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Full Paper

Synthesis, Acidity and Antioxidant Properties of Some Novel 3,4-disubstituted-4,5-dihydro-1*H*-1,2,4-triazol-5-one Derivatives

Muzaffer Alkan [1,*], Haydar Yüksek [2], Özlem Gürsoy-Kol [2] and Mustafa Calapoğlu [1]

[1] Education Faculty, Kafkas University, 36100 Kars, Turkey

[2] Department of Chemistry, Kafkas University, 36100 Kars, Turkey; E-mail: hyuksek61@gmail.com (Yüksek), ozlemgursoy@gmail.com (Gürsoy-Kol), mustafacalapoglu@yahoo.com (Calapoğlu)

* Author to whom correspondence should be addressed; E-mail: muzzo_61@yahoo.com; Fax (+90)-474-2121185

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Abstract: 3-Alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2a-g** reacted with 4-diethylaminobenzaldehyde to afford the corresponding 3-alkyl(aryl)-4-(4-diethyl-aminobenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3a-g**. The acetylation reactions of compounds **3a-e** were investigated and compounds **4a-e** were thus obtained. The new compounds were characterized using IR, ^{1}H-NMR, ^{13}C-NMR, UV and MS spectral data. In addition, the newly synthesized compounds **3a-g** were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents such as isopropyl alcohol, *tert*-butyl alcohol, acetone and *N*,*N*-dimethylformamide (DMF), and the half-neutralization potential values and the corresponding pK_a values were determined for all cases. Moreover, **3** and **4** type compounds were also screened for their antioxidant activities.

Keywords: 4,5-Dihydro-1*H*-1,2,4-triazol-5-ones, Schiff base, Acetylation, Antioxidant activity, pK_a, Potentiometric titrations.

Introduction

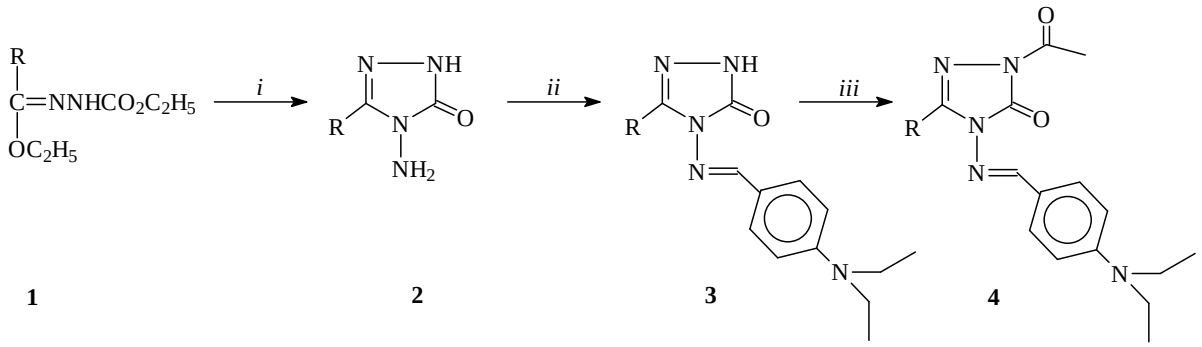
1,2,4-Triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are reported to possess a broad spectrum of biological activities such as antifungal, antimicrobial, hypoglycemic, antihypertensive, analgesic, antiparasitic, hypocholesteremic, antiviral, anti-inflammatory, antitumor, antioxidant and anti-HIV properties [1-7]. In addition, several articles reporting the synthesis of some *N*-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been published [6-17]. The acylation of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives has also been reported [3,5,9-12,17].

On the other hand, 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 tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents, and the pK_a values of the compounds were determined [6,7,9-14,18-21].

Furthermore, antioxidants are extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress. 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 [22]. 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 issue damage. Oxidative damages play a significant 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 a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [23].

In the present paper, the antioxidant activities of seven new 3-alkyl(aryl)-4-(4-diethylamino-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3a-g**, which were synthesized by the reactions of 3-alkyl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2a-g** with 4-diethylaminobenzaldehyde and five new 1-acetyl-3-alkyl(aryl)-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4a-e**, which were synthesized by the acetylations of **3a-e** were determined (Scheme 1).

Scheme 1.



i) N_2H_4, H_2O, reflux, 6 h; ***ii***) $Et_2NC_6H_4CHO$ (*p*-), AcOH, reflux, 1 h; ***iii***) Ac_2O, reflux, 1 h

a) R= CH_3, **b)** R= CH_2CH_3, **c)** R= $CH_2C_6H_5$, **d)** R= $CH_2C_6H_4.CH_3$ (*p*-), **e)** R= $CH_2C_6H_4.Cl$ (*p*-), **f)** R= C_6H_5, **g)** R= cyclopropyl

Moreover, we also examined the potentiometric titrations of the synthesized compounds **3** with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents (isopropyl alcohol, *tert*-butyl alcohol, acetone and DMF) to determine the corresponding half-neutralization potentials (HNP) and the corresponding pK_a values. 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 [9-14,18-21,24].

