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Synthesis and antimicrobial studies of some new [2-methoxy-4-(3-alkyl/ary)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-azomethinphenyl] acetates with their antioxidant activities

Haydar Yüksek[1], Abdülmelik Aras[1], Murat Beytur[1], Sevda Manap[1], Özlem Gürsoy-Kol[1*] and Muzaffer Alkan[2]

[1]Department of Chemistry, Kafkas University, 36100 Kars, Turkey
[2]Education Faculty, Kafkas University, 36100 Kars, Turkey

ABSTRACT

*In this study, nine novel [2-Methoxy-4-(3-alkyl/ary)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-azomethinphenyl acetates (**4**) were synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2**) with 3-methoxy-4-acetoxybenzaldehyde (3). The newly synthesized compounds were characterized using 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, including reducing power, free radical scavenging and metal chelating activity.*

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

INTRODUCTION

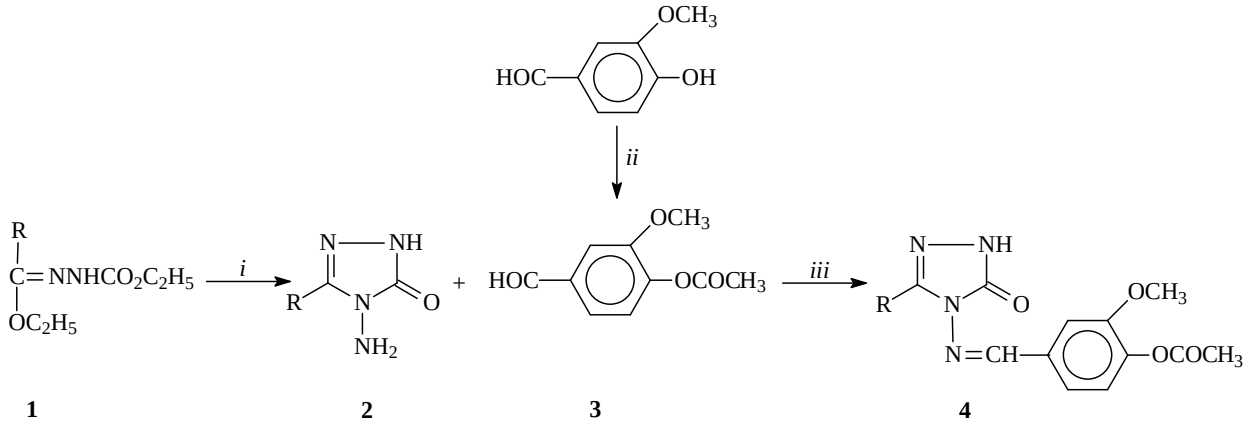
In the last two decades there has been a growing attention in the role of reactive oxygen species (ROS) and nitrogen species (RNS) in food, drugs, and even living system. Therefore, scientists in diverse disciplines have become more curious about naturally-occurring antioxidant as well as in related synthetic derivatives that could supply active components which prohibit or decrease the effect of oxidative stress [1].

External chemicals and internal metabolic processes in the human body or in the food system may generate highly reactive free radicals. At high concentrations, they could be important mediators of damage among cell structures, including lipids and membranes, proteins, and nucleic acids [2]. In this regard, it is important to search for and synthesize new classes of compounds that have antioxidant properties.

1,2,4-Triazole derivatives are recorded to own a wide variety of pharmacological activities like antibacterial [3], antioxidant [4], anti-inflammatory [5], antiparasitic [6], analgesic [7], antiviral [8], antitumor [9], anti-HIV [10], antihypertensive and diuretic [11] properties. Besides, a few articles declaring the synthesis of several N-arylidenamino-4, 5-dihydro-1H-1, 2, 4-triazol-5-one derivatives have been reported so far [3-4].

