# Synthesis and *In* vitro Antioxidant Activities of Novel 4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one Derivatives with Their Acidic Properties

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**Summary:** In the present study 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2a-i** reacted with 3-methyl-thiophene-2-carboxyaldehyde to afford the corresponding 3-alkyl(aryl)-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3a-i.** The acetylation reactions of compounds **3a, 3b, 3d, 3g** and **3i** were investigated and compounds **4a, 4b, 4d, 4g** and **4i** were thus obtained. The structures of fourteen new compounds are established from the spectral data. In addition, the synthesized **3** and **4** type compounds were analyzed for their *in* vitro potential antioxidant activities in three different methods including reducing power, free radical scavenging and metal chelating activity. Compound **3a** and **3d** showed the best activity for the iron binding. On the other hand, compounds **3a-i** were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in four nonaqueous solvents (isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethylformamide) because of the weak acidic properties of 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring N-H group. Also half-neutralization potential (HNP) values and the corresponding p$K_a$ values were determined in all cases.

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

## Introduction

In the last two decades, a great deal of research has been devoted to the study of different types of natural and synthetic antioxidant because of their capacity to protect organism and cell from damage that is induced by the oxidative stress. Scientists have become more interested in new compounds, they have either synthesized or obtained them from natural sources that could provide active components for preventing or reducing the impact of oxidative stress on cells [1]. Exogenous chemicals and endogenous metabolic processes in human body or in food system might produce highly reactive free radicals, especially oxygen, derived from radicals which are capable of oxidizing biomolecules that result in the cell death and issue damage. Oxidative damages significantly play a pathological role in serious human diseases. For example, cancer, emphysema, cirrhosis, atherosclerosis and arthritis which have all been correlated with oxidative damage. Also, the excessive generation of reactive oxygen species (ROS) 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 [2].

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 [3-11], and several papers have devoted with the synthesis of some *N*-arylidenamino derivatives of 4,5-dihydro-1*H*-1,2,4-triazol-5-one compounds [10-15]. The acetylation of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have also been reported [10-16]. In addition, it is known that 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one rings have weak acidic properties, so some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentiometrically with TBAH in non-aqueous solvents (isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethylformamide). The p$K_a$ and HNP values of the compounds 3a-i were determined [10-15, 17, 18].

In the present study, nine new 3-alkyl(aryl)-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(3a-i)** were synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(2a-i)** with 3-methyl-thiophene-2-carboxyaldehyde. The starting **2** type compounds were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones **(1)** with an aqueous solution of hydrazine hydrate as described in the literature [16, 19]. Besides, the reactions of compounds **3a, 3b, 3d, 3g** and **3i** with acetic anhydride gave **4** type compounds (Fig. 1).

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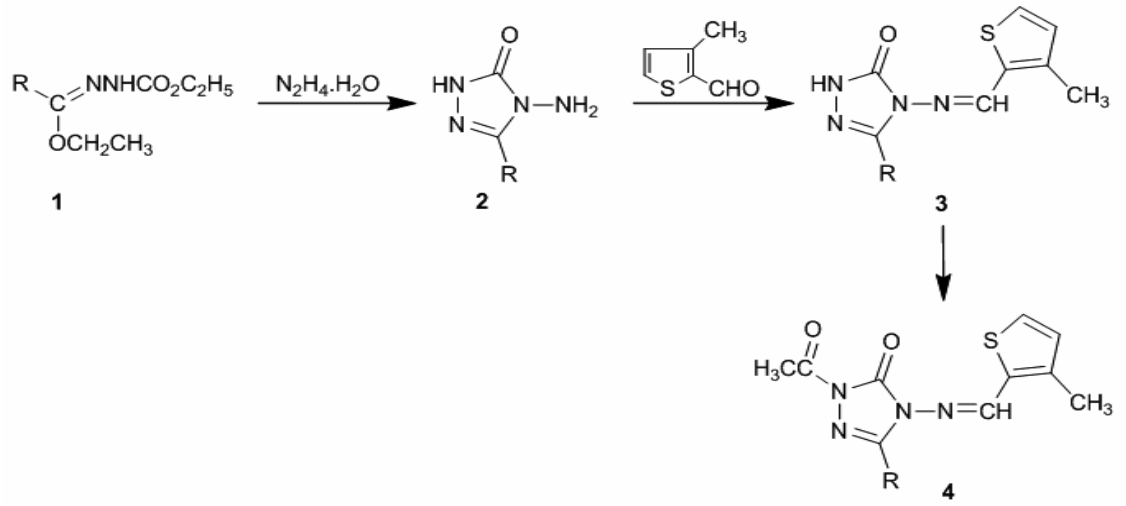

a) R= $CH_3$, b) R= $CH_2CH_3$, c) R= $CH_2CH_2CH_3$, d) R= $CH_2C_6H_5$, e) R= $CH_2C_6H_4.CH_3$ (*p*-), f) R= $CH_2C_6H_4.OCH_3$ (*p*-), g) R= $CH_2C_6H_4.Cl$ (*p*-), h) R= $CH_2C_6H_4.Cl$ (*m*-), i) R= $C_6H_5$

Fig. 1: Synthesis route of compounds **3, 4**

The structures of fourteen new compounds were identified using IR, $^1$H-NMR, $^{13}$C-NMR, UV and mass spectral data. Next part of the current study, due to a wide range applications and to find their the possible radical scavenging and antioxidant activity, the newly synthesized compounds were investigated using different antioxidant methodologies: 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging, reducing power and metal chelating activities. Moreover, we also examined the potentiometric titrations of the synthesized compounds **3a-i** with TBAH in four non-aqueous solvents (isopropyl alcohol, *t*-butyl alcohol, *N,N*-dimethylformamide, acetonitrile) to determine the corresponding HNP and the corresponding p*K*a values.