Results and Discussion

In this study, the structures of seven new 3-alkyl(aryl)-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3a-g** and five new 1-acetyl-3-alkyl(aryl)-4-(4-diethylamino-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4a-e** were identified using IR, ^{1}H-NMR, ^{13}C- NMR, UV and MS spectral data. In addition, the compounds **3a-g** and **4a-e** 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 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 α-tocopherol. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity [25]. 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 [26]. In this study, the reducing ability of compounds synthesized augmented with increasing concentration of samples. As can be seen in Figure 1, compound **3g** showed a moderate reducing activity for Fe^{3+} compared to the blank reaction. Compounds **4a** and **4b** also displayed a weak reducing activity. The other compounds showed lower absorbance than the blank, hence, no reductive activities were observed. Only compounds **3g**, **4a** and **4b** may reduce metal ions complexes to their lower oxidation state or to take part in electron transfer reaction. In other words, these compounds showed the ability of electron donor to scavenge free radicals.

DPPH$^{\bullet}$ 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 with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [27]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [28]. 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.

Figure 1. Total reductive potential of different concentrations (50-100-150 µg/mL) of **3g**, **4a**, **4b**, BHT and α-tocopherol. High absorbance at 700 nm indicates high reducing power.

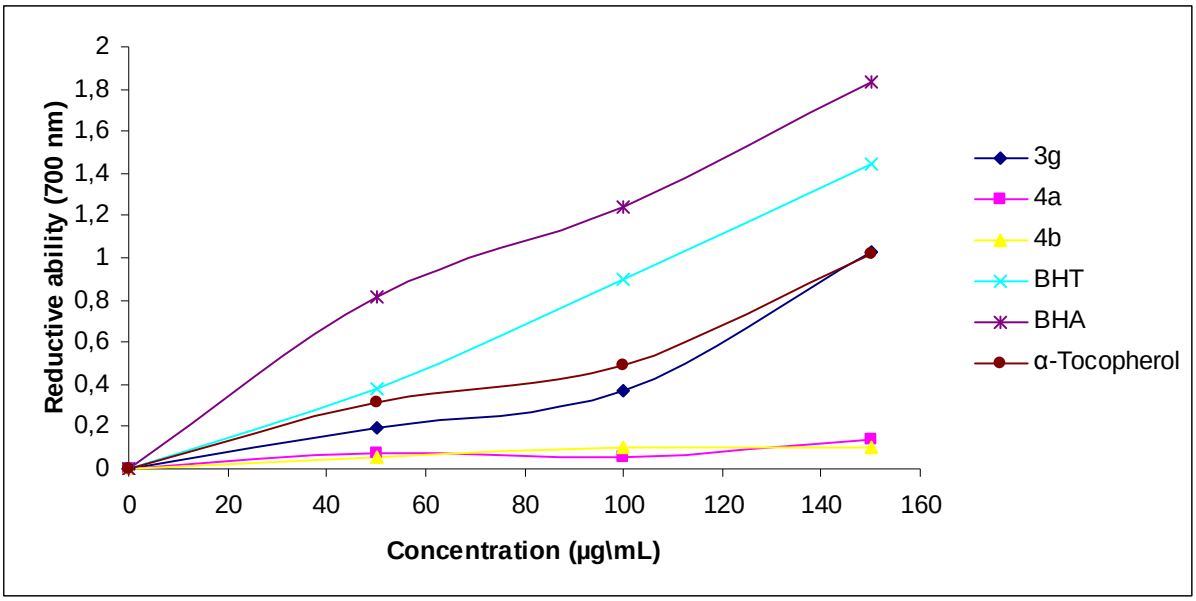
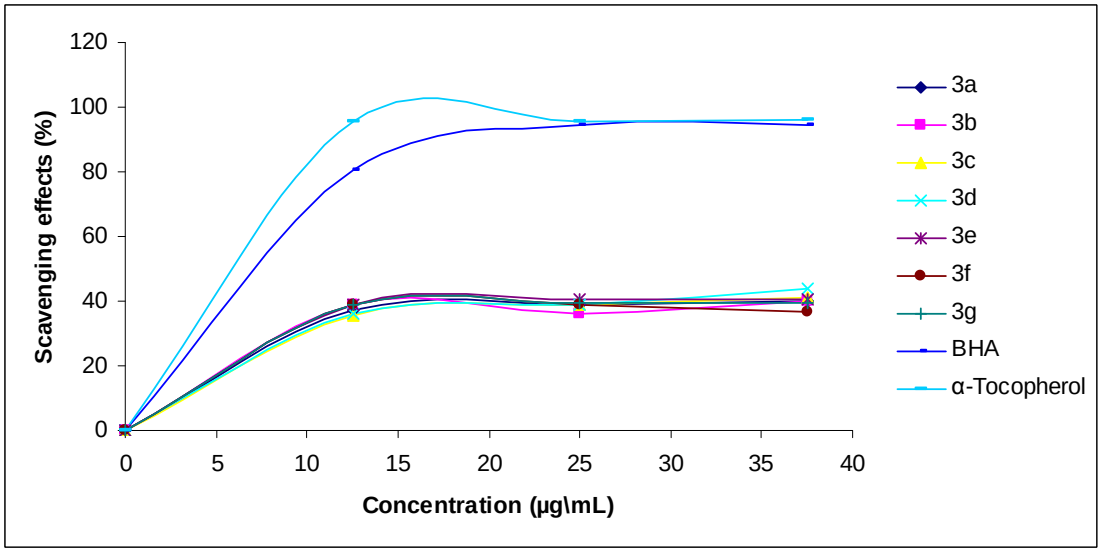
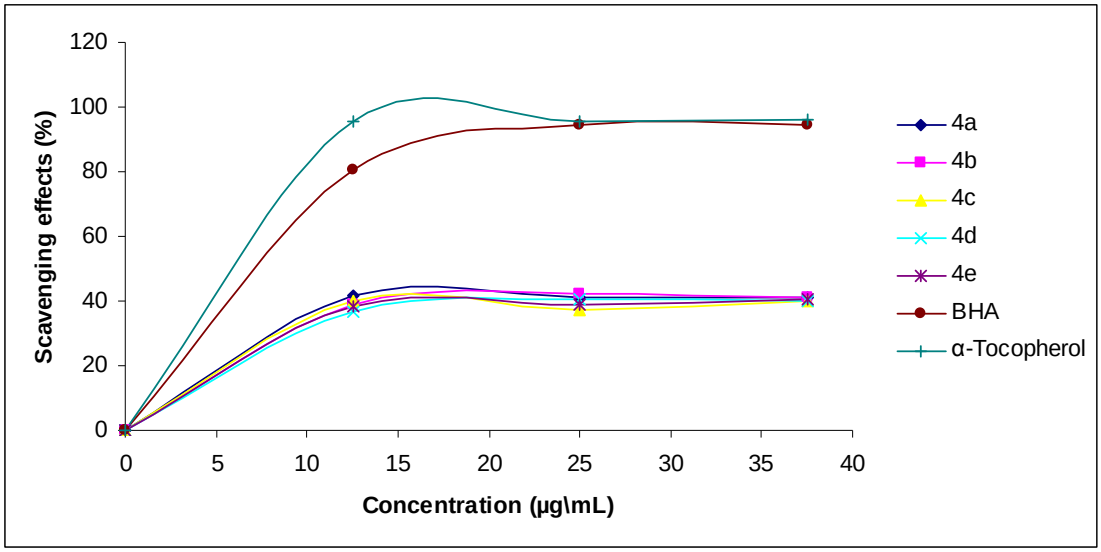


