In vitro Antioxidant and Acidic Properties of Novel 4-(5-Methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one Derivatives. Synthesis and Characterization

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In the present study 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones 2a-i reacted with 5-methylthiophene-2-carboxyaldehyde to afford the corresponding compounds 3a-i and the acetylation reactions of compounds 3a, 3b, 3d, 3g and 3i were investigated. The structures of fourteen new compounds are established from the spectral data. The synthesized 3 and 4 type compounds were analyzed for their in vitro potential antioxidant activities by three different methods. Besides, compounds 3a-i were titrated potentiometrically with tetrabutylammonium hydroxide in four nonaqueous solvents.

Keywords: 1,2,4-Triazol-5-one, Schiff base, Synthesis, Antioxidant activity, Acidity, Potentiometric titrations.

Antioxidants are extensively studied for their capacity to protect organism and cell from damage that is induced by the oxidative stress. A great deal of research has been devoted to the study of different types of natural and synthetic antioxidant. A large number of heterocyclic compounds, containing the 1,2,4-triazole ring, are associated with diverse biological properties such as antioxidant, anti-inflammatory, antimicrobial and antiviral activity [1-9], and several papers have been devoted to synthesis of some N-arylideneamino derivatives of 4,5-dihydro-1H-1,2,4-triazol-5-one compounds [8-13]. The acetylation of 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives have also been reported [8-14].

Scientists in various disciplines have become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cells [15]. Exogenous chemicals and endogenous metabolic processes in human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damages play a significantly pathological role in human diseases. For example, cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative damage. Also, excessive generation of ROS (reactive oxygen species) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [16].

In addition, it is known that 1,2,4-triazole and 4,5-dihydro-1H-1,2,4-triazol-5-one rings have weak acidic properties, thus some 1,2,4-triazole and 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents (isopropyl alcohol, t-butyl alcohol, acetonitrile and N,N-dimethylformamide). The pK<sub>a</sub> and HNP (half-neutralization potential) values of the compounds 3a-i were determined [8-13, 17, 18].

In the present study, nine new 3-alkyl(aryl)-4-(5-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives (3a-i) were synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones with 5-methylthiophene-2-carboxyaldehyde. The starting compounds 2 were prepared from the reactions of the corresponding ester ethoxycarbonyl-hydrazones (1) with an aqueous solution of hydrazine hydrate as described in the literature [14, 19]. Besides, the reactions of compounds 3a, 3b, 3d, 3g and 3i with acetic anhydride gave 4 type compounds (fig. 1). The structures of fourteen new compounds were identified using IR, 1H NMR, 13C NMR, UV and mass spectral data. In the next part of the current study, due to a wide range applications and to find their possible radical scavenging and antioxidant activity, the newly synthesized compounds were investigated using different antioxidant methodologies: 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, reducing power and metal chelating activities. Moreover, we also examined the potentiometric titrations of the synthesized compounds.

![Fig.1. Synthesis route of compounds 3, 4](image-url)
3a-i with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents (isopropyl alcohol, t-buty1 alcohol, N,N-dimethylformamide, acetonitrile) to determine the corresponding half-neutralization potentials (HNP) and the corresponding pK\textsubscript{a} values.

**Experimental part**

**Synthesis – General Procedures**

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points were determined in open glass capillaries using an Electrothermal 9100 digital melting point apparatus and are uncorrected. The IR spectra were obtained on a Perkin-Elmer Instruments Spectrum One FT-IR spectrometer. 1H and 13C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Varian Mercury spectrometer at 200 MHz and 50 MHz, respectively. UV absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a Shimadzu-1201 UV/VIS spectrometer. Extinction coefficients (ε) are expressed in L . mol\textsuperscript{-1} . cm\textsuperscript{-1}. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a Thermo Scientific TSQ Quantum Access Max Mass Spectrometer.