In this paper, we present the synthesis of a series of [2-Methoxy-4-(3-alkyl/ary)-4, 5-dihydro-1H-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetates **(4a-i)**. The starting compounds 3-alkyl/aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(2a-i)** were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones **(1a-i)** with an aqueous solution of hydrazine hydrate as described in the literature [12,13]. Compounds **4a-i** were obtained from the reactions of compounds **2a-i** with 3-methoxy-4-acetoxybenzaldehyde (**3**) [14], which was synthesized by the reaction of 3-methoxy-4-hydroxybenzaldehyde with acetic anhydride (Scheme **1**). In addition, due to a wide range of applications to find their possible antioxidant activity, the newly synthesized compounds were investigated

by using different antioxidant methodologies: 1, 1-diphenyl-2-picryl-hydrazyl (DPPH·) free-radical scavenging, reducing power and metal chelating activities.



1 **2** **3** **4**

i) $N_2H_4.H_2O$, reflux, 6 h; ii) Ac_2O, reflux, 1 h; iii) AcOH, reflux, 1.5 h

a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2CH_2CH_3$, d) R = $CH_2C_6H_5$, e) R = $CH_2C_6H_4CH_3$ (*p*-), f) R = $CH_2C_6H_4OCH_3$ (*p*-), g) R = $CH_2C_6H_4Cl$ (*p*-), h) R = $CH_2C_6H_4Cl$ (*m*-), i) R = C_6H_5

Scheme 1

EXPERIMENTAL SECTION

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points which were uncorrected were determined in open glass capillaries using a WRS-2A Microproceeor Melting-point apparatus. The IR spectra were recorded on an Perkin Elmer Instruments Spectrum One FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained 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 PG Instruments Ltd T80 UV/VIS spectrometer. Extinction coefficients (ε) are expressed in $L \cdot mol^{-1} \cdot cm^{-1}$.

General Method for the Synthesis of [2-Methoxy-4-(3-alkyl/ary)-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetates (4a-i)

3-Methoxy-4-hydroxybenzaldehyde (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 recrystallized from ethanol to afford compound **3**. m.p. 87 °C. IR (υ, cm^{-1}): 2842 and 2782 (CHO), 1763, 1677 (C=O), 1249 (COO). UV λmax (ε): 324 (3050), 258 (9740), 226 (12770). The corresponding compound **2** (0.01 mol) was dissolved in acetic acid (20 mL) and treated with 3-methoxy-4-acetoxybenzaldehyde (**3**) (0.01 mol). The mixture was refluxed for 2 h and then evaporated at 50–55 °C in *vacuo*. Several recrystallizations of the residue from ethanol gave pure compound **4** as colorless crystals.

[2-Methoxy-4-(3-methyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4a): Yield: 97%, m.p. 195-6°C. IR (υ, cm^{-1}): 3183 (NH), 1770, 1700 (C=O), 1596, 1578 (C=N), 1267 (COO). ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.30 (s, 3H, $OCOCH_3$), 3.80 (s, 3H, OCH_3), 6.83-7.51 (m, 3H, ArH), 9.80 (s, 1H, N=CH), 11.78 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 11.77 (CH_3), 20.77 ($OCO\underline{C}H_3$), 56.27 (OCH_3), 118.13, 119.89, 127.58, 127.68, 144.87, 152.10 (arom-C), 144.90 (triazole C_3), 148.93 (N=CH), 151.99 (triazole C_5), 169.05 (COO). UV λ_{max} (ε): 296 (10650), 264 (9830), 260 (8140), 234 (9560) nm.