Figure 2. Scavenging effect of compounds **3a-g**, BHA and α-tocopherol at different concentrations (12,5-25-37.5 µg/mL).



The decrease in absorbance of DPPH radical was caused by antioxidants, because of 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 [29]. BHA and α-tocopherol were used as a reference to antioxidant compounds. All the compounds tested with this method exhibited marked DPPH free radical scavenging activity in a concentration-dependent manner. Figures **2** and **3** illustrate a decrease in the concentration of DPPH radical due to the scavenging ability of these compounds. These results indicate that the newly synthesized compounds showed moderate activities as a radical scavenger, indicating that it has good activities as hydrogen donors.

Figure 3. Scavenging effect of compounds **4a-e**, BHA and α-tocopherol at different concentrations (12,5-25-37.5 µg/mL).



Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis [30]. Ferrozine can quantitatively form 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 [31]. 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 [32] 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 [33]. Also, the production of highly active ROS such as $O_2^{\cdot -}$, H_2O_2 and $OH^{\cdot}$ is 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 [34]. Ferrous ion chelating activities of the compounds, BHT and α-tocopherol are shown in Figure 4. 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

[35]. The data obtained from Figures 4 and 5 reveal that the compounds 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.

Figure 4. Metal chelating effect of different amount of the compounds **3a-g**, BHT and α-tocopherol on ferrous ions.

