

SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT PROPERTIES OF NEW 3-ALKYL (ARYL)-4-(3-HYDROXY-4-METHOXY-BENZYLIDENAMINO)-4,5-DIHYDRO-1H-1,2,4-TRIAZOL-5-ONES

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ABSTRACT

A series of novel 3-alkyl(aryl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3a-j**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2a-j**) with 3-hydroxy-4-methoxy-benzaldehyde (**3**). The new ten compounds were characterized using by IR, ¹H-NMR, ¹³C-NMR and UV spectral data. In addition, the synthesized compounds were analyzed for their in vitro potential antioxidant activities in three different methods. Compounds **3f** and **3h** showed best activity for the ferrous iron chelating activity and DPPH radical scavenging activity.

Keywords: 4,5-Dihydro-1H-1,2,4-triazol-5-one, Schiff base, antioxidant activity.

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INTRODUCTION

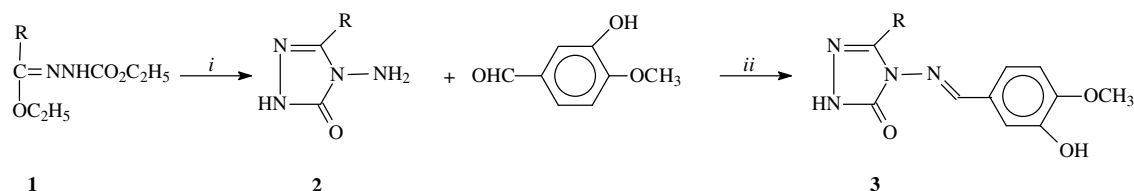
1,2,4-Triazole and their derivatives are reported to possess a broad spectrum of biological activities such as antimicrobial, anti-inflammatory, hypoglycemic, antiviral, antifungal, analgesic, antihypertensive, antitumor, anti-HIV and antioxidant properties¹⁻⁹. In addition, several articles reporting the synthesis of some *N*-arylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives have been published^{8,9}.

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, which could provide active components to prevent or reduce the impact of oxidative stress on cells¹⁰. Exogenous chemicals and endogenous metabolic processes in human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damages play a 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 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 cancer¹¹. In the present paper, the antioxidant activities of ten novel 3-alkyl(aryl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3a-j**), which were synthesized by the reactions of 3-alkyl-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2a-j**) with 3-hydroxy-4-methoxybenzaldehyde were determined (Scheme-1).

EXPERIMENTAL

Chemical reagents and all solvents used in this study were purchased from Fluka, Aldrich and Merck AG. Melting points which were uncorrected 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. ^1H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Ultrashield spectrometer at 200 MHz and 50 MHz, respectively. UV absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a Shimadzu-1201 UV/VIS spectrometer. Extinction coefficients (ϵ) are expressed in $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. The starting compounds **2a-j** were obtained from the reactions of the corresponding ester ethoxycarbonylhydrazones (**1a-j**) with an aqueous solution of hydrazine hydrate as described in the literature^{12, 13}.



(i) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, reflux, 6 h; (ii) AcOH, reflux, 1 h

(a) $\text{R} = \text{CH}_3$; (b) $\text{R} = \text{CH}_2\text{CH}_3$; (c) $\text{R} = \text{CH}_2\text{CH}_2\text{CH}_3$; (d) $\text{R} = \text{CH}_2\text{C}_6\text{H}_5$; (e) $\text{R} = \text{CH}_2\text{C}_6\text{H}_4\text{CH}_3$ (*p*-); (f) $\text{R} = \text{CH}_2\text{C}_6\text{H}_4\text{OCH}_3$ (*p*-); (g) $\text{R} = \text{CH}_2\text{C}_6\text{H}_4\text{Cl}$ (*p*-); (h) $\text{R} = \text{CH}_2\text{C}_6\text{H}_4\text{Cl}$ (*m*-); (i) $\text{R} = \text{C}_6\text{H}_5$; (j) $\text{R} = \text{cyclopropyl}$
Scheme-1

General procedure for the synthesis of 3-alkyl(aryl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (3a-j)

The corresponding compound **2** (0.01 mol) was dissolved in acetic acid (15 ml) and treated with 3-hydroxy-4-methoxybenzaldehyde (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-j** as colorless crystals.