**General Procedure for the Reaction of 3-alkyl(aryl)-4-(5-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (3)**

The corresponding compound (2) (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 5-methylthiophene-2-carboxyaldehyde (1.26 g, 0.01 mol). The mixture was refluxed for 1 h and then evaporated at 50-55°C in vacuo. Several recrystallizations of the residue from ethyl alcohol gave pure compounds 3a-i as colourless needles.

**Table 1**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Yield (%)</th>
<th>M.p. (°C)</th>
<th>m/z: 245 (48, M+23), 223 (59, M+1), 222 (12, M')</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>85.6</td>
<td>203</td>
<td>174 (52), 161 (58), 142 (30), 96 (100), 74 (12)</td>
</tr>
<tr>
<td>3b</td>
<td>92.4</td>
<td>182</td>
<td>m/z: 259 (59, M+23), 237 (5, M+1), 236 (4, M'), 234 (14), 185 (8), 169 (23), 142 (24), 96 (100), 69 (4)</td>
</tr>
<tr>
<td>3c</td>
<td>85.3</td>
<td>166</td>
<td>m/z: 273 (34, M+23), 251 (3, M+1), 250 (9, M'), 234 (11), 169 (23), 142 (23), 96 (100), 69 (3)</td>
</tr>
<tr>
<td>3d</td>
<td>98.3</td>
<td>214</td>
<td>m/z: 321 (57, M+23), 299 (46, M+1), 298 (3, M'), 279 (8), 234 (15), 184 (8), 160 (21), 142 (22), 96 (100), 60 (3)</td>
</tr>
<tr>
<td>3e</td>
<td>97.8</td>
<td>216</td>
<td>m/z: 335 (47, M+23), 313 (28, M+1), 285 (8), 273 (23), 239 (7), 185 (6), 169 (17), 142 (18), 111 (10), 96 (100), 69 (3)</td>
</tr>
<tr>
<td>3f</td>
<td>97.3</td>
<td>210</td>
<td>m/z: 351 (100, M+23), 329 (20, M+1), 309 (10), 279 (33), 236 (34), 234 (23), 169 (5), 142 (18), 96 (43)</td>
</tr>
<tr>
<td>3g</td>
<td>98.6</td>
<td>250</td>
<td>m/z: 357 (5, M+2+23), 355 (17, M+23), 334 (16, M+2), 333 (38, M+1), 315 (5), 169 (4), 142 (6), 96 (27), 74 (100), 60 (3)</td>
</tr>
<tr>
<td>3h</td>
<td>94.6</td>
<td>215</td>
<td>m/z: 357 (10, M+2+23), 355 (25, M+23), 334 (16, M+2), 333 (45, M+1), 280 (10), 234 (10), 185 (7), 169 (16), 142 (22), 96 (100), 74 (29), 60 (6)</td>
</tr>
<tr>
<td>3i</td>
<td>99.4</td>
<td>199</td>
<td>m/z: 307 (34, M+23), 285 (100, M+1), 284 (8, M'), 243 (20), 222 (5), 123 (7), 93 (3), 60 (3)</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>v (NH)</th>
<th>v (C=O)</th>
<th>v (C=N)</th>
<th>v (substituted benzenoid ring)</th>
<th>λ\textsubscript{max} nm (ε)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>3169</td>
<td>1708</td>
<td>1606</td>
<td>-</td>
<td>318 (14.028), 264 (5978), 218 (2788)</td>
</tr>
<tr>
<td>3b</td>
<td>3178</td>
<td>1698</td>
<td>1594</td>
<td>-</td>
<td>316 (13.509), 264 (7.299), 218 (3.504)</td>
</tr>
<tr>
<td>3c</td>
<td>3184</td>
<td>1711</td>
<td>1597</td>
<td>-</td>
<td>312 (14.976), 330 (13.729), 266 (9.635), 212 (8240)</td>
</tr>
<tr>
<td>3d</td>
<td>3170</td>
<td>1709</td>
<td>1595</td>
<td>760 and 698</td>
<td>320 (14.993), 264 (7.012), 218 (5.360)</td>
</tr>
<tr>
<td>3e</td>
<td>3173</td>
<td>1712</td>
<td>1595</td>
<td>831</td>
<td>316 (16.771), 308 (18.968), 266 (13.780), 218 (14.676)</td>
</tr>
<tr>
<td>3f</td>
<td>3171</td>
<td>1713</td>
<td>1612, 1596</td>
<td>854</td>
<td>318 (17.464), 268 (10.383), 226 (8.818), 210 (4.450)</td>
</tr>
<tr>
<td>3g</td>
<td>3168</td>
<td>1709</td>
<td>1594</td>
<td>825</td>
<td>320 (21.437), 266 (10.000), 222 (16.496)</td>
</tr>
<tr>
<td>3h</td>
<td>3177</td>
<td>1705</td>
<td>1594</td>
<td>789 and 688</td>
<td>316 (14.906), 266 (8.663), 214 (14.366)</td>
</tr>
<tr>
<td>3i</td>
<td>3166</td>
<td>1714</td>
<td>1588</td>
<td>761 and 684</td>
<td>322 (19.993), 264 (18.494), 218 (13.636)</td>
</tr>
</tbody>
</table>
General Method for the Preparation of 1-Acetyl-3-alkyl(aryl)-4-(5-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (4)