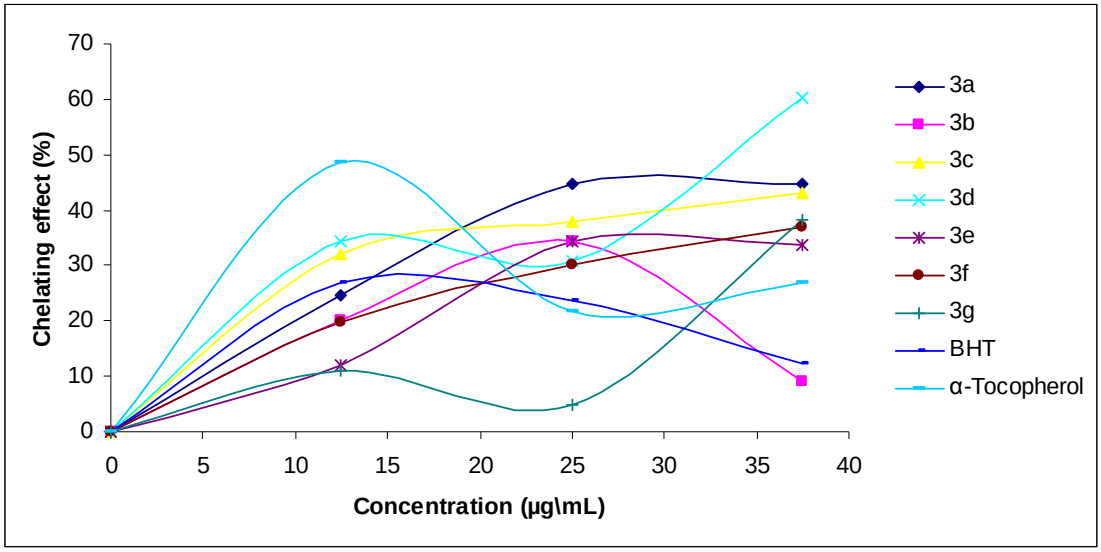
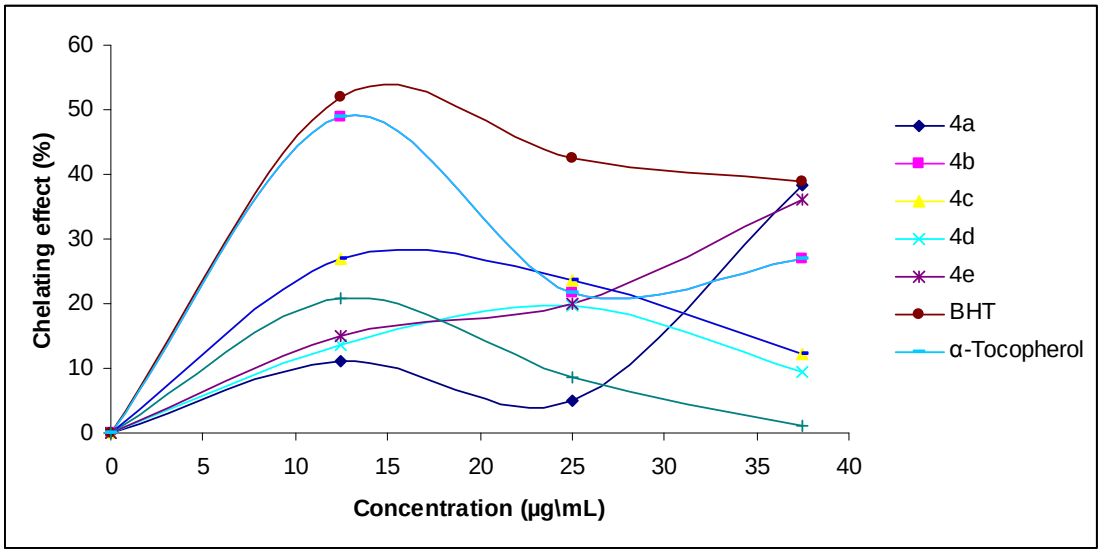


Figure 5. Metal chelating effect of different amount of the compounds **4a-e**, BHT and α-tocopherol on ferrous ions.

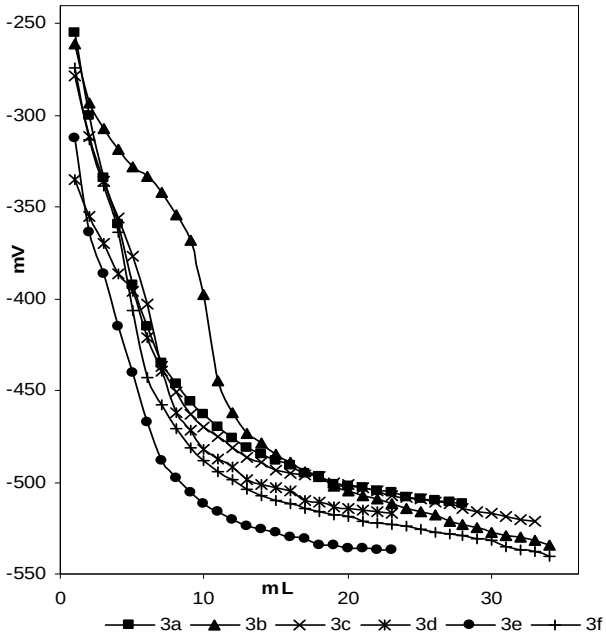


In conclusion, the data here reported could be of the possible interest because of the observed hydrogen donating, radical scavenging and metal chelating activities of yhr dtudied compounds could prevent redox cycling. Design and synthesis of novel small molecules which can specifically protective role in biological systems are in perspective in modern medicinal chemistry.

Potentiometric titrations

As a separate study, newly synthesized compounds **3** were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl and *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. As an example, the potentiometric titration curves for 0.001 M solutions of compounds **3a-g** titrated with 0.05 M TBAH in isopropyl alcohol are shown in Figure **6**.

Figure 6. Potentiometric titration curves of 0.001 *M* solutions of compound **3a-g** titrated with 0.05 *M* TBAH in acetone at 25°C.



The HNP values and the corresponding pK_a values of compounds **3a-g**, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, acetone and DMF, are presented in Table 1.

Table 1. The HNP and the corresponding pK_a values of compounds **3a-g** in isopropyl alcohol, *tert*-butyl alcohol, acetone and DMF.