3-Methyl-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3a)

Yield 95%, m.p. 280°C. IR (KBr, cm^{-1}): 3278 (OH), 3121 (NH), 1686 (C=O), 1606 and 1576 (C=N). ^1H NMR (DMSO- d_6 , δ): 2.24 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.00 (d, 1H, ArH, $J=8.34$ Hz), 7.17 (d, 1H, ArH, $J=8.20$ Hz), 7.31 (s, 1H, ArH), 9.50 (s, 1H, N=CH), 9.76 (s, 1H, OH), 11.78 (s, 1H, NH). ^{13}C NMR (DMSO- d_6 , δ): 11.57 (CH_3), 56.22 (OCH_3), [112.22, 112.74, 122.14, 126.64, 144.63, 151.80] (arom-C), 147.35 (triazole C_3), 151.34 (N=CH), 154.79 (triazole C_5). UV λ_{max} (ϵ , nm): 328 (13580), 318 (14235), 302 (14040), 234 (13950), 218 (12920).

3-Ethyl-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3b)

Yield 94%, m.p. 190°C. IR (KBr): 3297 (OH), 3180 (NH), 1682 (C=O), 1609 and 1581 (C=N) cm^{-1} . ^1H NMR (DMSO- d_6): δ 1.23 (t, 3H, CH_2CH_3 , $J=7.20$ Hz), 2.67 (q, 2H, CH_2CH_3 , $J=7.26$ Hz), 3.83 (s, 3H, OCH_3), 7.02 (d, 1H, ArH, $J=8.08$ Hz), 7.18-7.35 (m, 2H, ArH), 9.53 (s, 1H, N=CH), 9.38 (s, 1H, OH), 11.80 (s, 1H, NH). UV λ_{max} (ϵ): 322 (12610), 234 (9030), 216 (11410) nm.

3-*n*-Propyl-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3c)

Yield 91%, m.p. 188°C. IR (KBr): 3280 (OH), 3191 (NH), 1696 (C=O), 1601 and 1592 (C=N) cm^{-1} . ^1H NMR (DMSO- d_6): δ 0.94 (t, 3H, $\text{CH}_2\text{CH}_2\text{CH}_3$, $J=7.40$ Hz), 1.67 (sext, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$, $J=7.40$ Hz), 2.63 (t, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$, $J=7.30$ Hz), 3.82 (s, 3H, OCH_3), 7.00 (d, 1H, ArH, $J=8.36$ Hz), 7.16 (d, 1H, ArH, $J=8.31$ Hz), 7.31 (s, 1H, ArH), 9.49 (s, 1H, N=CH), 9.21 (s, 1H, OH), 11.75 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 13.94 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 19.36 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 27.27 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 56.05 (OCH_3), [112.24, 112.67, 122.12, 126.70, 147.30, 151.89] (arom-C), 147.41 (triazole C_3), 151.37 (N=CH), 154.77 (triazole C_5). UV λ_{max} (ϵ): 316 (13915), 314 (14265), 308 (14220), 294 (13575), 234 (14030), 218 (13020) nm.

3-Benzyl-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3d)

Yield 93%, m.p. 213°C. IR (KBr): 33050 (OH), 3181 (NH), 1718 (C=O), 1595 (C=N), 764 and 710 (monosubstituted aromatic ring) cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.82 (s, 3H, OCH₃), 4.02 (s, 2H, CH₂Ph), 7.00 (d, 1H, ArH, $J=8.25$ Hz), 7.15 (d, 1H, ArH, $J=8.34$ Hz), 7.22-7.32 (m, 6H, ArH), 9.48 (s, 1H, N=CH), 9.77 (s, 1H, OH), 11.80 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 31.56 (CH₂Ph), 56.07 (OCH₃), [112.25, 112.88, 122.11, 126.63, 146.65, 151.80] [127.18, 128.91 (2C), 129.27 (2C), 136.29] (arom-C), 147.38 (triazole C₃), 151.40 (N=CH), 154.62 (triazole C₅). UV λ_{max} (ϵ): 320 (15380), 234 (12630), 218 (14470) nm.

