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*Research Article*

# Synthesis and In Vitro Antioxidant Evaluation of New 1,3,5-Tri-{2-methoxy-4-[(4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene Derivatives

**H. Yuksek, O. Akyildirim, and O. Gursoy Kol**

*Department of Chemistry, Kafkas University, 36100 Kars, Turkey*

Correspondence should be addressed to O. Gursoy Kol; gursoyozlem@yahoo.com

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Nine new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were synthesized and characterized by elemental analyses and IR, $^{1}$H-NMR, $^{13}$C-NMR and UV spectral data. The synthesized compounds were analyzed for their *in vitro* potential antioxidant activities in three different methods. Those antioxidant activities were compared to standard antioxidants such as BHA, BHT and $\alpha$-tocopherol. Compounds **4e**, **5a** and **5d** showed best activity for iron binding. In addition, the compounds **4** were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents (isopropyl alcohol, *tert*-butyl alcohol, acetone and *N,N*-dimethyl formamide). Thus, the half-neutralization potential values and the corresponding p$K_a$ values were determined in all cases.

## 1. 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 antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer [1–9]. 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 [7, 8, 10–13]. The acetylation of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have also been reported [12–14].

Furthermore, antioxidants have extensively been studied for their capacity to protect organism and cell from damage that are induced by oxidative stress. Scientists have become more interested in new compounds; they have either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cell [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 issue damage. Oxidative damages significantly play a pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage. Also, excessive generation of ROS induced by various stimuli which exceeds the antioxidant capacity of the organism leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [16]. In the present study, due to a wide range of applications 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.

Besides, 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 that 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 nonaqueous solvents, and the p$K_a$ values of the compounds were determined [7, 8, 10, 12, 13, 17, 18].

## 2. Experimental

Chemical reagents used in this study were purchased from Merck AG, Aldrich, and Fluka. The starting materials **3a–g** were prepared from the reactions of the corresponding ester

ethoxycarbonylhydrazones **2a–g** with an aqueous solution of hydrazine hydrate as described in the literature [14, 19]. Melting points were determined in open glass capillaries using an electrothermal digital melting point apparatus and are uncorrected. The IR spectra were recorded 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 ($\varepsilon$) are expressed in $L \cdot mol^{-1} \cdot cm^{-1}$. Elemental analyses were carried out on an Leco 932 Elemental Combustion System (CHNS-O) for C, H, and N.

*2.1. General Procedure for the Synthesis of Compounds* **4**. 3-Methoxy-4-hydroxybenzaldehyde (0.03 mol) dissolved in ethyl acetate (100 mL) was treated with 1,3,5-benzenetricarbonyl chloride (0.01 mol), and to this solution was slowly added triethylamine (0.03 mol) with stirring at 0–5°C. Stirring was continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethanol to afford compound **1**, mp. 137°C; IR (KBr) ($\upsilon$, $cm^{-1}$): 2850 and 2739 (CHO); 1748, 1700 (C=O); 1274 (COO). $^1$H NMR (DMSO-$d_6$): $\delta$ 3.87 (s, 9H, $3OCH_3$), 7.56–7.64 (m, 9H, ArH), 9.01 (s, 3H, ArH), 9.99 (s, 3H, CHO). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 56.06 ($3OCH_3$), [111.90 (2C), 123.52 (3C), 123.63 (3C), 130.21 (4C), 135.46 (4C), 143.57 (4C), 151.26 (4C)] (Ar-C), 161.63 (3COO), 191.98 (3CHO). UV $\lambda_{max}$ ($\varepsilon$): 304 (9298), 256 (26386), 222 (39042), 212 (35722) nm. The corresponding compound **3** (0.003 mol) was dissolved in acetic acid (15 mL) and treated with 1, 3,5-tri-(2-methoxy-4-formylphenoxycarbonyl)-benzene **1** (0.001 mol). The mixture was refluxed for 1.5 h and then evaporated at 50–55°C *in vacuo*. Several recrystallizations of the residue from AcOH-$H_2O$ (1 : 3) gave pure compounds 1,3,5-tri-{2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-benzene **4** as colorless crystals.