**Results and Discussion**

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

*Antioxidant Activity*

The compounds **3** and **4** were screened for their *in-vitro* antioxidant activities. 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 by using the method of Oyaizu [20]. High absorbance at 700 nm indicates high reducing power. The reducing powers of the compounds were observed at different concentrations, and results were compared with butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity [21]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, such as prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [22]. In the study, all the amount of the compounds, except **3f** and **4d**, showed lower absorbance then 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, except **3f** and **4d**, did not show the reductive activities. Fig. 2 shows the reducing activity of compounds **3f** and **4d** and standards BHT, BHA and α-tocopherol using the potassium ferricyanide method. Reducing power of the compounds and the standards were found as following order: BHT > BHA > α-tocopherol > compound **3f** > compound **4d**.

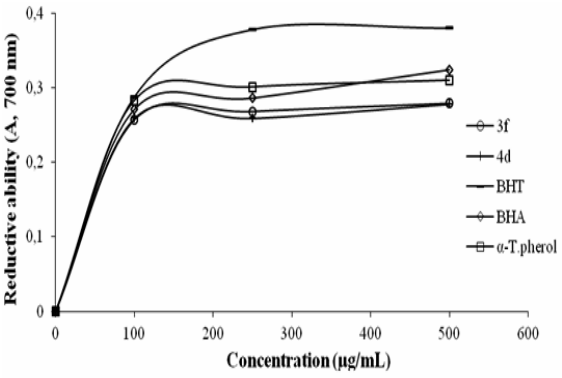


Fig. 2: Total reductive potential of different concentrations of compounds 3f, 4d, BHT, BHA and α-tocopherol.

*DPPH• Radical Scavenging Activity*

Free radical scavenging activity of compounds was measured via DPPH· by using the method of Blois [23]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [24]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [25]. The reduction capability of DPPH radical 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 of 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 the antioxidative activity of antioxidants [26]. 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 chelation of ferrous ions by the synthesized compounds and standards was estimated by the method of Dinis *et al*. [27]. 3-(2-Pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine) can form quantitatively complexes with $Fe^{2+}$. 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 ($Fe^{3+}$) is the relatively biologically inactive form of iron. However, it can be reduced to the active $Fe^{2+}$, depending on condition, particularly pH [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 may not activate metal ions and potentially inhibit the metal-dependent processes [30]. Also, the production of highly active ROS such as $O_2^{\cdot-}$, $H_2O_2$ and OH are also catalyzed by free iron though Haber-Weiss reactions:

$$O^{\cdot}_2 + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$$

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:

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

$Fe^{3+}$ ion also produces radicals from peroxides, although 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 [31]. Ferrous ion chelating activities of the compounds **3**, **4,** BHT, BHA and α-tocopherol are respectively shown in Fig. 3 and 4. In the 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].

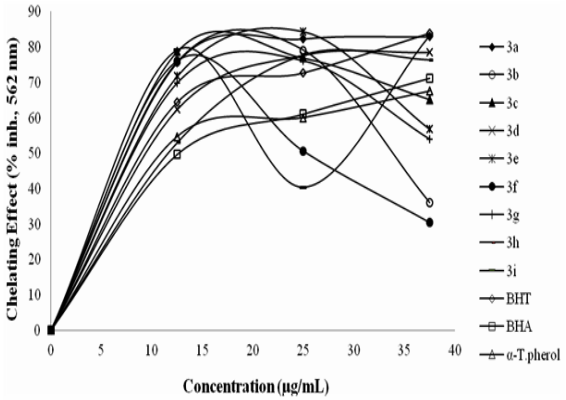


Fig. 3: Metal chelating effect of different amount of the compounds **3**, BHT, BHA and α-tocopherol on ferrous ions.

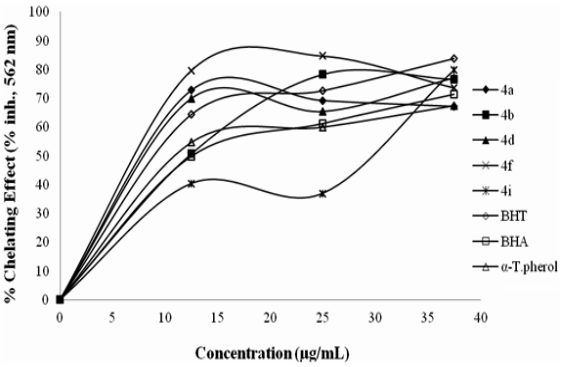


Fig. 4: Metal chelating effect of different amount of the compounds 4, BHT, BHA and α-tocopherol on ferrous ions.

It was reported that the compounds with structures containing two or more of the functional groups, such as -OH, -SH, -COOH, $-PO_3H_2$, C=O, $-NR_2$, -S-, -O- in a favourable structure–function configuration should have chelation activity [33-35]. In this respect, L-carnitine may chelate the ferrous ions with hydroxyl and carboxylate groups [35]. In this study; **3** and **4** type compounds contain -S- and C=O groups and we suggested the chelated configurations as can be seen in Fig. 5 and 6.

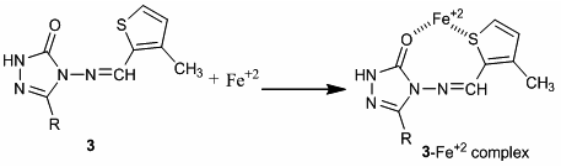


Fig. 5: The proposed chelating of ferrous ions reaction by 3 type compounds.

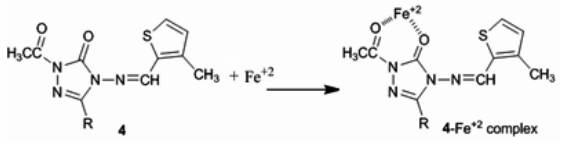


Fig. 6: The proposed chelating of ferrous ions reaction by 4 type compounds.