3-(*p*-Methylbenzyl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3e)

Yield 91%, m.p. 215°C. IR (KBr): 3423 (OH), 3173 (NH), 1701 (C=O), 1606 and 1575 (C=N), 810 (1, 4-disubstituted aromatic ring) cm^{-1} . ^1H NMR (DMSO- d_6): δ 2.24 (s, 3H, PhCH₃), 3.83 (s, 3H, OCH₃), 3.96 (s, 2H, CH₂Ph), 7.00 (d, 1H, ArH, $J=8.36$ Hz), 7.09-7.21 (m, 5H, ArH), 7.30 (s, 1H, ArH), 9.47 (s, 1H, N=CH), 9.18 (s, 1H, OH), 11.85 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 21.05 (PhCH₃), 31.17 (CH₂Ph), 56.08 (OCH₃), [112.26, 112.84, 122.11, 126.65, 146.78, 151.80] [129.14 (2C), 129.47 (2C), 133.18, 136.23] (arom-C), 147.39 (triazole C₃), 151.39 (N=CH), 154.53 (triazole C₅). UV λ_{max} (ϵ): 320 (17970), 314 (17920), 234 (16400), 222 (17440) nm.

3-(*p*-Methoxybenzyl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3f)

Yield 97%, m.p. 218°C. IR (KBr): 3524 (OH), 3188 (NH), 1704 (C=O), 1609 and 1587 (C=N), 808 (1, 4-disubstituted aromatic ring) cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.70 (s, 3H, *p*-OCH₃), 3.85 (s, 3H, OCH₃), 3.95 (s, 2H, CH₂Ph), 6.87 (d, 2H, ArH, $J=8.54$ Hz), 7.01 (d, 1H, ArH, $J=8.37$ Hz), 7.17 (d, 1H, ArH, $J=8.34$ Hz), 7.25 (d, 2H, ArH, $J=8.59$ Hz), 7.33 (s, 1H, ArH), 9.49 (s, 1H, N=CH), 9.37 (s, 1H, OH), 11.75 (s, 1H, NH). UV λ_{max} (ϵ): 318 (16200), 284 (11910), 228 (18565) nm.

3-(*p*-Chlorobenzyl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3g)

Yield 98%, m.p. 225°C. IR (KBr): 3423 (OH), 3177 (NH), 1703 (C=O), 1606 and 1576 (C=N), 811 (1, 4-disubstituted aromatic ring) cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.83 (s, 3H, OCH₃), 4.03 (s, 2H, CH₂Ph), 7.01 (d, 1H, ArH, $J=8.34$ Hz), 7.15 (d, 1H, ArH, $J=8.34$ Hz), 7.26-7.39 (m, 5H, ArH), 9.47 (s, 1H, N=CH), 9.39 (s, 1H, OH), 11.90 (s, 1H, NH). UV λ_{max} (ϵ): 320(17810), 234 (13460), 222 (20730) nm.

3-(*m*-Chlorobenzyl)-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3h)

Yield 98%, m.p. 198°C. IR (KBr): 3254 (OH), 3180 (NH), 1682 (C=O), 1608 and 1589 (C=N), 882 and 765 (1, 3-disubstituted aromatic ring) cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.83 (s, 3H, OCH₃), 4.05 (s, 2H, CH₂Ph), 7.01 (d, 1H, ArH, $J=8.36$ Hz), 7.17 (d, 1H, ArH, $J=8.29$ Hz), 7.28-7.39 (m, 5H, ArH), 9.48 (m, 2H, N=CH + OH), 11.70 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 31.11 (CH₂Ph), 56.11(OCH₃), [112.28, 113.02, 122.03, 126.56, 146.12, 151.75] [127.24, 128.14, 129.26, 130.73, 133.42, 138.66] (arom-C), 147.39 (triazole C₃), 151.39 (N=CH), 154.53 (triazole C₅). UV λ_{max} (ϵ): 318 (18140), 234 (15790), 222 (17785) nm.