*2.1.1. 1,3,5-Tri-{2-methoxy-4-[(3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4a**). Yield 0.67 g (74%). mp. 174°C. IR (KBr): 3223 (NH); 1751, 1715, 1705 (C=O); 1602 (C=N); 1271 (COO) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.29 (s, 9H, $3CH_3$), 3.86 (s, 9H, $3OCH_3$), 7.45–7.68 (m, 9H, Ar-H), 9.02 (s, 3H, Ar-H), 9.75 (s, 3H, 3N=CH), 11.86 (s, 3H, 3NH). UV $\lambda_{max}$ ($\varepsilon$): 310 (41762), 262 (37725), 222 (64713) nm.

*2.1.2. 1,3,5-Tri-{2-methoxy-4-[(3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4b**). Yield 0.72 g (76%). mp. 319°C. IR (KBr): 3220 (NH); 1750, 1703 (C=O); 1600 (C=N); 1271 (COO) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.18 (t, 9H, $3CH_3$, $J$ = 7.52 Hz), 2.70 (q, 6H, $3CH_2$, $J$ = 7.52 Hz), 3.88 (s, 9H, $3OCH_3$), 7.34–7.69 (m, 9H, Ar-H), 9.02 (s, 3H, Ar-H), 9.75 (s, 3H, 3N=CH), 11.89 (s, 3H, 3NH). UV $\lambda_{max}$ ($\varepsilon$): 310 (22888), 260 (27033), 224 (55717) nm.

*2.1.3. 1,3,5-Tri-{2-methoxy-4-[(3-n-propyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4c**). Yield 0.76 g (77%). mp. 168°C. IR (KBr): 3216 (NH); 1751, 1707 (C=O); 1598 (C=N); 1271 (COO) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 0.94 (t, 9H, $3CH_3$, $J$ = 7.52 Hz), 1.68 (sext., 6H, $3CH_2$, $J$ = 7.52 Hz), 3.84 (s, 9H, $3OCH_3$), 2.64 (q, 6H, $3CH_2$, $J$ = 7.52 Hz), 7.41–7.66 (m, 9H, Ar-H), 9.00 (s, 3H, Ar-H), 9.74 (s, 3H, 3N=CH), 11,89 (s, 3H, 3NH). UV $\lambda_{max}$ ($\varepsilon$): 310 (23732), 260 (26373), 218 (52676) nm.

*2.1.4. 1,3,5-Tri-{2-methoxy-4-[(3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4d**). Yield 1.03 g (91%). mp. 161°C. IR (KBr): 3214 (NH); 1750, 1718 (C=O); 1599 (C=N); 1272 (COO); 760 and 706 (monosubstituted benzenoid ring) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.85 (s, 9H, $3OCH_3$), 4.07 (s, 6H, $3CH_2$), 7.20–7.68 (m, 24H, Ar-H), 9.02 (s, 3H, Ar-H), 9.70 (s, 3H, 3N=CH), 12.03 (s, 3H, 3NH). UV $\lambda_{max}$ ($\varepsilon$): 310 (13919), 258 (17758), 216 (45657) nm.

*2.1.5. 1,3,5-Tri-{2-methoxy-4-[(3-p-methylbenzyl-4,5- dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4e**). Yield 1.15 g (98%). mp. 216°C. IR (KBr): 3219 (NH); 1751, 1705 (C=O); 1600 (C=N); 1272 (COO); 829 (1,4-disubstituted benzenoid ring) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.21 (s, 9H, $3CH_3$), 3.84 (s, 9H, $3OCH_3$), 4.00 (s, 6H, $3CH_2$), 7.06–7.65 (m, 21H, Ar-H), 9.02 (s, 3H, Ar-H), 9.69 (s, 3H, 3N=CH), 12.04 (s, 3H, 3NH). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 20.54 ($3PhCH_3$), 30.83 ($3CH_2Ph$), 56.07 ($3OCH_3$), 111.93, 120.94 (arom-C), 123.50 (2C), 123.59 (2C), 128.57 (6C), 128.99 (6C), 130.35 (arom-C), 130.70 (3C), 132.68, 133.03 (arom-C), 135.46 (3C), 135.79 (3C), 141.13 (3C), 143.60 (3C), 146.36 (3C), 151.19 (3C), 150.95 (3triazole $C_3$), 151.31 (N=CH), 151.91 (3triazole $C_5$), 161.69 (3COO). UV $\lambda_{max}$ ($\varepsilon$): 300 (10113), 258 (13837), 226 (66208), 216 (61806) nm. *Anal.* Calcd. for $C_{63}H_{54}N_{12}O_{12}$ (1171.19): C, 64.61; H, 4.65; N, 14.35. Found: C, 64.85; H, 6.22; N, 13.29.