Low absorbance at 562 nm indicates high metal chelating activity. The data obtained from Fig. 3 and 4 reveal that the metal chelating effects of the compounds **3a** and **3d** were concentration-dependent, the other compounds were not. Thus, the compounds **3a** and **3d** 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 the study, compounds **3a-i** were titrated potentiometrically with TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide. 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 p$K_a$ values were calculated. The HNP values and the corresponding p$K_a$ 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*-dimethyl formamide are presented in Table-1. Using all values in Table-1 for compounds **3a-i** in isopropyl alcohol, *t*-butyl alcohol, *N,N*-dimethylformamide and acetonitrile were drawn pH-mL (TBAH), mV-mL (TBAH), $\Delta E/\Delta V$-mL (TBAH), $\Delta^2E/\Delta V^2$-mL (TBAH) and $\Delta V/\Delta E$-mL (TBAH) graphic (Fig. 7-11 for **3i**). The pH of the weak acids is given by the following equation:

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

pH = p$K_a$ occurs when $[A^-]$ is equal to [HA] at the half-neutralization point. Therefore, the pH values can be regarded as p$K_a$ 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 ($\varepsilon$ = 36.7) > acetonitrile ($\varepsilon$ = 36.0) > isopropyl alcohol ($\varepsilon$ = 19.4) > *t*-butyl alcohol ($\varepsilon$ = 12.0). The acidity of a compound depends on several factors. The two most important factors are the solvent effect and molecular structure. Table-1 shows that the half neutralization potential (HNP) values and the corresponding p$K_a$ values obtained from potentiometric titrations depend on the type of non-aqueous solvents used and molecular structure of the compound.

As seen in Table-1, the acidic order for compounds **3a**, **3b, 3c, 3d, 3g** and **3i** is: isopropyl alcohol > *N,N*-dimethyl formamide > *t*-butyl alcohol > acetonitrile, for compounds **3f** and **3h** is: isopropyl alcohol > *t*-butyl alcohol > *N,N*-dimethyl formamide > acetonitrile, for compound **3e** is: isopropyl alcohol > *t*-butyl alcohol > *N,N*-dimethyl formamide > acetonitrile. In isopropyl alcohol, **3a**, **3b**, **3c**, **3d, 3e,**

**3f, 3g, 3h** and **3i** compounds show the strongest acidic properties. **3a**, **3b**, **3c**, **3d**, **3e, 3g** and **3i** compounds show the weakest acidic properties in acetonitrile, **3f** and **3h** compounds show the weakest acidic properties in *N,N*-dimethyl formamide.

In addition, acidity is determined in each solvent and is observed in the following order; **3i>3h>3g>3d>3a>3f>3b>3e>3c** in isopropyl alcohol, **3i>3h>3g>3f>3d=3a>3e>3b>3c** in *t*-butyl alcohol, **3i>3g>3d>3b>3a>3h>3e>3c>3f** in *N,N*-dimethyl formamide and **3i>3h>3d>3g>3e>3f>3a>3c>3b** in acetonitrile. According to these results, compound **3i** is showed the strongest acidic properties in all solvents, compound **3c is** showed the weakest acidic properties in isopropyl alcohol and *t*-butyl alcohol, compound **3f** is showed the weakest acidic properties in *N,N*-dimethyl formamide and compound **3b** is showed the weakest acidic properties in acetonitrile.

According to dielectric constant, 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), but this collocation is not seen in this work. This situation was observed due to the fact that different R groups connected in molecules. Because, molecules has been gotten different properties with connected R groups.

Table-1: Half-Neutralization potentials (HNP) and the corresponding p$K_a$ values of 3a-i compounds in isopropyl alcohol, *t*-butyl alcohol, *N,N*-dimethyl formamide and acetonitrile at 25 °C.

| Compound | Isopropyl alcohol | | *t*-Butyl alcohol | | *N,N*-Dimethyl formamide | | Acetonitrile | |
|---|---|---|---|---|---|---|---|---|
| No. | pKa | HNP (mV) | pKa | HNP (mV) | pKa | HNP (mV) | pKa | HNP (mV) |
| 3a | 13.02 ± 0.05 | -356.4 ± 3.2 | 14.89 ± 0.04 | -465.9 ± 3.0 | 14.88 ± 0.03 | -464.9 ± 3.2 | 15.24 ± 0.04 | -486.9 ± 2.6 |
| 3b | 13.36 ± 0.03 | -376.5 ± 2.9 | 15.01 ± 0,06 | -474.1 ± 2.6 | 14.47 ± 0.05 | -441.1 ± 3.0 | 15.45 ± 0.05 | -499.9 ± 2.3 |
| 3c | 13.58 ± 0.06 | -389.9 ± 3.1 | 15.23 ± 0.05 | -486.6 ± 2.8 | 15.22 ± 0.06 | -486.7 ± 2.8 | 15.35 ± 0.04 | -494.4 ± 3.1 |
| 3d | 13.01 ± 0.04 | -355.5 ± 2.8 | 14.89 ± 0.05 | -467.1 ± 3.2 | 14.26 ± 0.04 | -430.1 ± 2.6 | 14.96 ± 0.06 | -470.5 ± 2.8 |
| 3e | 13.43 ± 0.05 | -379.9 ± 2.9 | 14.92 ± 0.06 | -468.4 ± 2.7 | 15.07 ± 0.06 | -478.0 ± 2.5 | 15.08 ± 0.03 | -477.6 ± 2.5 |
| 3f | 13.18 ± 0.03 | -365.7 ± 3.0 | 14.88 ± 0.04 | -466.2 ± 2.4 | 15.30 ± 0.05 | -491.2 ± 3.1 | 15.12 ± 0.05 | -480.0 ± 3.0 |
| 3g | 12.81 ± 0.04 | -343.8 ± 3.2 | 14.65 ± 0.05 | -452.6 ± 3.1 | 14.16 ± 0.04 | -423.2 ± 2.5 | 15.03 ± 0.06 | -489.6 ± 3.2 |
| 3h | 12.68 ± 0.06 | -336.1 ± 2.6 | 14.47 ± 0.03 | -441.5 ± 3.0 | 15.01 ± 0.05 | -474.3 ± 3.0 | 14.90 ± 0.04 | -467.6 ± 2.9 |
| 3i | 12.50 ± 0.05 | -325.4 ± 2.8 | 13.97 ± 0.06 | -412.6 ± 2.8 | 13.81 ± 0.04 | -402.7 ± 2.7 | 14.28 ± 0.05 | -430.9 ± 3.1 |