[2-Methoxy-4-(3-ethyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4b): Yield: 95%, m.p. 184-5°C. IR (υ, cm^{-1}): 3178 (NH), 1773, 1709 (C=O), 1600, 1588 (C=N), 1253 (COO). ^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H, $CH_2\underline{CH}_3$, *J*=7.2 Hz), 2.30 (s, 3H, $OCOCH_3$), 2.64 (q, 2H, $\underline{CH}_2CH_3$, *J*=7.2 Hz), 3.82 (s, 3H, OCH_3), 6.84-7.50 (m, 3H, ArH), 9.81 (s, 1H, N=CH), 11.73 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 10.67 ($CH_2\underline{CH}_3$), 19.23 ($\underline{CH}_2CH_3$), 20.75 ($OCO\underline{C}H_3$), 56.59 (OCH_3), 118.27, 119.94, 127.62, 127.71, 139.93, 152.14 (arom-C), 148.63 (triazole C_3), 148.93 (N=CH), 152.10 (triazole C_5), 169.03 (COO). UV λ_{max} (ε): 296 (10080), 260 (9580), 234 (8710) nm.

[2-Methoxy-4-(3-*n*-propyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4c): Yield: 93%, m.p. 185-6°C. IR (υ, cm^{-1}): 3205 (NH), 1769, 1719 (C=O), 1590, 1576 (C=N), 1254 (COO). ^{1}H NMR (DMSO-d_6): δ 0.92 (s, 3H, $CH_2CH_2\underline{CH}_3$, *J*=7.4 Hz), 1.65 (sext, 2H, $CH_2\underline{CH}_2CH_3$, *J*=7.4 Hz), 2.30 (s, 3H, $OCOCH_3$),

2.60 (t, 2H, $\underline{CH_2}CH_2CH_3$, *J*=7.4 Hz), 3.81 (s, 3H, OCH_3), 6.80-7.51 (m, 3H, ArH), 9.79 (s, 1H, N=CH), 11.80 (s,1H, NH). UV λ_{max} (ε): 294 (12540), 270 (10940), 260 (10380), 232 (12280) nm.

[2-Methoxy-4-(3-benzyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4d): Yield: 92%, m.p. 198-9°C. IR (υ, cm^{-1}): 3188 (NH), 1773, 1718 (C=O), 1591, 1575 (C=N), 1277 (COO), 779 and 704 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, $OCOCH_3$), 3.79 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2Ph), 6.81-7.51 (m, 8H, ArH), 9.78 (s, 1H, N=CH), 11.98 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.74 ($OCO\underline{C}H_3$), 31.67 (CH_2Ph), 56.80 (OCH_3), 117.76, 127.38, 127.42, 127.65, 127.67, 129.12 (2C), 129.39, 129,46 (2C), 138.38, 152.08 (arom-C), 146.84 (triazole C_3), 148.65 (N=CH), 151.96 (triazole C_5), 169.06 (COO). UV λ_{max} (ε): 294 (12480), 268 (10865), 232 13510), 216 (12050) nm.

[2-Methoxy-4-(3-*p*-methylbenzyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4e): Yield: 97%, m.p. 194-6°C. IR (υ, cm^{-1}): 3188 (NH), 1777, 1715 (C=O), 1592 (C=N), 1281 (COO), 827 (1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 3H, $PhCH_3$), 2.27 (s, 3H, $OCOCH_3$), 3.78 (s, 3H, OCH_3), 3.95 (s, 2H, CH_2Ph, 6.79-7.48 (m, 7H, ArH), 9.76 (s, 1H, N=CH), 11.91 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.76 ($OCO\underline{C}H_3$), 21.29 ($PhCH_3$), 31.29 (CH_2Ph), 56.77 (OCH_3), 115.91, 117.71, 120.01, 127.74, 129.33 (2C), 129,72 (2C), 133.25, 136.51, 139.95, 152.05 (arom-C), 147.87 (triazole C_3), 148.77 (N=CH), 151.97 (triazole C_5), 169.14 (COO). UV λ_{max} (ε): 294 (10060), 268 (9210), 232 (9840), 214 (8150) nm.