*2.1.6. 1,3,5-Tri-{2-methoxy-4-[(3-p-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4f**). Yield 0.89 g (72%). mp. 230°C. IR (KBr): 3210 (NH); 1750, 1717 (C=O); 1598 (C=N); 1271 (COO); 824 (1,4-disubstituted benzenoid ring) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.88 (s, 9H, $3OCH_3$), 4.09 (s, 6H, $3CH_2$), 7.35–7.56 (m, 24H, Ar-H), 9.02 (s, 3H, Ar-H), 9.71 (s, 3H, 3N=CH), 12.04 (s, 3H, 3NH). UV $\lambda_{max}$ ($\varepsilon$): 310 (30831), 258 (47779), 222 (73777), 208 (58290) nm.

*2.1.7. 1,3,5-Tri-{2-methoxy-4-[(3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene* (**4g**). Yield 1.06 g (98%). mp. 243°C. IR (KBr): 3210 (NH); 1750, 1716 (C=O); 1602, 1585 (C=N); 1268 (COO); 768 and 693 (monosubstituted benzenoid ring) $cm^{-1}$. $^1$H NMR

(DMSO-$d_6$): $\delta$ 3.84 (s, 9H, $3OCH_3$), 7.50–7.64 (m, 18H, Ar-H), 7.90–7.94 (m, 6H, Ar-H), 9.02 (s, 3H, Ar-H), 9.72 (s, 3H, 3N=CH), 12.41 (s, 3H, 3NH). UV $\lambda_{max}$ ($\varepsilon$): 308 (24520), 258 (44728), 234 (67930), 216 (64853) nm.

*2.2. General Procedure for the Synthesis of Compound* **5**. The corresponding compound **4** (0.001 mol) was refluxed with acetic anhydride (20 mL) for 0.5 h. After the addition of absolute ethanol (100 mL), the mixture was refluxed for 1 h. Evaporation of the resulting solution at 40–45°C *in vacuo* and several recrystallizations of the residue from EtOH gave pure compounds **5** as colorless needles.

*2.2.1. 1,3,5-Tri-{2-methoxy-4-[(1-acetyl-3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene (***5a**). Yield 0.66 g (64%). mp. 207°C. IR (KBr): 1728 (C=O); 1623, 1582 (C=N); 1268 (COO) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.36 (s, 9H, $3CH_3$), 2.50 (s, 9H, $3COCH_3$), 3.85 (s, 9H, $3OCH_3$), 7.50–7.64 (m, 9H, Ar-H), 8.85 (s, 3H, Ar-H), 9.59 (s, 3H, 3N=CH). UV $\lambda_{max}$ ($\varepsilon$): 310 (20060), 258 (20237), 220 (63380), 216 (58300) nm.

*2.2.2. 1,3,5-Tri-{2-methoxy-4-[(1-acetyl-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-Benzene (***5d**). Yield 0.88 g (71%). mp. 182°C. IR (KBr): 1746 (C=O); 1602, 1590 (C=N); 1217 (COO); 746 and 710 (monosubstituted benzenoid ring) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.50 (s, 9H, $3COCH_3$), 3.85 (s, 9H, $3OCH_3$), 4.16 (s, 6H, $3CH_2$), 7.23–7.58 (m, 24H, Ar-H), 9.01 (s, 3H, Ar-H), 9.58 (s, 3H, 3N=CH). UV $\lambda_{max}$ ($\varepsilon$): 310 (45812), 296 (45995), 258 (47147), 230 (83429), 224 (81387) nm.

*2.3. Antioxidant Activity: Chemicals.* Butylated hydroxytoluene (BHT) was obtained from E. Merck (Merck KGaA, Darmstadt, Germany). Ferrous chloride, $\alpha$-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH$^\bullet$), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany).