The corresponding compound 3 (0.01 mol) was refluxed with acetic anhydride (15 mL) for 0.5 h. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h more. Evaporation of the resulting solution at 40-45 °C in vacuo and several recrystallizations of the residue from ethyl alcohol gave pure compounds 4a, 4b, 4d, 4g and 4i as colourless crystals.

Table 3

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Aliphatic C</th>
<th>Aromatic C</th>
<th>Triazole C3</th>
<th>N=CH</th>
<th>Triazole C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>11.66 (CH3), 16.08 (ArCH3)</td>
<td>127.45; 134.83;</td>
<td>145.68</td>
<td>149.67</td>
<td>151.99</td>
</tr>
<tr>
<td>3b</td>
<td>10.67 (CH3), 16.08 (ArCH3), 19.22 (CH2)</td>
<td>136.55; 144.54;</td>
<td>148.33</td>
<td>149.50</td>
<td>152.12</td>
</tr>
<tr>
<td>3c</td>
<td>13.45 (CH3CH2CH3), 15.39 (ArCH3), 18.85 (CH3CH2CH3), 26.72 (CH3CH2CH3)</td>
<td>126.77; 134.11; 135.84</td>
<td>146.51</td>
<td>148.80</td>
<td>151.34</td>
</tr>
<tr>
<td>3d</td>
<td>16.15 (CH3), 31.80 (CH2)</td>
<td>127.42; 127.50; 129.11 (2C);</td>
<td>146.56</td>
<td>149.38</td>
<td>151.96</td>
</tr>
<tr>
<td>3e</td>
<td>15.45 (CH3), 20.57 (PhCH3), 30.69 (CH3Ph)</td>
<td>126.80; 128.73 (2C); 128.96 (2C); 132.52;</td>
<td>146.00</td>
<td>148.59</td>
<td>151.23</td>
</tr>
<tr>
<td>3f</td>
<td>15.96 (CH3), 30.72 (CH3Ph), 54.48 (OCH3)</td>
<td>145.65; 158.56;</td>
<td>146.19</td>
<td>149.11</td>
<td>151.95</td>
</tr>
<tr>
<td>3g</td>
<td>16.14 (CH3), 31.16 (CH2)</td>
<td>127.51; 129.03 (2C); 131.46 (2C); 132.15;</td>
<td>145.82</td>
<td>149.15</td>
<td>151.70</td>
</tr>
<tr>
<td>3h</td>
<td>15.93 (CH3), 31.25 (CH3Ph)</td>
<td>127.28; 127.31; 128.11; 129.49;</td>
<td>146.27</td>
<td>152.04</td>
<td>152.13</td>
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</table>