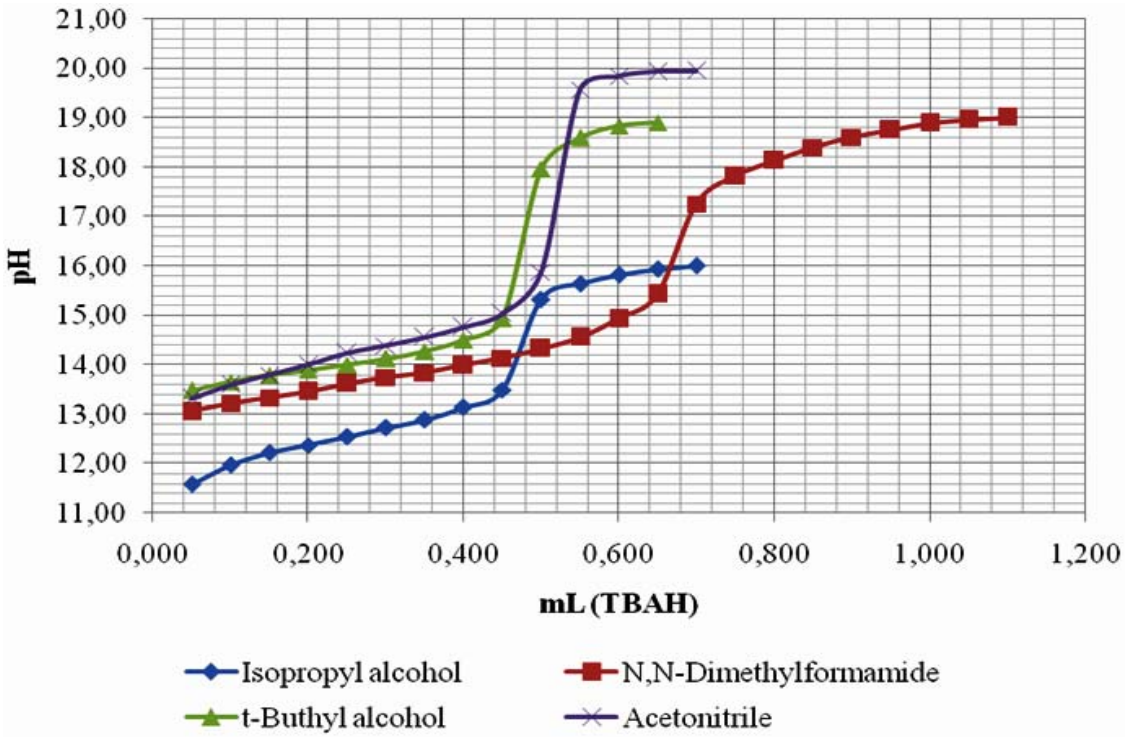


Fig. 7: pH - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **3i** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C.

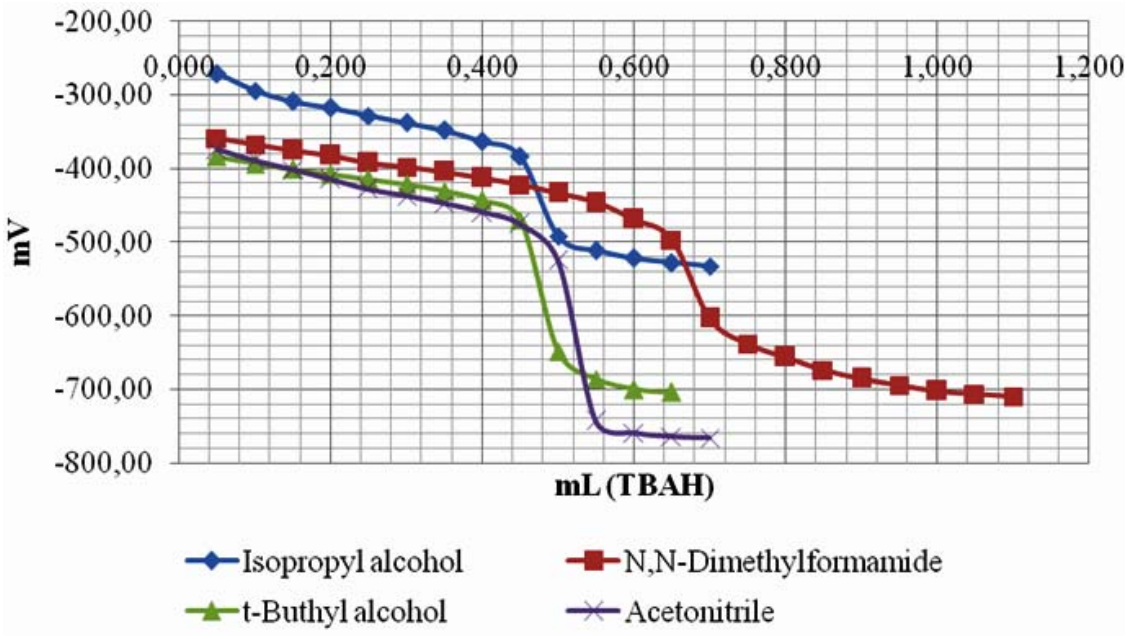


Fig. 8: mV - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **3i** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C.