*2.4. 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 ×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. Higher absorbance of the reaction mixture indicated greater reducing power.

*2.5. Free Radical Scavenging Activity.* Free radical scavenging activity of compounds was measured by DPPH$^\bullet$, using the method of Blois [21]. Briefly, 0.1 mM solution of DPPH$^\bullet$ 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 in a spectrophometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH$^\bullet$ concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

$$\text{Absorbance} = 0.0003 \times \text{DPPH}^\bullet - 0.0174. \quad (1)$$

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

$$\text{DPPH}^\bullet \text{ scavenging effect } (\%) = \left(A_0 - \frac{A_1}{A_0}\right) \times 100, \quad (2)$$

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

*2.6. 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 $\mu$g/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 then the mixture was shaken vigorously and left standing at the room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-$Fe^{2+}$ complex formation was given by the formula: %Inhibition = $(A_0 - A_1/A_0) \times 100$, 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.

*2.7. Potentiometric Titrations.* A Jenco model ion analyzer and an Ingold pH electrode were used for potentiometric titrations. For each compound that was 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 graph.

## 3. Results and Discussion

The 1,3,5-tri-{2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azo-methine]-phenoxycarbonyl}-benzene **4a–g** were prepared. The starting compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2a–g** were prepared from the reactions of the corresponding ester of ethoxycarbonylhydrazones **1a–g** with an aqueous solution of hydrazine hydrate as described in the literature

[14, 19, 23]. Compounds **4** were obtained from the reactions of compounds **3** with 1,3,5-tri-(2-methoxy-4-formylphenoxycarbonyl)-benzene **1**, which were synthesized by the reactions of 3-methoxy-4-hydroxybenzaldehyde with 1,3,5-benzenetricarbonyl chloride by using triethylamine. Then the reactions of compounds **4a** and **4d** with acetic anhydride were investigated, and compounds **5a** and **5d** were prepared (Scheme 1).

The structures of seven new 1,3,5-tri-{2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-benzenes **4a–g** and two new 1,3,5-tri-{2-methoxy-4-[(1-acetyl-3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethine]-phenoxycarbonyl}-benzenes **5a** and **5d** were characterized using IR, $^1$H NMR, $^{13}$C NMR, UV, and elemental analyses data.

*3.1. Antioxidant Activity.* The antioxidant activities of 9 new compounds **4a–g**, **5a**, and **5d** were determined. Several methods have been used to determine antioxidant activities, and the methods used in the study are given below.

*3.1.1. 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 using the method of Oyaizu [20]. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT, and $\alpha$-tocopherol. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [24]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging [25]. In this study, all the amount 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.

*3.1.2. DPPH Radical Scavenging Activity.* The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [26]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [27]. 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 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, $DPPH^{\bullet}$ is usually used as a substrate to evaluate antioxidative activity of antioxidants [28]. In the study, antiradical activities of compounds and standard antioxidants such as BHA and $\alpha$-tocopherol were determined by using $DPPH^{\bullet}$ method. Scavenging effect values of compounds **4a, 4c, 4e, 4f,** BHA and $\alpha$-tocopherol at different concentrations are given in Figure 1. The newly synthesized compounds showed no activity as a radical scavenger.