Table 4

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Yield (%)</th>
<th>M.p. (°C)</th>
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</thead>
<tbody>
<tr>
<td>4a</td>
<td>87.3</td>
<td>194</td>
</tr>
<tr>
<td>4b</td>
<td>81.7</td>
<td>151</td>
</tr>
<tr>
<td>4d</td>
<td>83.8</td>
<td>187</td>
</tr>
<tr>
<td>4g</td>
<td>96.8</td>
<td>176</td>
</tr>
<tr>
<td>4i</td>
<td>81.0</td>
<td>175</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Experimental and MS data for compounds 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>m/z: 245 (74), 223 (18), 147 (100), 142 (61), 124 (7), 120 (33), 115 (11), 106 (6), 96 (5)</td>
</tr>
<tr>
<td>4b</td>
<td>m/z: 259 (21), 238 (10), 237 (100), 142 (4), 124 (3), 120 (5), 114 (3)</td>
</tr>
<tr>
<td>4d</td>
<td>m/z: 321 (32), 300 (16), 299 (100), 279 (3), 147 (8), 142 (27), 120 (10), 115 (4)</td>
</tr>
<tr>
<td>4g</td>
<td>m/z: 357 (6), 355 (17), 355 (17), 335 (36), 333 (100), 277 (2), 255 (3), 226 (3), 209 (3), 145 (3), 142 (12), 120 (13)</td>
</tr>
<tr>
<td>4i</td>
<td>m/z: 307 (18), 286 (15), 285 (100), 147 (37), 142 (21), 120 (8), 115 (3)</td>
</tr>
</tbody>
</table>
Experimental and mass spectral data, IR and UV, $^1$H NMR, $^{13}$C NMR spectral data of the compounds 4 are presented in Table 5, 6, 7 and 8, respectively.

**Antioxidant Activity: Chemicals**

Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, $\alpha$-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA) and trichloroacetic acid (TCA) were bought from Sigma.

**Reducing power**

The reducing power of the synthesized compounds was determined according to the method of Oyaizu [20]. Different concentrations of the samples (50-250 $\mu$g/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min, after which a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl$_3$ (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured by a spectrophotometer. Higher absorbance of the reaction mixture indicated higher reducing power.

**Free radical scavenging activity**

Free radical scavenging activity of compounds was measured by DPPH, using the method of Blois [21]. Briefly, 0.1 mM solution of DPPH in ethanol was prepared, and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50-250 $\mu$g/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

$$\text{Absorbance} = 0.0003 \times \text{DPPH} - 0.0174$$

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect} (\%) = \left( \frac{A_0 - A_1}{A_0} \right) \times 100$$

where $A_0$ is the absorbance of the control reaction and $A_1$ is the absorbance in the presence of the samples or standards.

**Metal chelating activity**

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method...
of Dinis et al. [22]. Briefly, the synthesized compounds (50-250 μg/mL) were added to a 2 mM solution of FeCl₂ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm by a spectrophotometer. All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe²⁺ complex formation was given by the formula: % Inhibition = (A₀ – A₁/A₀) x 100, where A₀ is the absorbance of the control, and A₁ is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

**Potentiometric Titrations**

For potentiometric titrations an Orion 720A model pH-ionmeter equipped with a combined pH electrode (Ingold) and indicator electrode were used. A magnetic stirrer, a semi-micro burette and a 25 mL beaker were also used in titrations. Before potentiometric titrations, the pH meter was calibrated according to the instructions supplied by the manufacturers of the pH meter. During the titrations, the titrant was added in increments of 0.05 mL after each stable reading and mV values were recorded. The necessary chemicals were supplied from Fluka and Merck. After purifications, isopropyl alcohol was used to prepare a 0.05 N tetra-butylammonium hydroxide. For all potentiometric titrations, 0.05 N tetra-butylammonium hydroxide in isopropyl alcohol, which was prepared from 0.1 N tetra-butylammonium hydroxide (TBAH) by dilution, was used. The 0.05 M solution of TBAH in isopropyl alcohol, which is widely used in the titration of acids, was used as titrant. The half-neutralization potentials and the corresponding pKₐ values of all compounds, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, t-butyl alcohol, N,N-dimethylformamide and acetonitrile, are presented in table 9. Finally, the half-neutralization potential (HNP) values were determined by drawing the mV-mL (TBAH) graphic. From the titration curves, the HNP values were measured and the corresponding pKₐ values were calculated.