3-Phenyl-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3i)

Yield 96%, m.p. 217°C. IR (KBr): 3377 (OH), 3184 (NH), 1714 (C=O), 1602 and 1579 (C=N), 771 and 692 (monosubstituted aromatic ring) cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.85 (s, 3H, OCH₃), 7.04 (d, 1H, ArH, $J=8.36$ Hz), 7.22 (d, 1H, ArH, $J=8.32$ Hz), 7.30 (s, 1H, ArH), 7.52-7.55 (m, 3H, ArH), 7.90-7.93 (m, 2H, ArH), 9.43 (m, 2H, N=CH + OH), 12.32 (s, 1H, NH). UV λ_{max} (ϵ): 322 (15070), 280 (12790), 238 (16840), 218 (16870) nm.

3-Cyclopropyl-4-(3-hydroxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (3j)

Yield 95%, m.p. 217°C. IR (KBr): 3306 (OH), 3190 (NH), 1693 (C=O), 1607 and 1587 (C=N) cm^{-1} . ^1H NMR (DMSO- d_6): δ 0.89-1.00 (m, 4H, CH_2CH_2), 2.03-2.12 (m, 1H, CH), 3.83 (s, 3H, OCH_3), 7.01 (d, 1H, ArH, $J=8.35$ Hz), 7.20 (d, 1H, ArH, $J=8.31$ Hz), 7.33 (s, 1H, ArH), 9.50 (s, 1H, N=CH), 9.40 (s, 1H, OH), 11.75 (s, 1H, NH). UV λ_{max} (ϵ): 314 (14340), 284 (12225), 236 (13740), 224 (13550) nm.

Antioxidant Activity

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

Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu¹⁴. Different concentrations of the samples (100, 250 and 500 $\mu\text{g}/\text{mL}$) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. after which a portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl_3 (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity

Free radical scavenging activity of compounds was measured by DPPH \cdot , using the method of Blois¹⁵. Briefly, 0.1 mM solution of DPPH \cdot in ethanol was prepared, and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50-250 $\mu\text{g}/\text{mL}$). 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 spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH \cdot concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

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

The capability to scavenge the DPPH radical was calculated using the following equation (2):

$$\text{DPPH}\cdot \text{ scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100 \quad (2)$$

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

Metal chelating activity

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis *et al*¹⁶. Shortly, the synthesized compounds (50-250 $\mu\text{g}/\text{mL}$) were added to a 2 mM solution of FeCl_2 (50 μL). The reaction was initiated by the addition of 5 mM ferrozine (200 μL) 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 (Equation-3):

$$\% \text{ Inhibition} = (A_0 - A_1/A_0) \times 100 \quad (3)$$

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.

RESULTS AND DISCUSSION

In this study, the structures of ten new 3-alkyl(aryl)-4-(3-hydroxy-4-methoxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3a-j**) were identified using by IR, ¹H-NMR, ¹³C-NMR and UV spectral data.

Antioxidant Activity

The synthesized compounds (**3a-j**) were screened for their *in-vitro* antioxidant activities. Several different 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 synthesized compounds are assessed by the extent of conversion of the Fe³⁺/ferricyanide complex to the Fe²⁺/ferrous form. According to this study, 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 activity¹⁷. 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 scavenging¹⁸.

In the present study, all of the compounds had a lower absorbance than the control. Hereby, no activity was observed for reducing metal ion complexes to their lower oxidation state or for any electron transfer reaction. Therefore, the compounds did not exhibit any reductive activity.

DPPH• radical scavenging activity

The scavenging of the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability¹⁹. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule²⁰. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants.