Compd	Isopropyl alcohol		*tert*-butyl alcohol		DMF		Acetone	
	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a
3a	-300	12.45	-445	14.91	-477	15.49	-493	15.69
3b	-362	13.87	-473	15.36	-387	14.30	-483	15.49
3c	-336	13.30	-454	15.46	-373	13.97	-557	17.36
3d	-375	14.06	-473	15.86	-507	16.52	-583	17.89
3e	-313	12.75	-489	15.97	-482	15.89	-428	14.88
3f	-328	12.99	-403	14.27	-432	14.72	-258	11.75
3g	-	-	-556	16.02	-575	17.06	-488	15.26

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: DMF (ε=36.7) > acetone (ε=36) > isopropyl alcohol (ε=19.4) > *tert*-butyl alcohol (ε=12). As seen in Table 1, the acidity order for compounds **3a** and **3d** is: isopropyl alcohol > *tert*-butyl alcohol > DMF > acetone; for compounds **3b** and **3c** it is: isopropyl alcohol > DMF > *tert*-butyl alcohol > acetone; for compound **3e** it is: isopropyl alcohol > acetone > DMF > *tert*-butyl alcohol and for compound **3f**, it is: acetone > isopropyl alcohol > *tert*-butyl alcohol > DMF, while the ranking for compound **3g** is: acetone > *tert*-butyl alcohol > DMF. In isopropyl alcohol, all these compounds show the strongest acidic properties.

The degree to which a pure solvent ionizes was represented by its *autoprotolysis constant*, K_{HS}.

$$2HS = H_2S^+ + S^-$$

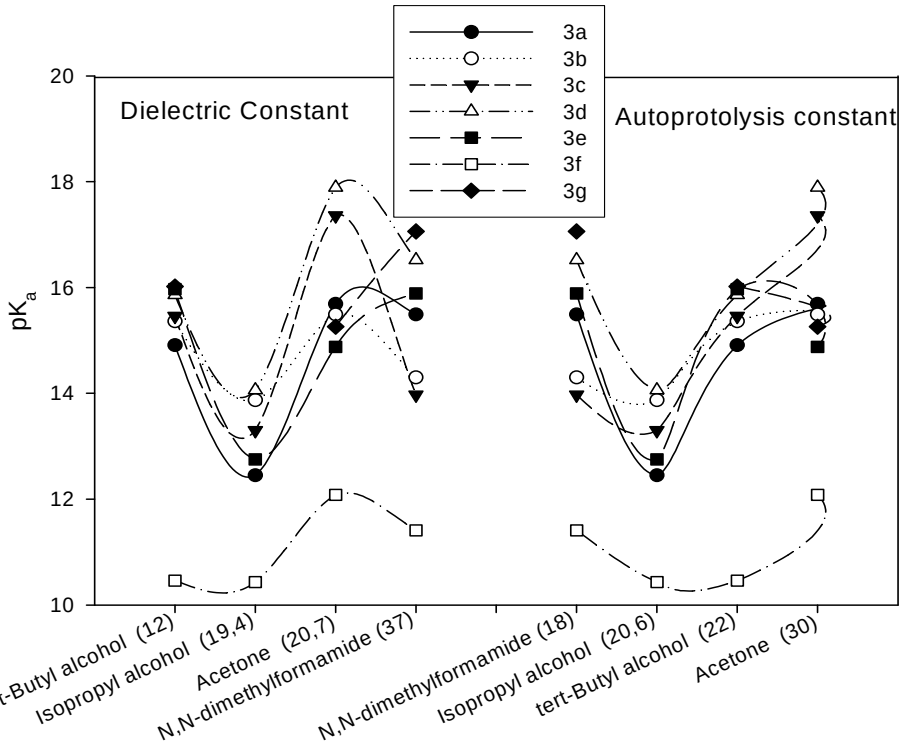
For the above reaction the constant is defined by

$$K_{HS} = [H_2S^+][S^-]$$

Autoprotolysis is an acid-base reaction between identical solvent molecules is which some act as an acid and others as a base. Consequently, 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 [36]. The exchange of the pK_a values with autoprotolysis constant and dielectric constant are given in Figure **7**.

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 [9-14,18-21,24]. Table 1 and Figure 6 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.

Figure 7. The variation of the pK_a values for synthesized componds **3a-g** with auto-protolysis constant and dielectric constant.



Experimental

General

Melting points were taken on an Electrothermal 9100 digital melting point apparatus and are uncorrected. IR spectra were registered on a Perkin-Elmer Spectrum One FT-IR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard on 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 Unicam UV/VIS spectrometer. Extinction coefficients (ε) are expressed in $L \cdot mol^{-1} \cdot cm^{-1}$. The starting compounds **2a-g** were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones **1a-g** with an aqueous solution of hydrazine hydrate as described in the literature [17,37].

General Method for the Preparation of 3-alkyl(aryl)-4-(4-diethylaminobenzylideneamino)-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 4-diethylaminobenzaldehyde (1.77 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 an appropriate solvent gave pure compounds **3a-g** as colourless crystals.

