, 708 cm⁻¹ (monosubstituted benzoid ring); ¹H NMR (DMSO-d₆): δ 2.38 (s, 3H, PhCH₃), 3.58 (s, 3H, OCH₃), 3.99 (s, 2H, CH₂Ph), 7.16 (d, 1H, ArH), 7.22, 7.25 (m, 1H, ArH), 7.28, 7.33 (m, 4H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.67 (d, 1H, ArH), 7.74 (d, 2H, ArH), 9.57 (s, 1H, N=CH), 11.96 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 21.57 (PhCH₃), 31.63 (CH₃Ph), 56.41 (OCH₃), 113.98, 121.51, 126.75, 127.25, 128.72 (2C), 128.93 (2C), 129.32 (2C), 129.91, 130.34 (2C), 132.52, 136.16, 138.49, 146.18, 154.27 (arom-C), 146.63 (triazole C₅), 151.66 (N=CH), 152.42 (triazole C₃); UV-Vis λmax (ε): 308 (13190), 220 (19700) nm; MS (70 eV): m/z (%) 502 (M+1+23), 501 (M+23, 100), 480 (M+2), 479 (M+1, 72), 328, 320, 315, 299, 244, 243, 187.

3-p-Methylbenzyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 4e: Yield 4.75 g (97%). m.p.209-10°C. IR: 3122 (NH), 1695 (C=O), 1588 (C=N), 1357, 1180 (SO₂), 835 cm⁻¹ (1,4-disubstituted benzenoid ring); ¹H NMR (DMSO-d₆): δ 2.25 (s, 3H, PhCH₃), 2.39 (s, 3H, PhCH₃), 3.59 (s, 3H, OCH₃), 3.93 (s, 2H, CH₂Ph), 7.10, 7.12 (m, 2H, ArH), 7.16, 7.18 (m, 3H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.66, 7.69 (m, 1H, ArH), 7.75 (d, 2H, ArH), 9.56 (s, 1H, N=CH), 11.94 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 21.09 (CH₃PhCH₂), 21.59 (PhCH₃), 31.22 (CH₂PhCH₃), 56.43 (OCH₃), 114.02, 121.48, 126.76, 128.73 (2C), 129.18 (2C), 129.50 (2C), 129.94, 130.36 (2C), 132.51, 133.05, 136.30, 138.50, 146.21, 154.26 (arom-C), 146.78 (triazole C₅), 151.61 (N=CH), 152.42 (triazole C₃); UV-Vis λmax (ε): 308 (24750), 226 (28400), 214 (24638) nm; MS (70 eV): m/z (%) 516 (M+1+23), 515 (M+23, 100), 494 (M+2), 493 (M+1, 70), 491, 437, 408, 393, 355, 329, 327, 322, 306, 244, 243, 187.

3-p-Chlorobenzyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 4f: Yield 4.95 g (97%). m.p.207-209°C. IR: 3161 (NH), 1698 (C=O), 1602 (C=N); 1355, 1183 (SO₂), 833 cm⁻¹ (1,4-disubstituted benzenoid ring); ¹H NMR (DMSO-d₆): δ 2.39 (s, 3H, PhCH₃), 3.58 (s, 3H, OCH₃), 4.00 (s, 2H, CH₂Ph), 7.17 (d, 1H, ArH), 7.31, 7.38 (m, 4H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.67, 7.69 (m, 1H, ArH), 7.74 (d, 2H, ArH), 9.58 (s, 1H, N=CH), 11.98 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 21.59 (PhCH₃), 30.94 (OCH₃), 56.44 (OCH₃), 114.05, 121.57, 126.69, 128.75 (2C), 128.86 (2C), 129.95, 130.35 (2C), 131.25 (2C), 131.93, 132.47, 135.16, 138.46, 146.21, 154.29 (arom-C), 146.30 (triazole C₅), 151.63 (N=CH), 152.60 (triazole C₃); UV-Vis λmax (ε): 308 (17381), 224 (27737) nm; MS (70 eV): m/z (%) 537 (M+2+23, 20), 535 (M+23, 45), 514 (M+2), 513 (M+1), 493, 453, 424, 402, 362, 361, 337, 332, 329 (100), 324, 307, 267, 258, 250, 229, 215, 104.