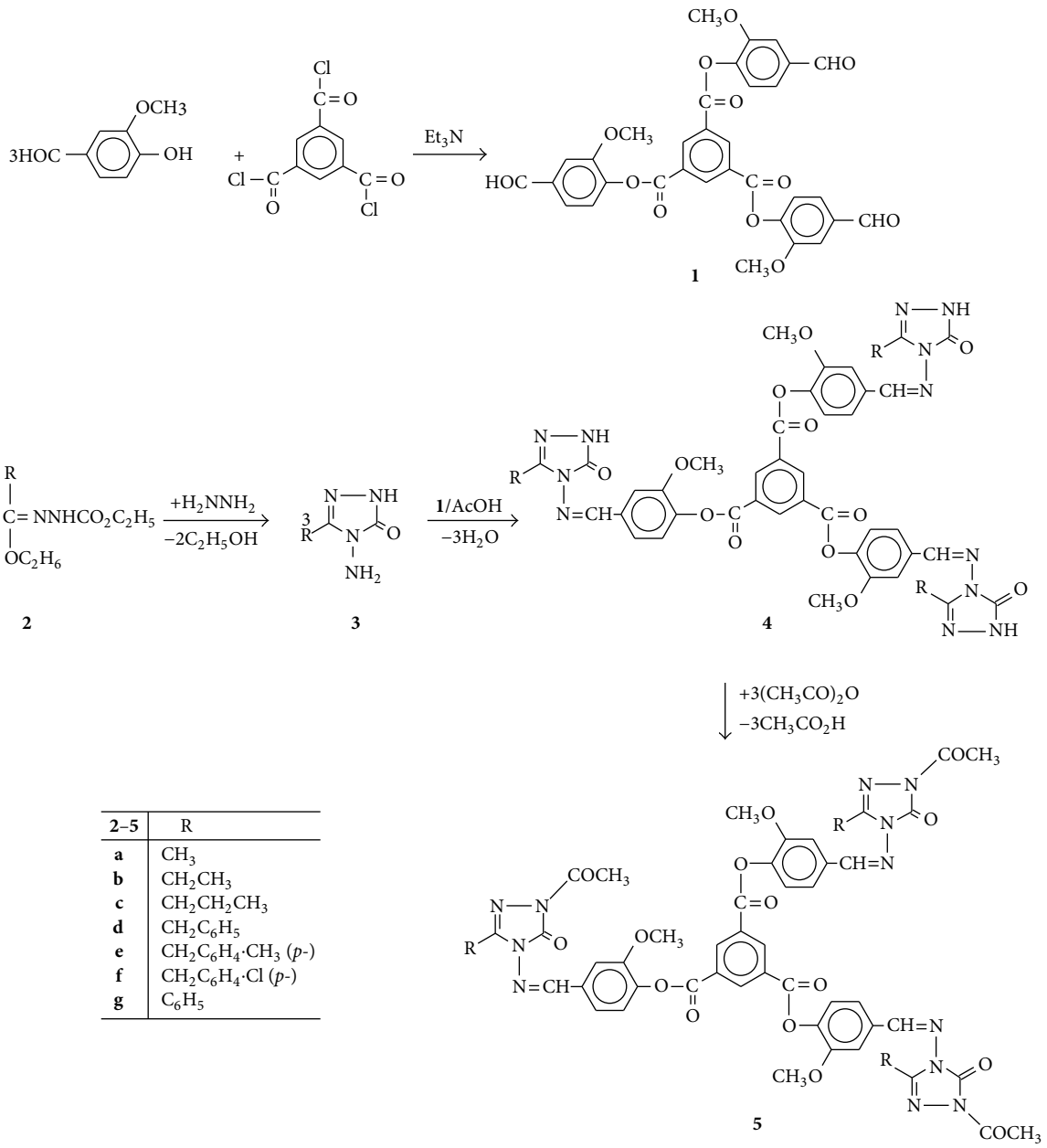
*3.1.3. Ferrous Ion Chelating Activity.* The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis [22]. Ferrozine can quantitatively form complexes with $Fe^{2+}$. In the presence of chelating agents, the complex formation is disrupted with 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 [29]. 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 [30] 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 [31]. Also, the production of highly active ROS such as $O_2^{\bullet-}$, $H_2O_2$, and $OH^{\bullet}$ is also catalyzed by free iron though Haber-Weiss reactions:

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + OH^- + OH^{\bullet}. \quad (3)$$

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:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}. \quad (4)$$

$Fe^{3+}$ ion also produces radicals from peroxides, even though the rate is tenfold less than that of $Fe^{2+}$ ion, which is the most powerful pro-oxidant among the various types of metal ions [32]. Ferrous ion chelating activities of the compounds **4**, **5**, BHT, and BHA are shown in Figures 2 and 3, respectively. 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 $\sigma$-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of metal ion [33]. The data obtained from Figures 2 and 3 reveal that the compounds, especially **4e, 5a**, and **5d** 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,




| 2–5 | R |
|---|---|
| **a** | $CH_3$ |
| **b** | $CH_2CH_3$ |
| **c** | $CH_2CH_2CH_3$ |
| **d** | $CH_2C_6H_5$ |
| **e** | $CH_2C_6H_4{\cdot}CH_3$ (*p*-) |
| **f** | $CH_2C_6H_4{\cdot}Cl$ (*p*-) |
| **g** | $C_6H_5$ |

Scheme 1

the compound-iron complex may also be active, since it can participate in iron-catalyzed reactions.