3-Methyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3a**). Yield 84 %; mp. 211 °C (EtOH); ^{1}H-NMR: δ 1.09 (t, 6H, $2CH_2\underline{CH_3}$), 2.20 (s, 3H, CH_3), 3.37 (q, 4H, $2\underline{CH_2}CH_3$), 6.69 (d, 2H, *J*=9.2 Hz, Ar-H), 7.57 (d, 2H, *J*=8.8 Hz, Ar-H), 9.37 (s, 1H, N=CH), 11.66 (s, 1H, NH); ^{13}C-NMR: δ 11.88 (CH_3), 13.06 ($2CH_3$), 44.47 ($2CH_2$), 111.67 (2C), 120.17, 130.25 (2C), 144.76 (4-diethylaminophenyl carbons), 150.51 (triazole C_3), 152.19 (N=CH), 156.10 (triazole C_5); IR: 3180 (NH), 1688 (C=O), 1594 (C=N), 818 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 357 (25324), 230 (9898), 207 (16875) nm; MS: m/z 273 (M^+), 274 (M+1).

3-Ethyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3b**). Yield 94 %; mp. 186 °C (EtOH); ^{1}H-NMR: δ 1.08 (t, 6H, $2CH_2\underline{CH_3}$), 1.17 (t, 3H, $CH_2\underline{CH_3}$), 2.61 (q, 2H, $\underline{CH_2}CH_3$), 3.37 (q, 4H, $2\underline{CH_2}CH_3$), 6.69 (d, 2H, *J*=9.2 Hz, Ar-H), 7.56 (d, 2H, *J*=8.8 Hz, Ar-H), 9.37 (s, 1H, N=CH), 11.68 (s, 1H, NH); ^{13}C-NMR: δ 10.79 (CH_3), 13.05 ($2CH_3$), 19.34 (CH_2), 44.47 ($2CH_2$), 111.69 (2C), 120.23, 130.20 (2C), 148.58 (4-diethylaminophenyl carbons), 150.50 (triazole C_3), 152.33 (N=CH), 156.07 (triazole C_5); IR: 3179 (NH), 1683 (C=O), 1613, 1589 (C=N), 818 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 358 (33660), 229 (12280), 207 (22775) nm.

3-Benzyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3c**). Yield 97 %; mp. 202 °C (EtOH); ^{1}H-NMR: δ 1.09 (t, 6H, $2CH_2\underline{CH_3}$), 3.98 (s, 2H, CH_2), 3.37 (q, 4H, $2\underline{CH_2}CH_3$), 6.69 (d, 2H, *J*=8.8 Hz, Ar-H), 7.19-7.29 (m, 5H, Ar-H), 7.54 (d, 2H, *J*=8.8 Hz, Ar-H), 9.35 (s, 1H, N=CH), 11.82 (s, 1H, NH); ^{13}C-NMR: δ 13.05 ($2CH_3$), 31.84 (CH_2), 44.47 ($2CH_2$), 111.70 (2C), 120.16, 130.25 (2C), 146.78 (4-diethylaminophenyl carbons), 127.34, 129.09 (2C), 129.46 (2C),

136.68 (phenyl carbons), 150.53 (triazole C_3), 152.18 (N=CH), 155.74 (triazole C_5); IR: 3165 (NH), 1700 (C=O), 1610, 1588 (C=N), 817 (1,4-disubstituted Ar), 770 and 683 (monosubstituted Ar) cm^{-1}; UV λ_{max} (ε): 358 (17280), 209 (19526) nm.

3-(4-Methylbenzyl)-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3d**). Yield 94 %; mp. 198 °C (EtOH); ^{1}H-NMR: δ 1.09 (t, 6H, $2CH_2\underline{CH_3}$), 2.22 (s, 3H, CH_3), 3.92 (s, 2H, CH_2), 3.37 (q, 4H, $2\underline{CH_2}CH_3$), 6.69 (d, 2H, *J*=8.8 Hz, Ar-H), 7.07 (d, 2H, *J*=8.1 Hz, Ar-H), 7.17 (d, 2H, *J*=8.1 Hz, Ar-H), 7.55 (d, 2H, *J*=8.8 Hz, Ar-H), 9.35 (s, 1H, N=CH), 11.79 (s, 1H, NH); ^{13}C-NMR: δ 13.07 ($2CH_3$), 21.78 (CH_3), 31.43 (CH_2), 44.47 ($2CH_2$), 111.70 (2C), 120.20, 130.24 (2C), 146.93 (4-diethylaminophenyl carbons), 129.33 (2C), 129.65 (2C), 133.57, 136.36 (4-methylphenyl carbons), 150.52 (triazole C_3), 152.19 (N=CH), 155.68 (triazole C_5); IR: 3173 (NH), 1702 (C=O), 1611, 1589 (C=N), 829, 817 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 358 (30127), 210 (24382) nm.