3-m-Chlorobenzyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 4h: Yield 4.90 g (96%). m.p.184-86°C. IR: 3168 (NH), 1702 (C=O), 1594 (C=N), 1364, 1185 (SO₂), 826 (1,4-disubstituted benzenoid ring), 785, 687 cm⁻¹ (monosubstituted benzoid ring); ¹H NMR (DMSO-d₆): δ 2.38 (s, 3H, PhCH₃), 3.59 (s, 3H, OCH₃), 4.03 (s, 2H, CH₂Ph), 7.17 (d, 1H, ArH), 7.26, 7.37 (m, 4H, ArH), 7.45, 7.47 (m, 2H, ArH), 7.58 (m, 1H, ArH), 7.68, 7.70 (m, 1H, ArH), 7.74 (d, 2H, ArH), 9.57 (s, 1H, N=CH), 11.99 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 21.58 (PhCH₃), 31.14 (CH₂Ph), 56.44 (OCH₃), 114.03, 121.66, 126.66, 127.32, 128.20, 128.74 (2C), 129.26, 129.86, 130.35 (2C), 130.74, 132.51, 133.45, 138.49, 138.53, 146.12, 154.34 (arom-C), 146.18 (triazole C₅), 151.61 (N=CH), 152.72 (triazole C₃); UV-Vis λmax (ε): 308 (16280), 222 (21370) nm; MS (70 eV): m/z (%) 537 (M+2+23, 18), 535 (M+23, 55), 514 (M+2), 513 (M+1), 477, 410, 353, 346, 345.
3-Phenyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzilidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 4i: Yield 4.00 g (86%). m.p. 185-86°C. IR: 3161 (NH), 1702 (C=O), 1600 (C=N), 1372, 1180 (SO₂), 842 (1,4-disubstituted benzenoid ring), 765, 716 cm⁻¹ (monosubstituted benzenoid ring); ¹H NMR (DMSO-d₆): δ 2.36 (s, 3H, PhCH₃), 3.59 (s, 3H, OCH₃), 7.20 (d, 1H, ArH), 7.41, 7.43 (m, 2H, ArH), 7.51, 7.59 (m, 4H, ArH), 7.70, 7.74 (m, 3H, ArH), 7.86, 7.88 (m, 1H, ArH), 9.56 (s, 1H, N=CH), 12.37 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 21.58 (PhCH₃), 56.45 (OCH₃), 114.15, 122.03, 126.58, 127.07, 128.33 (2C), 128.69 (2C), 128.96 (2C), 129.82, 130.32 (2C), 130.61, 132.32, 138.43, 144.90, 155.50 (arom-C), 146.19 (triazole C₆), 151.80 (N=CH), 154.44 (triazole C₆); UV-Vis λ_max (e): 308 (19869), 274 (22464), 232 (29095) nm; MS (70 eV): m/z (%) 468 (M+23, 100), 457 (M+2), 456 (M+1), 437, 417, 404, 384, 362, 329, 305, 289, 284, 276, 243, 227.

General procedure for the synthesis of compounds 5

The corresponding compound 4 (0.01 mol) was refluxed with acetic anhydride (15 mL) for 30 min. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h more. Evaporation of solvent from the resulting solution at 40-45°C in vacuo and several recrystallizations of the residue from EtOH gave pure compounds 5a-e and 5g as colourless crystals.

1-Acetyl-3-ethyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzilidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 5b: Yield 3.90 g (85%). m.p. 144-46°C. IR: 1711, 1695 (C=O), 1604 (C=N), 1371, 1184 (SO₂), 805 cm⁻¹ (1,4-disubstituted benzenoid ring); ¹H NMR (DMSO-d₆): δ 1.23 (t, 3H, CH₂CH₃), 2.44 (s, 3H, PhCH₃), 2.50 (s, 3H, COCH₃), 2.68 (q, 2H, CH₂CH₃), 3.62 (3H, OCH₃), 7.22 (d, 1H, ArH), 7.49 (d, 2H, ArH), 7.56 (s, 1H, ArH), 7.70, 7.78 (m, 3H, ArH), 9.48 (s, 1H, N=CH); ¹³C NMR (DMSO-d₆): δ 9.87 (CH₂CH₃), 19.01 (CH₂CH₃), 21.62 (PhCH₃), 23.91 (COCH₃), 56.53 (OCH₃), 114.14, 121.61, 126.19, 128.73 (2C), 130.34 (2C), 130.39, 132.49, 138.54, 146.22, 154.87 (arom-C), 148.54 (triazole C₆), 150.53 (N=CH), 154.77 (triazole C₆), 166.45 (COCH₃); UV-Vis λ_max (e): 308 (20848), 292 (18293), 226 (25087) nm; MS (70 eV): m/z (%) 482 (M+1+23), 481 (M+23, 100), 460 (M+2), 719, 745 (M+1), 439, 417, 404, 384, 362, 329, 305, 289, 284, 276, 243, 227.