*3.1.4. Potentiometric Titrations.* In order to determine the p$K_a$ values of the compounds **4a–g**, they were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl alcohol, *tert*-butyl alcohol, acetone, and DMF. The mV values read in each titration were plotted against 0.05 M TBAH volumes (mL) added, and potentiometric titration curves were obtained for all the cases. From the titration curves, the HNP values were measured, and the corresponding p$K_a$ values were calculated. The data obtained from the potentiometric titrations was interpreted, and the effect of the C-3 substituent in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring as well as solvent effects was studied [7, 8, 10, 12, 13, 17, 18].

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

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: DMF ($\varepsilon$ = 36.7) > acetone ($\varepsilon$ = 36) > isopropyl alcohol ($\varepsilon$ = 19.4) >*tert*-butyl alcohol ($\varepsilon$ = 12). As seen in Table 1, the acidity order for compound **4a** is: *tert*-butyl alcohol > acetone, for compound **4b** it is acetone > DMF > *tert*-butyl

Table 1: The HNP and the corresponding p$K_a$ values of compounds 4a-g in isopropyl alcohol, *tert*-butyl alcohol, DMF, and acetone.

| Comp. | Isopropyl alcohol | | *tert*-Butyl alcohol | | DMF | | Acetone | |
|---|---|---|---|---|---|---|---|---|
| | HNP (mV) | p$K_a$ | HNP (mV) | p$K_a$ | HNP (mV) | p$K_a$ | HNP (mV) | p$K_a$ |
| **4a** | — | — | −142 | 10.00 | — | — | −214 | 10.07 |
| **4b** | −377 | 14.80 | −343 | 14.17 | −336 | 14.06 | −146 | 9.81 |
| **4c** | −172 | 9.73 | — | — | −400 | 15.20 | — | — |
| **4d** | −253 | 11.47 | — | — | −364 | 14.67 | — | — |
| **4e** | −432 | 16.08 | −195 | 10.45 | −398 | 15.34 | — | — |
| **4f** | −244 | 11.75 | — | — | −467 | 15.66 | −609 | — |
| **4g** | — | — | — | — | −730 | — | −160 | 9.87 |

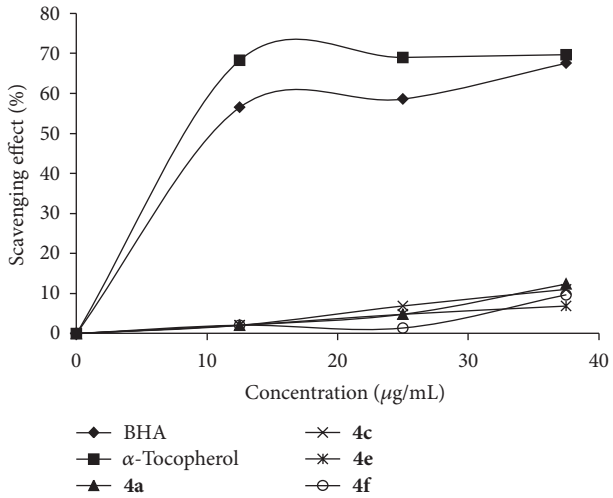



Figure 1: Scavenging effect of compounds **4a**, **4c**, **4e**, **4f**, BHA, and $\alpha$-tocopherol at different concentrations (12.5–25–37.5 $\mu$g/mL).