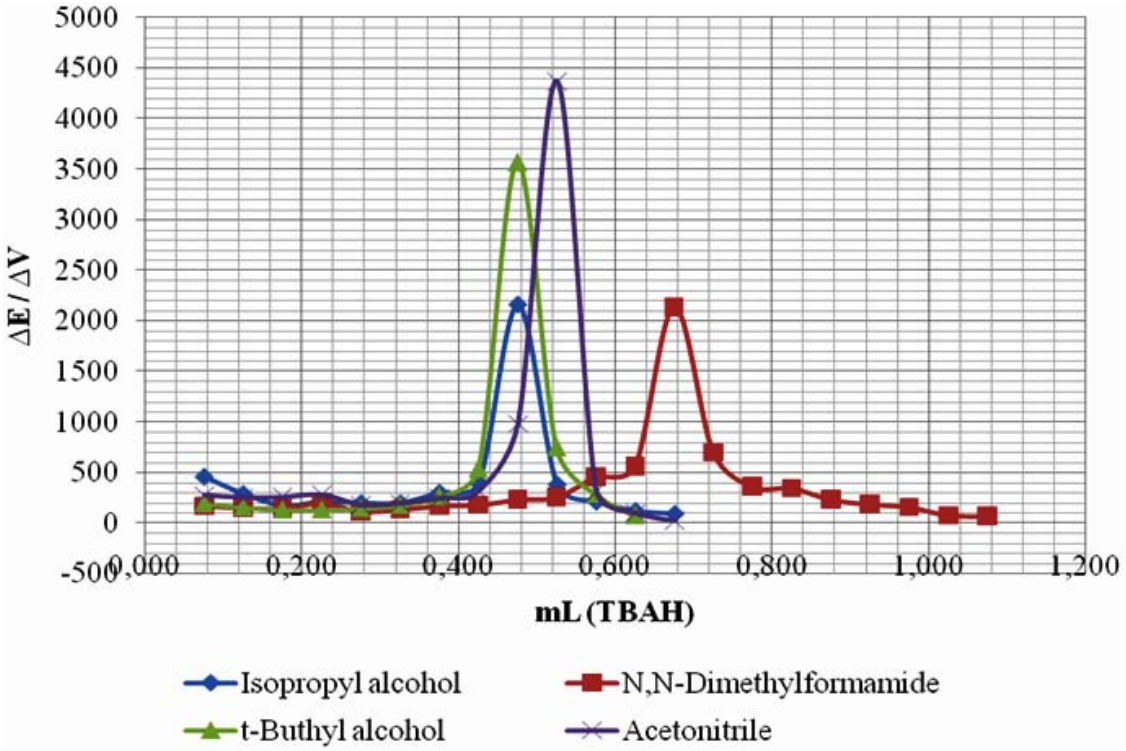


Fig. 9: ΔE / ΔV - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **3i** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C.

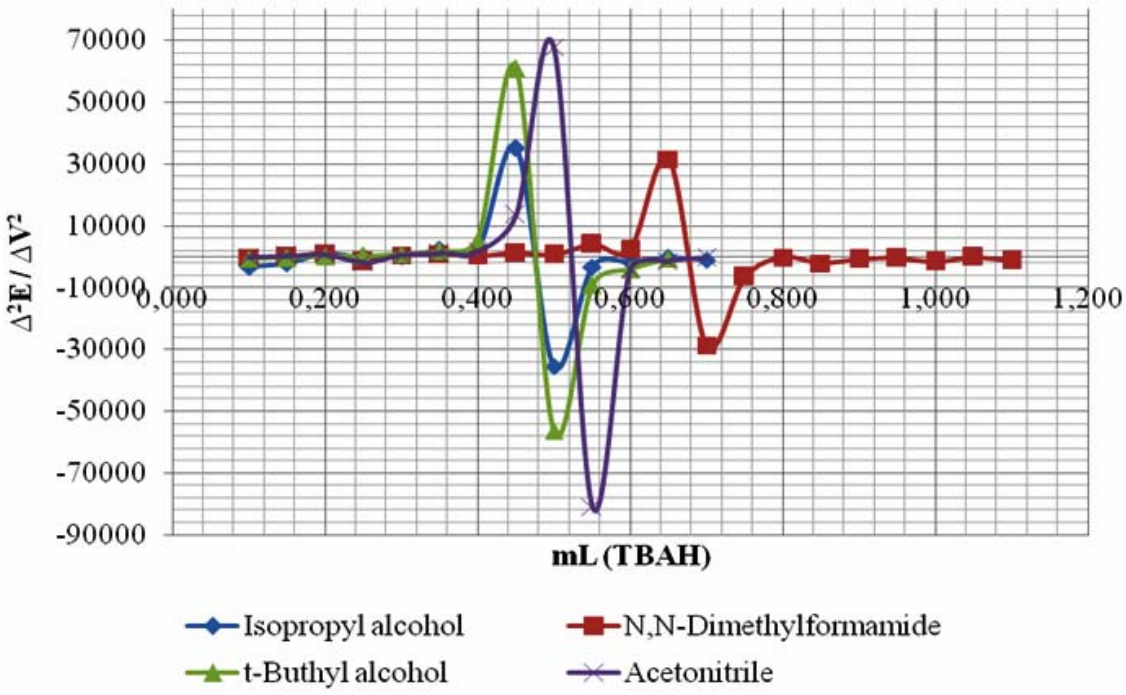


Fig. 10: $\Delta^2E / \Delta V^2$ - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound 3i titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C.

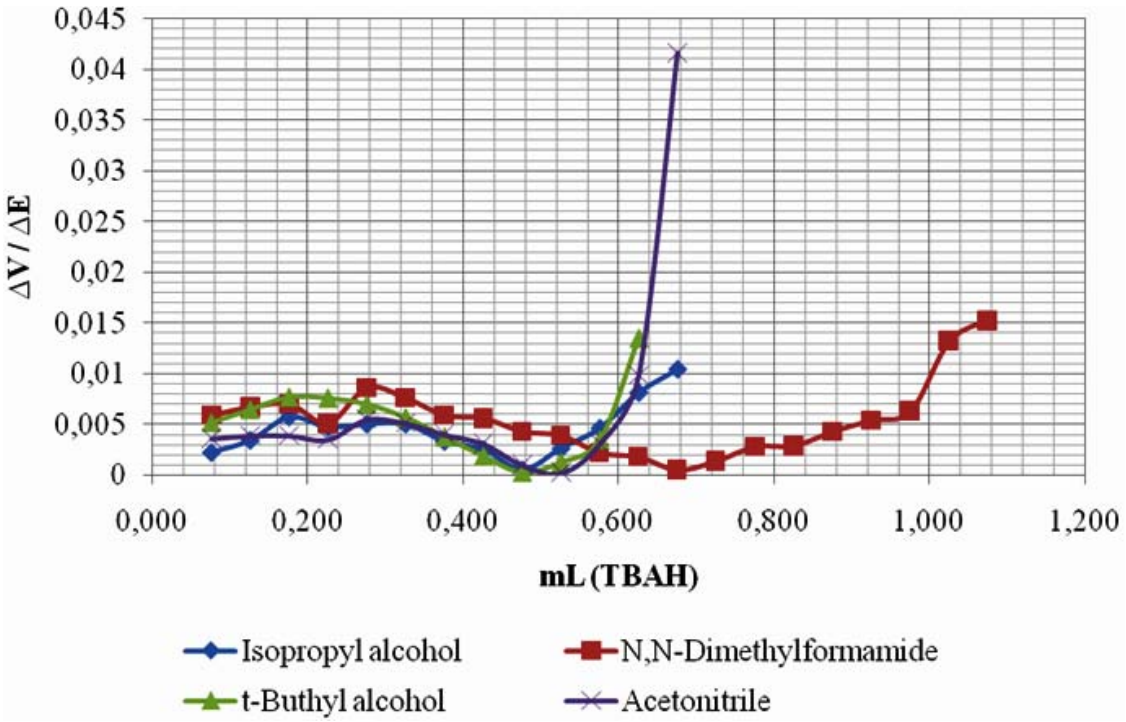


Fig. 11: ΔV / ΔE - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound 3i titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C.