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 antioxidants²¹. As a reference to antioxidant compounds were used BHT, BHA and α -tocopherol. Compounds **3e**, **3f** and **3h** showed activity at moderate value closer to standard BHT antioxidant. Therefore, these compounds tested with this method exhibited DPPH free radical scavenging activity in a concentration-dependent manner. Figure-1 illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of synthesized these compounds. The other compounds did not show activity in a concentration-dependent manner. The radical scavenging effect of the compounds and standards decreased in the order of α -tocopherol > BHA > BHT > **3e** > **3h** > **3f** which were 77.8, 76.5, 53.7, 24.1, 22.8, 21.6 (%), at the highest concentration, respectively.

Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe²⁺. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of color reduction therefore allows estimation of the chelating activity of the coexisting chelator²². Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe³⁺) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe²⁺, depending on condition, particularly pH²³ 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 processes²⁴.

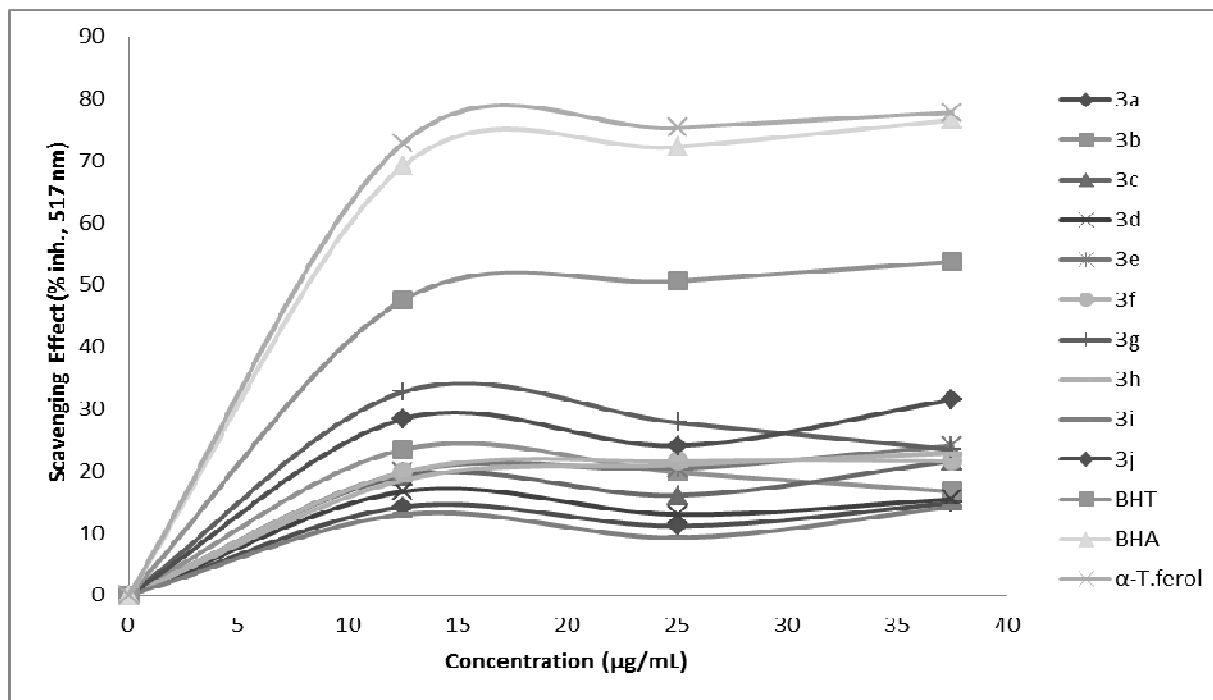
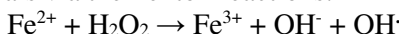


Fig.- 1: Scavenging effect of compounds 3a-j, BHT, BHA and α -tocopherol at different concentrations (12.5-25-37.5 $\mu\text{g/mL}$).

Also, the production of highly active ROS such as $\text{O}_2^{\cdot-}$, H_2O_2 and OH^{\cdot} is also catalyzed by free iron through Haber-Weiss reactions:



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^{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 ions²⁵.