**Results and discussions**

In this study, the structures of 9 new 3-alkyl(aryl)-4-(5-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (3a-i) and 5 new 1-acetyl-3-alkyl(aryl)-4-(5-methyl-2-thienyl-methyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (4a, 4b, 4d, 4g, 4k) were identified using IR, 1H NMR, 13C NMR, UV and mass spectral data.

**Antioxidant Activity**

The compounds 3 and 4 were screened for their in-vitro antioxidant activities. Several methods are used to determine antioxidant activities. The methods used in this study are discussed below.

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

The reductive capabilities of compounds are assessed by the extent of conversion of the Fe³⁺/ferricyanide complex to the Fe²⁺/ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and α-tocopherol. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity [23]. The antioxidant activity of a 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 [24]. In this study, all of the amounts of the compounds showed lower absorbance than blank. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction. In other words, compounds did not show the reductive activities.

**DPPH• radical scavenging activity**

The scavenging of the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [25]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [26]. 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 antioxidants, because the reaction between antioxidant molecules and radical, progresses, which result 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 [27]. BHA, BHT and α-tocopherol were used as a reference to antioxidant compounds. The newly synthesized compounds showed no activity as a radical scavenger.

**Ferrous ion chelating activity**

The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis [22]. Ferrozine can quantitatively form complexes with Fe²⁺. 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 [28]. Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe³⁺) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe²⁺, depending on condition, particularly pH [29] and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents could not activate metal ions and potentially inhibit the metal-dependent processes [30]. Also, the production of highly active ROS such as O₂⁻, H₂O₂ and OH⁻ is also catalyzed by free iron through 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:

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

**Figure 4.** pH - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound 3a titrated with 0.05 M TBAH in isopropyl alcohol, t-butyl alcohol, acetonitrile and N,N-dimethyl formamide at 25 °C.

**Figure 5.** mV - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound 3a titrated with 0.05 M TBAH in isopropyl alcohol, t-butyl alcohol, acetonitrile and N,N-dimethyl formamide at 25 °C.
Fe³⁺ ion also produces radicals from peroxides, although the rate is tenfold less than that of Fe²⁺ ion, which is the most powerful pro-oxidant among the various types of metal ions [31]. Ferrous ion chelating activities of the compounds, BHT, BHA and α-tocopherol are shown in Figures 2 and 3. 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 [32].

The data obtained from Figures 2 and 3 reveal that the compounds, especially 3d, 3f, 4a, 4b, 4d and 4i, demonstrate a marked capacity for iron binding, 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 this study, compounds 3a-i were titrated potentiometrically with TBAH in isopropyl alcohol, t-butyl alcohol, acetonitrile and N,N-dimethylformamide. The mV values read in each titration were drawn against TBAH volumes (mL) added and potentiometric titration curves were formed for all the cases. From the titration curves, the HNP values were measured and the corresponding pKₐ values were calculated. The half-neutralization potential (HNP) values and the corresponding pKₐ values of all triazole derivatives, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, t-butyl alcohol, acetonitrile and N,N-dimethylformamide are presented in table 9. Using all values in table 9 for compounds 3a-i in isopropyl alcohol, t-butyl alcohol, N,N-dimethylformamide and acetonitrile were drawn pH-mL (TBAH), mV-mL (TBAH), ΔE/ΔV-mL (TBAH), Δ²E/ΔV²-mL (TBAH) and ΔV/ΔE-mL (TBAH) graphic (figs. 4-8 for 3a). The pH of the weak acids is given by the following equation:

\[ pH = pK_a + \log [A^-]/[HA] \]

pH = pKₐ occurs when [A⁻] is equal to [HA] at the half-neutralization point. Therefore, the pH values can be regarded as pKₐ at the half-neutralization points. When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: N,N-dimethyl formamide (ε = 36.7) > acetonitrile (ε = 36.0) > isopropyl alcohol (ε = 19.4) > t-butyl alcohol (ε = 12.0).