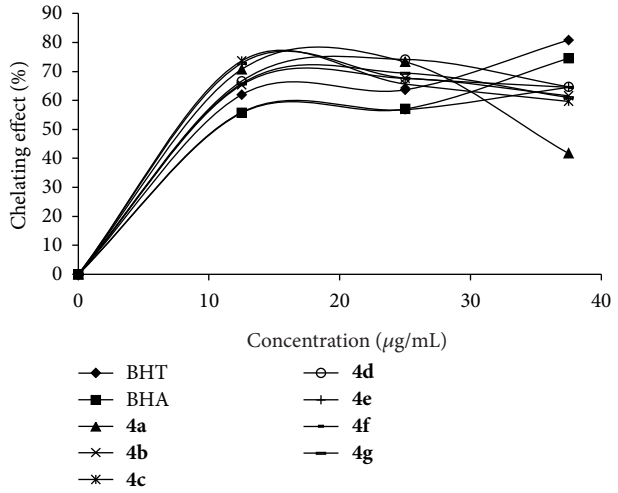



Figure 2: Metal chelating effect of different amount of the compounds **4a–g**, BHT, and BHA on ferrous ions.

alcohol > isopropyl alcohol, for compounds **4c** and **4d** it is isopropyl alcohol > DMF, for compound **4e** it is *tert*-butyl alcohol > DMF > isopropyl alcohol, for compound **4f** it is: isopropyl alcohol > DMF > acetone, while the order for compound **4 g** is acetone > DMF. Moreover, as seen in Table 1, for compounds **4a** and **4 g** in isopropyl alcohol, for compound **4a** in DMF, for compounds **4c, 4d, 4f,** and **4 g** in *tert*-butyl alcohol, and for compounds **4c, 4d,** and **4e** in acetone, the HNP values and the corresponding p$K_a$ values were not obtained.

As it is well known, the acidity of a compound depends on some factors. The two most important factors are the solvent effect and molecular structure [7, 8, 10, 12, 13, 17, 18, 34]. Table 1 and Figure 4 show that the HNP values and corresponding p$K_a$ 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.

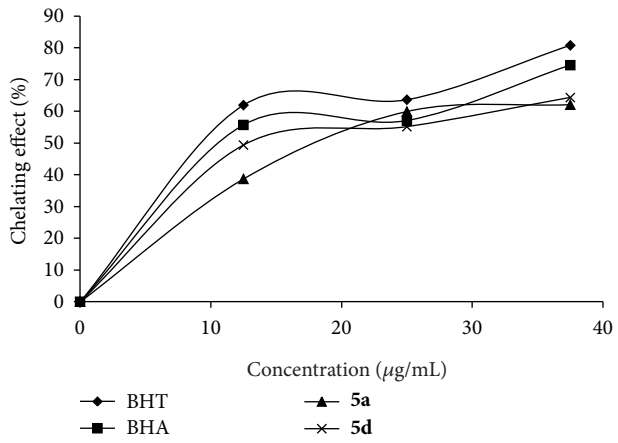



Figure 3: Metal chelating effect of different amount of the compounds **5a** and **5d**, BHT, and BHA on ferrous ions.

## 4. Conclusion

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 reported with regard to the observed radical scavenging and 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

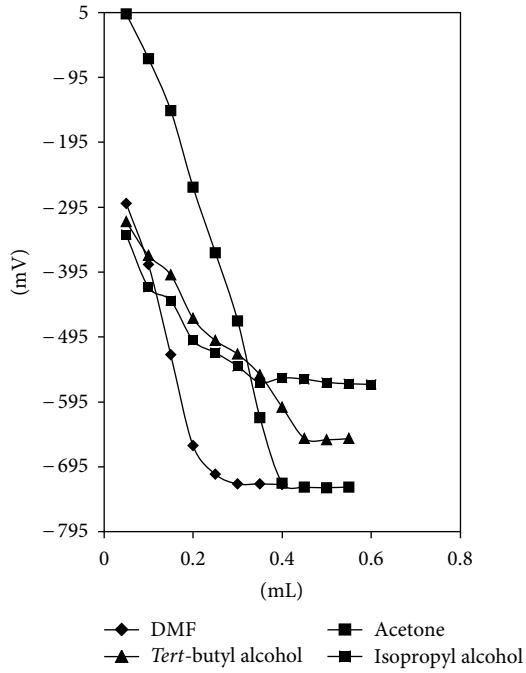



Figure 4: Potentiometric titration curves of 0.001 M solutions of compound **4b** titrated with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF, and acetone at 25°C.

chemistry. These results may also provide some guidance for the development of novel triazole-based therapeutic target.

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