[2-Methoxy-4-(3-*p*-methoxybenzyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4f): Yield: 95%, m.p. 165-6°C. IR (υ, cm^{-1}): 3181 (NH), 1775, 1717 (C=O), 1592 (C=N), 1249 (COO), 826 (1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H, $OCOCH_3$), 3.67 (s, 3H, *p*-$PhOCH_3$), 3.78 (s, 3H, OCH_3), 3.93 (s, 2H, CH_2Ph, 6.83 (d, 2H, ArH, J=7.8 Hz), 7.03-7.46 (m, 3H, ArH), 7.19 (d, 2H, ArH, J=8.6 Hz), 9.76 (s, 1H, N=CH), 11.89 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.78 ($OCO\underline{C}H_3$), 30.81 (CH_2Ph), 55.65 (*p*-$PhOCH_3$), 56.77 (OCH_3), 114.53 (2C), 115.91, 118.06, 120.02, 127.59, 1597 (triazole C_5), 169.17 (COO). UV λ_{max} (ε): 294 (11220), 270 (10890), 234 (12370), 216(10675) nm.

[2-Methoxy-4-(3-*p*-chlorobenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-azomethinphenyl acetate (4g): Yield: 97%, m.p. 178-9°C. IR (υ, cm^{-1}): 3182 (NH), 1777, 1714 (C=O), 1603, 1589 (C=N), 1251 (COO), 850 (1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3H, $OCOCH_3$), 3.79 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2Ph, 6.81-7.46 (m, 7H, ArH), 9.78 (s, 1H, N=CH), 11.97 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.75 ($OCO\underline{C}H_3$), 31.00 (CH_2Ph), 56.81 (OCH_3), 116.02, 117.77, 127.61, 127.68, 129.06 (2C), 131.33, 131.40 (2C), 133.35, 135.45, 152.09 (arom-C), 146.50 (triazole C_3), 148.75 (N=CH), 151.93 (triazole C_5), 169.04 (COO). UV λ_{max} (ε): 294 (10895), 270 (9500), 232 (12370), 218 (11120) nm.

[2-Methoxy-4-(3-*m*-chlorobenzyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4h): Yield: 94%, m.p. 187-9°C. IR (υ, cm^{-1}): 3188 (NH), 1766, 1699 (C=O), 1592 (C=N), 1266 (COO), 779 and 693 (1,3-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, $OCOCH_3$), 3.78 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2Ph, 6.83-7.44 (m, 7H, ArH), 9.76 (s, 1H, N=CH), 12.01 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.79 ($OCO\underline{C}H_3$), 31.27 (CH_2Ph), 56.78 (OCH_3), 116.00, 117.68, 120.62, 127.49, 128.25, 129.65, 131.01, 133.61, 138.75, 139.98, 146.38, 152.05 (arom-C), 147.89 (triazole C_3), 148.78 (N=CH), 151.96 (triazole C_5), 169.15 (COO). UV λ_{max} (ε): 294 (9895), 270(8670), 232 (10140), 214 (8510) nm.

[2-Methoxy-4-(3-phenyl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-one-4-yl)-azomethinphenyl acetate (4i): Yield: 98%, m.p. 258-9°C. IR (υ, cm^{-1}): 3164 (NH), 1770, 1708 (C=O), 1600 (C=N), 1251 (COO), 778 and 693 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.18 (s, 3H, $OCOCH_3$), 3.79 (s, 3H, OCH_3), 6.80-7.39 (m, 3H, ArH), 7.48-7.50 (m, 3H, ArH), 7.83 (m, 2H, ArH), 9.91 (s, 1H, N=CH), 12.32 (s,1H, NH). UV λ_{max} (ε): 270 (18110), 226 (25650), 218 (25220) nm.

Antioxidant Activity

Chemicals: Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA) 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 [15]. 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 $FeCl_3$ (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˙ using the method of Blois [16]. 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 spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH˙ concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

Absorbance = 0.0003 x DPPH˙ – 0.0174

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

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

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*. [17]. Briefly, 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 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. 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.