As far as autoprotolysis constant (*N,N*-dimethyl formamide (pKs = 18.0) > acetonitrile (pKs = 33.0) > isopropyl alcohol (pKs = 20.6) > *t*-butyl alcohol (pKs = 22.0)), all compounds show the weakest acidic properties in *N,N*-dimethyl formamide, isopropyl alcohol and *t*-butyl alcohol but show the strongest acidic properties in acetonitrile. Finally, it was not observed leveling effect of each solvents.

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 is which some act as an acid and others as a base.

## Experimental

*Synthesis – General Procedures*

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points which were uncorrect were determined in open glass capillaries using an Electrothermal 9100 digital melting point apparatus. The IR spectra were obtained on a Perkin-Elmer Instruments Spectrum One FT-IR spectrometer. $^{1}$H and $^{13}$C 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 Schimadzu-1201 UV/VIS spectrometer. Extinction coefficients (ε) are expressed in $L \cdot mol^{-1} \cdot cm^{-1}$. 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-(3-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 3-methyl-thiophene-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.

*3-Methyl-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3a)**

Yield 96.9%, mp. 223 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3173 (NH), 1714 (C=O), 1608, 1603 (C=N); $^{1}$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.19 (s, 3H, $CH_3$), 2.32 (s, 3H, $CH_3$), 7.01 (d, 1H, arH, *J*=5.12 Hz), 7.68 (d, 1H, arH, *J*=4.76 Hz), 9.84 (s, 1H, N=CH), 11.79 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 11.72 ($CH_3$), 14.47 ($CH_3$), ar-C: [130.59, 131.95, 132.26, 143.63], 144.55 (triazole-$C_3$), 148.59 (N=CH), 152.08 (triazole-$C_5$); UV $\lambda_{max}$ (ε): 316 (28556), 284 (18486), 218 (8458) nm; MS: m/z 222 ($M^+$, 8), 223 (M+1, 32).

*3-Ethyl-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3b)**

Yield 95.3%, mp. 190 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3181 (NH), 1702 (C=O), 1594 (C=N); $^{1}$H-NMR (DMSO-$d_6$, $\delta$ ppm): 1.16 (t, 3H, $CH_3$, *J*=7.69 Hz), 2.32 (s, 3H, $CH_3$), 2.58 (q, 2H, $CH_2$, *J*=7.69 Hz), 7.00 (d, 1H, arH, *J*=5.12 Hz), 7.66 (d, 1H, arH, *J*=5.12 Hz), 9.84 (s, 1H, N=CH), 11.82 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 10.64 ($CH_3$), 14.45 ($CH_3$), 19.22 ($CH_2$), ar-C: [130.53, 131.93, 132.39, 143.52], 144.33 (triazole-$C_3$), 148.37 (N=CH), 152.22 (triazole-$C_5$); UV $\lambda_{max}$ (ε): 316 (13447), 286 (9162), 220 (11900) nm; MS: m/z 236 ($M^+$, 8), 237 (M+1, 5).

*3-n-Propyl-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3c)**

Yield 85.3%, mp. 182 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3175 (NH), 1708 (C=O), 1593 (C=N); $^{1}$H-NMR (DMSO-$d_6$, $\delta$ ppm): 0.94 (t, 3H, $CH_2CH_2\underline{CH_3}$), 1.67 (sext, 2H, $CH_2\underline{CH_2}CH_3$), 2.35 (s, 3H, $CH_3$), 2.57 (t, 2H, $\underline{CH_2}CH_2CH_3$), 7.04 (d, 1H, arH), 7.71 (d, 1H, arH), 9.86 (s, 1H, N=CH), 11.85 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 13.95 ($CH_2CH_2\underline{CH_3}$), 14.25 (Ph-$CH_3$), 19.34 ($CH_2\underline{CH_2}CH_3$), 27,22 ($\underline{CH_2}CH_2CH_3$) ar-C: [130.35, 131.74, 132.20, 143.29], 147.01 (triazole-$C_3$), 148.16 (N=CH), 151.94 (triazole-$C_5$); UV $\lambda_{max}$ (ε): 312 (11805), 294 (9625), 270 (8398), 210 (6371) nm; MS: m/z 251 (M+1, 8).

*3-Benzyl-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3d)**

Yield 97.3%, mp. 196 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3169 (NH), 1706 (C=O), 1590 (C=N), 770 and 699 (monosubstituted benzenoid ring); $^{1}$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.29 (s, 3H, $CH_3$), 3.94 (s, 2H, $CH_2$), 7.00 (d, 1H, arH, *J*=5.12 Hz), 7.20-7.29 (m, 5H, arH), 7.68 (d, 1H, arH, *J*=5.12 Hz), 9.81 (s, 1H, N=CH), 11.96 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.43 ($CH_3$), 31.83 ($CH_2$), ar-C: [127.43, 129.11 (2C), 129.57 (2C), 130.75, 131.93, 132.41, 136.33, 143.64], 146.55 (triazole-$C_3$), 148.04 (N=CH), 152.06 (triazole-$C_5$); UV $\lambda_{max}$ (ε): 316 (19329), 280 (12385), 220 (10994) nm; MS: m/z 298 ($M^+$, 1), 299 (M+1, 8).