3-(4-Chlorobenzyl)-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3e**). Yield 96 %; mp. 170 °C (EtOH); ^{1}H-NMR: δ 1.09 (t, 6H, $2CH_2\underline{CH_3}$), 3.98 (s, 2H, CH_2), 3.37 (q, 4H, $2\underline{CH_2}CH_3$), 6.68 (d, 2H, *J*=8.8 Hz, Ar-H), 7.32 (q, 4H, Ar-H), 7.53 (d, 2H, *J*=9.2 Hz, Ar-H), 9.36 (s, 1H, N=CH), 11.85 (s, 1H, NH); ^{13}C-NMR: δ 13.07 ($2CH_3$), 31.18 (CH_2), 44.47 ($2CH_2$), 111.70 (2C), 120.13, 130.27 (2C), 146.43 (4-diethylaminophenyl carbons), 129.02 (2C), 131.38 (2C), 132.05, 135.64 (4-chlorophenyl carbons), 150.54 (triazole C_3), 152.17 (N=CH), 155.78 (triazole C_5); IR: 3176 (NH), 1701 (C=O), 1602, 1587 (C=N), 846, 812 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 360 (20757), 221 (13254), 210 (16274) nm.

3-Phenyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one **(3f).** Yield 84 %; mp. 232 °C (EtOH); ^{1}H-NMR: δ 1.08 (t, 6H, $2CH_2\underline{CH_3}$), 3.36 (q, 4H, $2\underline{CH_2}CH_3$), 6.70 (d, 2H, *J*=8.8 Hz, Ar-H), 7.47-7.48 (m, 3H, Ar-H), 7.56-7.59 (m, 2H, Ar-H), 7.90 (d, 2H, *J*=9.2 Hz, Ar-H), 9.24 (s, 1H, N=CH), 12.24 (s, 1H, NH); ^{13}C-NMR: δ 13.05 ($2CH_3$), 44.50 ($2CH_2$), 111.75 (2C), 119.90, 130.56 (2C), 144.99 (4-diethylaminophenyl carbons), 127.10, 128.35 (2C), 129.19 (2C), 130.56 (phenyl carbons), 150.73 (triazole C_3), 152.35 (N=CH), 159.33 (triazole C_5); IR: 3160 (NH), 1687 (C=O), 1609, 1588 (C=N), 812 (1,4-disubstituted Ar), 769 and 690 (monosubstituted Ar) cm^{-1}; UV λ_{max} (ε): 364 (36264), 240 (21813), 210 (25875) nm.

3-Cyclopropyl-4-(4-diethylaminobenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3g**). Yield 52 %; mp. 194 °C (EtOH-water, 1:3); ^{1}H-NMR: δ 0.85-0.98 (m, 4H, CH_2CH_2), 1.08 (t, 6H, $2CH_2\underline{CH_3}$), 1.99-2.06 (m, 1H, CH), 3.31-3.43 (m, 4H, $2\underline{CH_2}CH_3$), 6.65-6.71 (m, 2H, Ar-H), 7.58 (d, 2H, *J*=8.7 Hz, Ar-H), 9.33 (s, 1H, N=CH), 11.63 (s, 1H, NH); IR: 3170 (NH), 1695 (C=O), 1598 (C=N), 818 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 359 (20400), 240 (6100) nm.

General Method for the Preparation of 1-Acetyl-3-alkyl(aryl)-4-(4-diethylaminobenzylideneamino)-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 EtOH gave pure compounds **4a-e** as colourless crystals.

1-Acetyl-3-methyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4a**). Yield 94 %; mp. 147 °C (EtOH); ^{1}H-NMR: δ 1.09 (t, 6H, $2CH_2\underline{CH_3}$), 2.26 (s, 3H, CH_3), 2.44 (s, 3H, $COCH_3$), 3.38 (q, 4H, $2\underline{CH_2}CH_3$), 6.70 (d, 2H, *J*=8.8 Hz, Ar-H), 7.59 (d, 2H, *J*=8.4 Hz, Ar-H), 9.17 (s, 1H, N=CH); ^{13}C-NMR: δ 11.97 (CH_3), 13.04 ($2CH_3$), 24.11 ($CO\underline{C}H_3$), 44.51 ($2CH_2$), 111.67 (2C), 119.44, 130.69 (2C), 147.36 (4-diethylaminophenyl carbons), 148.83 (triazole C_3), 150.92 (N=CH), 158.75 (triazole C_5), 166.68 ($\underline{C}OCH_3$); IR: 1768, 1696 (C=O), 1597, 1588 (C=N), 836 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 361 (22015), 230 (9972), 209 (14910) nm; MS: m/z 315 (M^+), 316 (M+1).

1-Acetyl-3-ethyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4b**). Yield 85 %; mp. 127 °C (EtOH); ^{1}H-NMR: δ 1.06-1.23 (m, 9H, $3CH_2\underline{CH_3}$), 2.45 (s, 3H, $COCH_3$), 2.64 (q, 2H, $\underline{CH_2}CH_3$), 3.39 (m, 4H, $2\underline{CH_2}CH_3$), 6.69 (d, 2H, *J*=8.8 Hz, Ar-H), 7.57 (d, 2H, *J*=8.1 Hz, Ar-H), 9.16 (s, 1H, N=CH); ^{13}C-NMR: δ 9.33 (CH_3), 12.25 ($2CH_3$), 18.61 (CH_2), 23.35 ($CO\underline{C}H_3$), 43.73 ($2CH_2$), 110.85 (2C), 118.64, 129.86 (2C), 150.09 (4-diethylaminophenyl carbons), 148.26 (triazole C_3), 150.10 (N=CH), 157.89 (triazole C_5), 165.88 ($\underline{C}OCH_3$); IR: 1765, 1693 (C=O), 1605, 1586 (C=N), 835 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 361 (22862), 232 (9455), 209 (13357) nm; MS: m/z 329 (M^+), 330 (M+1).