Antimicrobial Activity

Simple susceptibility screening test using agar-well diffusion method [18] as adapted earlier [19] was used for determination of antimicrobial activities of **4a-i** compounds. All test microorganisms were obtained from the Microbiologics Environmental Protection Laboratories Company in France and are as follows; Bs: *Bacillus subtilis* (ATCC-11774), Bc: *Bacillus cereus* (ATCC-11778), Pa: *Pseudomonas aeruginosa* (ATCC-27853), Kp: *Klebsiella pneumoniae* (ATCC-4352), Sa: *Staphylococcus aureus* (ATCC-6538), Ec: *Escherichia coli* (ATCC-25922). All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 1 mg/ml.

Each microorganism was suspended in Mueller-Hinton Broth and diluted to 106 colony forming unit (cfu) per ml. They were "flood-inoculated" onto the surface of Mueller Hinton Agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 250–5000 µg/50 µl of the chemical substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Dimethylsulphoxide was used as solved control.

RESULTS AND DISCUSSION

In this study, the structures of nine novel [2-methoxy-4-(3-alkyl/ary)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-azomethinphenyl acetates **(4a-i)** were characterized using by IR, ^{1}H NMR, ^{13}C NMR and UV spectral data.

Antioxidant Activity

The compounds **4a-i** 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 reducing power of the compounds **4a-i** and standard antioxidants was determined as explained in [20,21]. In this study, all the amount of the compounds showed lower absorbance than standard antioxidants. 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, synthesized compounds did not show the reductive activities, but compounds **4a-c** showed higher activities than blank and the other compounds also their reductive ability was concentration-dependent as seen in Figure **1**.

Reducing power of the compounds and the standards were found as following order: BHA > BHT > α-tocopherol > **4c** > **4b** > **4a** for the highest concentration.