Ferrous ion chelating activities of the newly synthesized compounds, BHA, BHT and α -tocopherol are shown in Figure-2. In the present study, metal chelating activity 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 ion²⁶.

The results obtained from Figure-2 reveal that the synthesized compounds, especially **3f**, **3h**, and **3i** demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. The metal chelating effect of the compounds and references decreased in the order of **3h** > **3i** > **3f** > BHA > α -tocopherol > BHT, which were 75.5, 74.2, 65.3, 54.8, 54.3, 53.7 (%), at the highest concentration, respectively.

CONCLUSION

The synthesis of ten new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are described. They were characterized by using IR, ^1H NMR, ^{13}C NMR and UV spectral data. In addition to, synthesized newly

compounds evaluated antioxidant activity in three different methods (reducing power, free radical scavenging and metal chelating activity), were drawn their graphs and their results were interpreted. According to the data obtained antioxidant activity, especially compounds **3f** and **3h** demonstrate a marked capacity for the antioxidant activity. 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 chemistry.

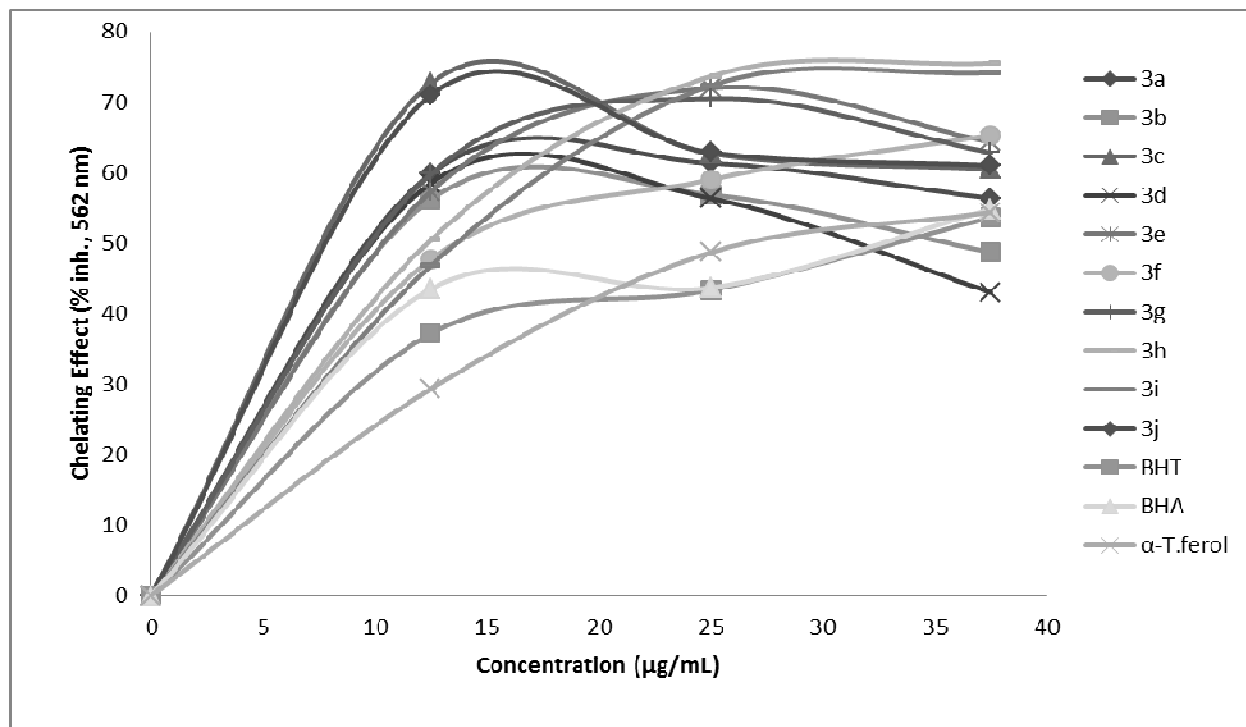


Fig.- 2: Metal chelating effect of different amount of the compounds 3a-j, BHT, BHA and α -tocopherol on ferrous ions.

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