1-Acetyl-3-benzyl-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4c**). Yield 91 %; mp. 146 °C (EtOH); ^{1}H-NMR: δ 1.09 (t, 6H, $2CH_2\underline{CH_3}$), 2.47 (s, 3H, $COCH_3$), 4.06 (s, 2H, CH_2), 3.34-3.41 (m, 4H, $2\underline{CH_2}CH_3$), 6.71 (d, 2H, *J*=9.1 Hz, Ar-H), 7.26-7.33 (m, 5H, Ar-H), 7.57 (d, 2H, *J*=8.7 Hz, Ar-H), 9.16 (s, 1H, N=CH); ^{13}C-NMR: δ 13.07 ($2CH_3$), 24.25 ($CO\underline{C}H_3$), 31.77 (CH_2), 44.51 ($2CH_2$), 111.69 (2C), 119.38, 130.74 (2C), 149.07 (4-diethylaminophenyl carbons), 127.63, 129.19 (2C), 129.69 (2C), 135.48 (phenyl carbons), 149.07 (triazole C_3), 150.92 (N=CH), 158.45 (triazole C_5), 166.73 ($\underline{C}OCH_3$); IR: 1737, 1729 (C=O), 1604, 1585 (C=N), 820 (1,4-disubstituted Ar), 764 and 689 (monosubstituted Ar) cm^{-1}; UV λ_{max} (ε): 362 (28665), 235 (11040), 210 (22050) nm; MS: m/z 391 (M^+), 392 M+1).

1-Acetyl-3-(4-methylbenzyl)-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4d**). Yield 91 %; mp. 142 °C (EtOH); ^{1}H-NMR: δ 1.10 (t, 6H, $2CH_2\underline{CH_3}$), 2.23 (s, 3H, CH_3), 2.47 (s, 3H, $COCH_3$), 4.00 (s, 2H, CH_2), 3.39 (q, 4H, $2\underline{CH_2}CH_3$), 6.71 (d, 2H, *J*=8.8 Hz, Ar-H), 7.09 (d, 2H, *J*=7.7 Hz, Ar-H), 7.21 (d, 2H, *J*=8.1 Hz, Ar-H), 7.57 (d, 2H, *J*=8.8 Hz, Ar-H), 9.15 (s, 1H, N=CH); ^{13}C-NMR: δ 13.07 ($2CH_3$), 21.31 (CH_3), 24.22 ($CO\underline{C}H_3$), 31.39 (CH_2), 44.51 ($2CH_2$), 111.73 (2C), 119.45, 130.72 (2C), 149.02 (4-diethylaminophenyl carbons), 129.56 (2C), 129.74 (2C), 132.33, 136.71 (4-methylphenyl carbons), 149.20 (triazole C_3), 150.20 (N=CH), 158.46 (triazole C_5), 166.70 ($\underline{C}OCH_3$); IR: 1737, 1728 (C=O), 1604, 1587 (C=N), 843, 820 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 362 (27633), 240 (9881), 209 (18927) nm.

1-Acetyl-3-(4-chlorobenzyl)-4-(4-diethylaminobenzylideneamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4e**). Yield 88 %; mp. 164 °C (EtOH); ^{1}H-NMR: δ 1.10 (t, 6H, $2CH_2\underline{CH_3}$), 2.47 (s, 3H, $COCH_3$), 4.07 (s, 2H, CH_2), 3.39 (q, 4H, $2\underline{CH_2}CH_3$), 6.71 (d, 2H, *J*=9.2 Hz, Ar-H), 7.36 (s, 4H, Ar-H), 7.56 (d, 2H, *J*=8.8 Hz, Ar-H), 9.17 (s, 1H, N=CH); IR: 1739, 1701 (C=O), 1605, 1585 (C=N), 817, 802 (1,4-disubstituted Ar) cm^{-1}; UV λ_{max} (ε): 363 (36264), 222 (21813), 210 (25875) nm.

Antioxidant Activity: Chemicals

Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA) and trichloracetic acid (TCA) were bought from Sigma (Sigma –Aldrich GmbH, Sternheim,Germany).

Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu [38]. 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^{o}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 $FeCI_3$ (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured in a spectrophometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity

Free radical scavenging activity of compounds was measured by DPPH·, using the method of Blois [39]. 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 stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophometer. 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):

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

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

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis *et al*. [30]. Briefly, the synthesized compounds (50-250 µg/mL) were added to a 2 mM solution of $FeCI_2$ (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 standing 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 run 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

A Jenway 3040-model ion analyzer and an Ingold pH electrode were used for potentiometric titrations. For each compound that would be titrated, the 0.001 M solution was separately prepared in each non-aqueous solvent. The 0.05 M solution of TBAH in isopropyl alcohol, which is widely used in the titration of acids, was used as titrant. The mV values, that were obtained in pH-meter, were recorded. Finally, the HNP values were determined by drawing the mL (TBAH)-mV graphic.

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Sample availability: Contact the authors.