*3-p-Methylbenzyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3e)**

Yield 96.2%, mp. 189 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3167 (NH), 1707 (C=O), 1592 (C=N), 831 (1,4-disubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.25 (s, 3H, $CH_3$), 2.32 (s, 3H, $CH_3$), 3.91 (s, 2H, $CH_2$), 7.03 (d, 1H, arH), 7.11 (d, 2H, arH), 7.42 (d, 2H, arH), 7.72 (d, 1H, arH), 9.83 (s, 1H, N=CH), 11.96 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.22 ($CH_3$), 21.07 ($CH_3$), 31.23 ($CH_2$), ar-C: [129.23 (2C), 129.45 (2C), 130.53, 131.71, 132.21, 133.01, 136.27, 143.40], 146.50 (triazole-$C_3$), 147.80 (N=CH), 151.85 (triazole-$C_5$); UV $\lambda_{max}$ ($\varepsilon$): 314 (17627), 272 (12887), 216 (14785) nm; MS: m/z 313 (M+1, 5).

*3-p-Methoxybenzyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3f)**

Yield 94.0%, mp. 207 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3166 (NH), 1707 (C=O), 1612, 1593 (C=N), 833 (1,4-disubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.29 (s, 3H, $CH_3$), 3.77 (s, 3H, $OCH_3$), 3.96 (s, 2H, $CH_2$), 6.92 (d, 2H, arH), 7.09 (d, 1H, arH), 7.29 (d, 2H, arH), 7.78 (d, 1H, arH), 9.90 (s, 1H, N=CH), 12.01 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.22 ($CH_3$), 30.76 ($CH_2$), 55.48 ($OCH_3$), ar-C: [114.30 (2C), 127.90, 130.42 (2C), 130.50, 131.72, 132.24, 143.40, 158.56], 146.35 (triazole-$C_3$), 147.78 (N=CH), 151.86 (triazole-$C_5$); UV $\lambda_{max}$ ($\varepsilon$): 314 (21222), 284 (18687), 272 (16259), 226 (19262), 212 (16858) nm; MS: m/z 328 ($M^+$, 4), 329 (M+1, 50).

*3-p-Chlorobenzyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3g)**

Yield 99.3%, mp. 244 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3165 (NH), 1705 (C=O), 1589 (C=N), 833 (1,4-disubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.29 (s, 3H, $CH_3$), 3.95 (s, 2H, $CH_2$), 7.00 (d, 1H, arH, *J*=4.67 Hz), 7.29-7.35 (m, 4H, arH), 7.68 (d, 1H, arH, *J*=4.76 Hz), 9.81 (s, 1H, N=CH), 11.96 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.43 ($CH_3$), 31.20 ($CH_2$), ar-C: [129.03 (2C), 130.78, 131.48 (2C), 131.93, 132.11, 132.35, 135.30, 143.70], 146.19 (triazole-$C_3$), 148.11 (N=CH), 152.05 (triazole-$C_5$); UV $\lambda_{max}$ ($\varepsilon$): 316 (19057), 282 (12505), 224 (15823) nm; MS: m/z 333 (M+1, 5), 334 (M+2, 3).

*3-m-Chlorobenzyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3h)**

Yield 74.3%, mp. 165 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3184 (NH), 1703 (C=O), 1584 (C=N), 787 and 689 (1,3-disubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.32 (s, 3H, $CH_3$), 4.01 (s, 2H, $CH_2$), 7.03 (d, 1H, arH), 7.26-7.37 (m, 3H, arH), 7.41 (s, 1H, arH), 7.73 (d, 1H, arH), 9.48 (s, 1H, N=CH), 12.01 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.22 ($CH_3$), 31.26 ($CH_2$), ar-C: [127.27, 128.12, 129.43, 130.68, 130.71, 131.73, 132.11, 133.41, 138.50, 143.53], 145.81 (triazole-$C_3$), 147.96 (N=CH), 151.82 (triazole-$C_5$); UV $\lambda_{max}$ ($\varepsilon$): 314 (15246), 274 (11610), 214 (16190) nm; MS: m/z 332 ($M^+$, 3), 333 (M+1, 38), 334 (M+2, 16).

*3-Phenyl-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(3i)**

Yield 96.8%, mp. 202 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 3166 (NH), 1700 (C=O), 1578 (C=N), 769 and 691 (monosubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.35 (s, 3H, $CH_3$), 7.02 (d, 1H, arH, *J*=4.76 Hz), 7.48-7.49 (m, 3H, arH), 7.69 (d, 1H, arH, *J*=4.76 Hz), 7.86-7.89 (m, 2H, arH), 9.76 (s, 1H, N=CH), 12.35 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.48 ($CH_3$), ar-C: [127.32, 128.43 (2C), 129.13 (2C), 130.76, 131.07, 132.02, 132.24, 144.12], 144.79 (triazole-$C_3$), 151.11 (N=CH), 152.21 (triazole-$C_5$); UV $\lambda_{max}$ ($\varepsilon$): 318 (18224), 276 (20016), 226 (15201) nm; MS: m/z 284 ($M^+$, 3), 285 (M+1, 10).

*General Method for the Preparation of 1-Acetyl-3-alkyl(aryl)-4-(3-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 colorless crystals.

*1-Acetyl-3-methyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(4a)**

Yield 75.8%, mp. 199 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 1726, 1715 (C=O), 1622, 1586 (C=N); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.25 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 2.45 (s, 3H, $COCH_3$), 7.04 (d, 1H, arH, *J*=4.76 Hz), 7.74 (d, 1H, arH, *J*=4.76 Hz), 9.66 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 11.83 ($CH_3$), 14.48 ($CH_3$), 24.12 ($COCH_3$), ar-C: [131.59 (2C), 132.10 (2C)], 144.90 (triazole-$C_3$), 147.01 (N=CH), 151.17 (triazole-$C_5$), 166.40 (C=O); UV $\lambda_{max}$ ($\varepsilon$): 316 (15949), 276 (9885), 222 (5858) nm.