The acidity of a compound depends on several factors. The two most important factors are the solvent effect and molecular structure. Table 9 shows that the half neutralization potential (HNP) values and the corresponding pKₐ values obtained from potentiometric titrations depend on the type of non-aqueous solvents used and molecular structure of the compound.

As seen in table 9, the acidic order for compounds 3a, 3b and 3e is: isopropyl alcohol > N,N-dimethyl formamide > t-butyl alcohol > acetonitrile, for compounds 3c, 3d and 3g is: isopropyl alcohol > t-butyl alcohol > N,N-dimethyl formamide > acetonitrile, for compounds 3f and 3h is: isopropyl alcohol > t-butyl alcohol > acetonitrile > N,N-
Table 9

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Isopropyl alcohol pH&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Isopropyl alcohol HNP (mV)</th>
<th>t-Butyl alcohol pH&lt;sub&gt;a&lt;/sub&gt;</th>
<th>t-Butyl alcohol HNP (mV)</th>
<th>N,N-Dimethylformamide pH&lt;sub&gt;a&lt;/sub&gt;</th>
<th>N,N-Dimethylformamide HNP (mV)</th>
<th>Acetonitrile pH&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Acetonitrile HNP (mV)</th>
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</thead>
<tbody>
<tr>
<td><strong>3a</strong></td>
<td>13.12 ± 2.8</td>
<td>-362.5 ± 14.92</td>
<td>14.29 ± 2.7</td>
<td>-468.9 ± 14.94</td>
<td>14.79 ± 2.8</td>
<td>-459.9 ± 15.37</td>
<td>15.37 ± 2.8</td>
<td>-495.3 ± 2.8</td>
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<tr>
<td>0.05</td>
<td>2.8</td>
<td>3.2</td>
<td>0.06</td>
<td>3.0</td>
<td>0.03</td>
<td>2.8</td>
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<td></td>
</tr>
<tr>
<td><strong>3b</strong></td>
<td>13.28 ± 2.7</td>
<td>-370.9 ± 15.04</td>
<td>14.50 ± 2.5</td>
<td>-475.5 ± 14.94</td>
<td>14.94 ± 2.5</td>
<td>-469.7 ± 15.45</td>
<td>15.45 ± 2.7</td>
<td>-500.1 ± 2.7</td>
</tr>
<tr>
<td>0.05</td>
<td>2.7</td>
<td>2.5</td>
<td>0.05</td>
<td>3.2</td>
<td>0.05</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3c</strong></td>
<td>13.27 ± 2.6</td>
<td>-371.0 ± 15.08</td>
<td>14.58 ± 2.7</td>
<td>-478.1 ± 15.10</td>
<td>15.10 ± 2.7</td>
<td>-479.5 ± 15.37</td>
<td>15.37 ± 2.8</td>
<td>-495.1 ± 2.8</td>
</tr>
<tr>
<td>0.06</td>
<td>3.0</td>
<td>2.8</td>
<td>0.04</td>
<td>2.3</td>
<td>0.06</td>
<td>3.4</td>
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</tr>
<tr>
<td><strong>3d</strong></td>
<td>13.24 ± 2.6</td>
<td>-369.0 ± 14.66</td>
<td>14.66 ± 2.7</td>
<td>-453.2 ± 14.69</td>
<td>14.69 ± 2.7</td>
<td>-453.9 ± 15.14</td>
<td>15.14 ± 2.7</td>
<td>-481.7 ± 2.5</td>
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<tr>
<td>0.04</td>
<td>3.1</td>
<td>3.6</td>
<td>0.03</td>
<td>2.6</td>
<td>0.07</td>
<td>2.5</td>
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<tr>
<td><strong>3e</strong></td>
<td>12.90 ± 2.6</td>
<td>-348.5 ± 14.81</td>
<td>14.81 ± 2.4</td>
<td>-462.3 ± 14.66</td>
<td>14.66 ± 2.7</td>
<td>-453.5 ± 15.13</td>
<td>15.13 ± 2.8</td>
<td>-480.2 ± 2.5</td>
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<tr>
<td>0.04</td>
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<td>2.4</td>
<td>0.07</td>
<td>2.8</td>
<td>0.04</td>
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<tr>
<td><strong>3f</strong></td>
<td>13.31 ± 2.5</td>
<td>-373.1 ± 14.77</td>
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<td>-459.4 ± 14.52</td>
<td>15.42 ± 2.7</td>
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<td>3.3</td>
<td>0.05</td>
<td>3.3</td>
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<tr>
<td><strong>3g</strong></td>
<td>13.03 ± 2.6</td>
<td>-356.7 ± 14.51</td>
<td>14.51 ± 2.7</td>
<td>-443.9 ± 14.52</td>
<td>14.52 ± 2.7</td>
<td>-441.7 ± 15.07</td>
<td>15.07 ± 2.7</td>
<td>-476.7 ± 2.7</td>
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<td>3.1</td>
<td>0.05</td>
<td>2.5</td>
<td>0.06</td>
<td>3.1</td>
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</tr>
<tr>
<td><strong>3h</strong></td>
<td>12.70 ± 2.6</td>
<td>-337.3 ± 14.20</td>
<td>14.20 ± 2.5</td>
<td>-425.8 ± 14.87</td>
<td>14.87 ± 2.7</td>
<td>-465.7 ± 14.77</td>
<td>14.77 ± 2.8</td>
<td>-459.9 ± 2.7</td>
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<tr>
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<td>3.2</td>
<td>3.2</td>
<td>0.04</td>
<td>3.1</td>
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<td>2.9</td>
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<tr>
<td><strong>3i</strong></td>
<td>12.81 ± 2.6</td>
<td>-343.9 ± 14.48</td>
<td>14.48 ± 2.5</td>
<td>-424.5 ± 14.36</td>
<td>13.69 ± 2.7</td>
<td>-395.2 ± 14.28</td>
<td>14.28 ± 2.8</td>
<td>-430.8 ± 2.7</td>
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<td>2.3</td>
<td>2.7</td>
<td>0.04</td>
<td>2.4</td>
<td>0.05</td>
<td>2.8</td>
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</tr>
</tbody>
</table>