1-Acetyl-3-n-propyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzilidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 5c: Yield 3.87g (82%). m.p. 161-65°C. IR: 1691 (C=O), 1593 (C=N), 1368, 1183 (SO₂), 810 cm⁻¹ (1,4-disubstituted benzenoid ring); ¹H NMR (DMSO-d₆): δ 0.96 (t, 3H, CH₂CH₂CH₃), 1.70 (sext, 2H, CH₂CH₂CH₃), 2.43 (3H, PhCH₃), 2.51 (s, 3H, COCH₃), 2.61 (t, 2H, CH₂CH₂CH₃), 3.61 (3H, OCH₃), 7.22 (d, 1H, ArH), 7.48 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.73, 7.79 (m, 3H, ArH), 9.49 (s, 1H, N=CH); ¹³C NMR (DMSO-d₆): δ 13.91 (CH₂CH₂CH₃), 19.94 (CH₂CH₂CH₃), 21.63 (PhCH₃), 23.95 (COCH₃), 27.09 (CH₂CH₂CH₃), 56.53 (OCH₃), 114.19, 121.80, 126.22, 128.73 (2C), 130.24, 130.39 (2C), 132.48, 138.52, 146.22, 155.01 (arom-C), 148.50 (triazole C₆), 149.42 (N=CH), 154.74 (triazole C₆), 166.48 (COCH₃); UV-Vis λ_max (e): 308 (12954), 268 (19648), 224 (17880) nm; MS (70 eV): m/z (%) 496 (M+1+23), 495 (M+23, 95), 474 (M+2), 473 (M+1), 453 (100), 431, 426, 404, 349, 329, 307, 291, 283, 250, 234.

1-Acetyl-3-benzyl-4-[4-methoxy-3-(p-toluenesulfonyloxy)-benzilidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one, 5d: Yield 4.78 g (92%). m.p. 164-66°C. IR: 1779, 1720 (C=O), 1602 (C=N), 1374, 1182 (SO₂), 798 (1,4-disubstituted benzenoid ring), 756, 704 cm⁻¹ (monosubstituted benzenoid ring); ¹H NMR (DMSO-d₆): δ 2.39 (s, 3H, PhCH₃), 2.50 (s, 3H, COCH₃), 3.59 (3H, OCH₃), 4.08 (2H, CH₂Ph), 7.18 (d, 1H, ArH), 7.26, 7.34 (m, 5H, ArH), 7.46 (d, 2H, ArH), 7.59 (s, 1H, ArH), 7.71, 7.75 (m, 3H, ArH).
ArH), 9.45 (s, 1H, N=CH); $^{13}$C NMR (DMSO-d$_6$): δ 21.59 (PhCH$_3$), 23.98 (COCH$_3$), 31.58 (CH$_2$Ph), 56.48 (OCH$_3$), 114.07, 121.87, 126.20, 127.51, 128.72 (2C), 128.93, 129.00 (2C), 129.53 (2C), 130.36 (2C), 132.48, 135.03, 138.49, 146.23, 154.67 (arom-C), 148.70 (triazole C$_3$), 151.65 (N=CH), 152.45 (triazole C$_5$), 166.50 (COCH$_3$); UV-Vis $\lambda_{\text{max}}$ (e): 308 (25684), 226 (30618), 222 (29803) nm; MS (70 eV): m/z (%) 544 (M+1+23), 543 (M+23, 75), 501, 479, 349, 328, 307, 289, 243, 236, 204, 195 (90), 163.

1-Acetyl-3-p-methylbenzyl-4-[4-methoxy-3-(p-toluenesulfonyl)oxy]-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one, 5e: Yield 4.84 g (89%). m.p. 190-92°C. IR: 1725 (C=O), 1602 (C=N), 1371, 1182 (SO$_2$), 576 (SO$_3$). Each microorganism was suspended in Mueller Hifzissihha Institute of Refik Saydam (Ankara, Turkey)

Antimicrobial activity

All bacterial and yeast strains were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: (Escherichia coli ATCC 25922, Yersinia pseudotuberculosis ATCC 911, Pseudomonas aeruginosa ATCC 43288, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Bacillus cereus 702 Roma, Mycobacterium smegmatis ATCC607, Candida albicans ATCC 60193, Saccharomyces cerevisiae). Simple susceptibility screening test using agar well diffusion method was used. Each microorganism was suspended in Mueller...
Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately 10^6 colony forming unit (cfu)/mL. They were ‘flood-inoculated’ onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI, USA) and then dried. For *C. albicans*, SDA was used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the sample solutions 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. Ampicillin (10 µg) and fluconazole (5 µg) were standard antibacterial and antifungal agents, respectively. DMSO was used as solvent control.

**Acknowledgements**

This work was supported by the Scientific Research Projects Coordination Unit of Kafkas University (Project Number: 2011-FEF-32). The authors thank Dr. Z. Ocak for the determination of pKₐ values and Dr. M. Calapoglu for antioxidant activities.

**References**