*1-Acetyl-3-ethyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(4b)**

Yield 84.5%, mp. 145 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 1733, 1719 (C=O), 1611, 1575 (C=N); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 1.20 (t, 3H, $CH_3$, *J*=7.41 Hz), 2.34 (s, 3H, $CH_3$), 2.46 (s, 3H, $COCH_3$), 2.64 (q, 2H, $CH_2$, *J*=7.32 Hz), 7.03 (d, 1H, arH, *J*=5.12 Hz), 7.73 (d, 1H, arH, *J*=5.12 Hz), 9.67 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 10.02 ($CH_3$), 14.47 ($CH_3$), 19.30 ($CH_2$), 24.11 ($COCH_3$), ar-C: [131.52, 131.70, 132.06, 144.77], 148.96 (triazole-$C_3$), 150.53 (N=CH), 150.73 (triazole-$C_5$), 166.63 (C=O); UV $\lambda_{max}$ ($\varepsilon$): 316 (10104), 294 (7254), 220 (4260) nm; MS: m/z 279 (M+1, 3).

*1-Acetyl-3-benzyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(4d)**

Yield 82.4%, mp. 145 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 1735 (C=O), 1610, 1595 (C=N), 755 and 677 (mono-substituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.31 (s, 3H, $CH_3$), 2.47 (s, 3H, $COCH_3$), 4.02 (s, 2H, $CH_2$), 7.01 (d, 1H, arH, *J*=5.12 Hz), 7.22-7.35 (m, 5H, arH), 7.74 (d, 1H, arH, *J*=4.76 Hz), 9.65 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.44 ($CH_3$), 24.20 ($COCH_3$), 31.78 ($CH_2$), ar-C: [127.69, 129.18 (2C), 129.78 (2C), 131.67, 131.74, 132.04, 135.15, 144.84], 148.64 (triazole-$C_3$), 148.89 (N=CH), 150.29 (triazole-$C_5$), 166.65 (C=O); UV $\lambda_{max}$ ($\varepsilon$): 316 (18281), 290 (12448), 222 (13393) nm; MS: m/z 340 ($M^+$, 7), 341 (M+1, 12).

*1-Acetyl-3-p-chlorobenzyl-4-(3-methyl-2-thienyl-methyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(4g)**

Yield 82.7%, mp. 141 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 1731 (C=O), 1612 (C=N), 832 (1,4-disubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.31 (s, 3H, $CH_3$), 2.47 (s, 3H, $COCH_3$), 4.04 (s, 2H, $CH_2$), 7.03 (d, 1H, arH, *J*=4.76 Hz), 7.36 (s, 4H, arH), 7.75 (d, 1H, arH, *J*=5.12 Hz), 9.65 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.53 ($CH_3$), 24.20 ($COCH_3$), 31.15 ($CH_2$), ar-C: [129.09 (2C), 131.68 (3C), 131.74, 132.08, 132.40, 134.17, 144.91], 148.32 (triazole-$C_3$), 148.90 (N=CH), 150.47 (triazole-$C_5$), 166.93 (C=O); UV $\lambda_{max}$ ($\varepsilon$): 316 (18290), 280 (11511), 224 (19496) nm.

*1-Acetyl-3-phenyl-4-(3-methyl-2-thienylmethylene-amino)-4,5-dihydro-1H-1,2,4-triazol-5-ones* **(4i)**

Yield 76.6%, mp. 172 °C; IR (KBr) ($\upsilon$, $cm^{-1}$): 1770, 1747 (C=O), 1585 (C=N), 777 and 701 (monosubstituted benzenoid ring); $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 2.37 (s, 3H, $CH_3$), 2.48 (s, 3H, $COCH_3$), 7.05 (d, 1H, arH, *J*=5.12 Hz), 7.54-7.58 (m, 3H, arH), 7.75 (d, 1H, arH, *J*=5.12 Hz), 7.90-7.93 (m, 2H, arH), 9.61 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.49 ($CH_3$), 24.11 ($COCH_3$), ar-C: [125.96, 129.17 (2C), 129.29 (2C), 131.56, 131.99 (2C), 132.16, 145.30], 146.30 (triazole-$C_3$), 149.07 (N=CH), 153.54 (triazole-$C_5$), 166.93 (C=O); UV $\lambda_{max}$ ($\varepsilon$): 300 (11440), 284 (11977), 220 (8823) nm; MS: m/z 327 (M+1, 1).

*Antioxidant Activity: Chemicals*

BHT was purchased from E. Merck. Ferrous chloride, α-tocopherol, DPPH·, ferrozine, BHA and trichloroacetic acid (TCA) were bought from Sigma.

*Reducing Power*

Different concentrations of the samples (50-250 µg/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 TCA (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 in a spectrophometer.

*Free Radical Scavenging Activity*

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 µg/mL). The mixture was shaken vigorously and allowed to remain at the room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophometer. The DPPH concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

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

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

$$\text{DPPH}\cdot \text{ scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100$$

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*

Shortly, the synthesized compounds (50-250 µg/mL) were added to a 2 mM solution of $FeCl_2$

(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 remaining at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in a spectrophotometer. All test and analyses were carried out in triplicate and averaged. The percentage of inhibition of ferrozine-$Fe^{2+}$ complex formation was given by the formula: % Inhibition = $(A_0 - A_1/A_0)$ x 100, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

*Potentiometric Titrations*

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 manufactures 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 0.05 N TBAH. For all potentiometric titrations, 0.05 N tetrabutylammonium hydroxide in isopropyl alcohol, which was prepared from 0.1 N 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 HNP and the corresponding p$K_a$ values for all compounds, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, *N,N*-dimethylformamide and acetonitrile. The mV values that were obtained in pH-meter were recorded. The HNP values and the corresponding p$K_a$ values of all compounds, 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-1. Finally, the half-neutralization potential values were determined by drawing the mV-mL (TBAH) graphic. From the titration curves, the HNP values were measured and the corresponding p$K_a$ values were calculated.

## Conclusion

The synthesis and *in-vitro* antioxidant evaluation of new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are described. All of the compounds demonstrate a marked capacity for the iron binding. The data here reported could be of the possible interest because of the observed metal chelating activities of the studied compounds could prevent redox cycling. Design and synthesis of novel small molecules can play specifically a protective role in biological systems and 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 instrinsic 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 values and corresponding p$K_a$ values obtained from the potentiometric titrations rely on the non-aqueous solvents used and the substituents at C-3, in triazole ring.

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