dimethyl formamide, for compound 3i is: isopropyl alcohol > N,N-dimethylformamide > acetoneitrile > t-butyl alcohol. In isopropyl alcohol, 3a - i compounds show the strongest acidic properties. 3a - e and 3g compounds show the weakest acidic properties in acetonitrile, 3f and 3h compounds show the weakest acidic properties in N,N-dimethylformamide, compound 3i shows the weakest acidic properties in t-butyl alcohol. This situation may be attributed to the hydrogen bonding between the negative ions formed and the solvent molecules in the amphiprotic neutral solvents. Autoprotolysis is an acid-base reaction between identical solvent molecules in which some act as an acid and others as a base.

Conclusions

The synthesis and in vitro antioxidant evaluation of new 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives are described. All of the compounds demonstrate a marked capacity for iron binding. The data here reported could be of possible interest because of the observed radical scavenging and metal chelating activities of the studied compounds could prevent redox cycling. Design and synthesis of novel small molecules which can have a specifically protective role in biological systems are in perspective in modern medicinal chemistry. These results may also provide some guidance for the development of novel triazole-based therapeutic target.

The extent of an autoprotolysis reaction depends both on the intrinsic acidity and the intrinsic basicity of the solvent. The importance of the autoprotolysis constant in titrations lies in its effect on the completeness of a titration reaction. Half-neutralization potential (HNP) values and corresponding pK<sub>a</sub> values obtained from the potentiometric titrations rely on the non-aqueous solvents used and the substituents at C-3, in triazole ring.

References


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