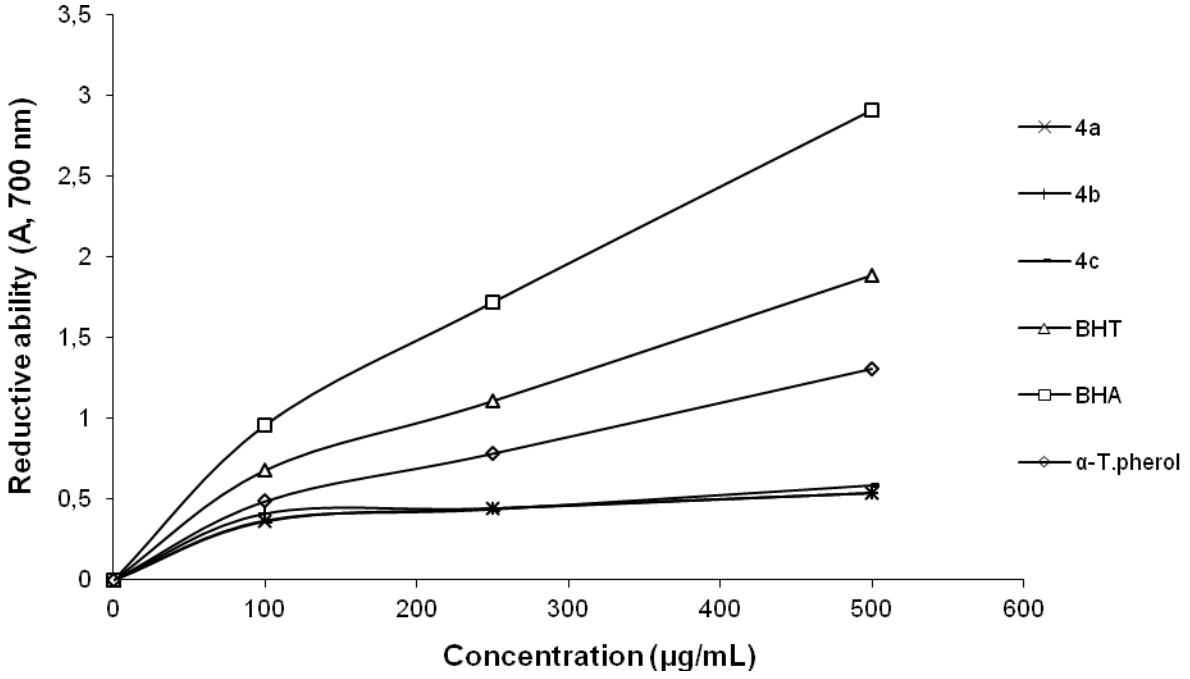


Figure 1. Total reductive potential of different concentrations of compounds 4a-c, BHT, BHA and α-tocopherol

DPPH• radical scavenging activity: Free radical scavenging effect of the compounds **4a-i** was estimated by DPPH·, as explained in [22,23]. BHT and BHA were used as a reference to antioxidant compounds. Compounds **4a, 4b, 4d, 4h** and **4i** tested with this method exhibited moderate DPPH· scavenging activity with concentration-dependent manner as seen in Figure **2**. The radical scavenging effect of the compounds and the standards were found as following order: BHA > BHT > **4b** > **4a** > **4h** > **4d** > **4i**.

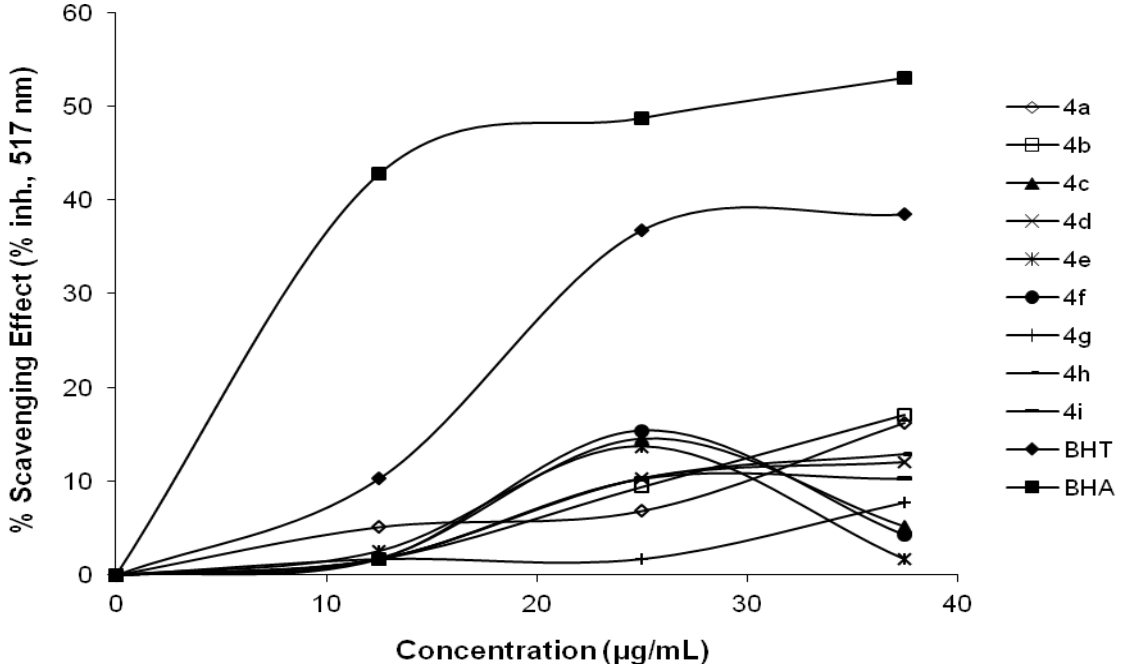


Figure 2. Scavenging effect of compounds 4a-i, BHT and BHA at different concentrations (12,5-25-37.5 µg/mL)

Ferrous ion chelating activity: The chelating of ferrous ions by the compounds **4a-i** and references was measured as explained in [23-25]. Ferrous ion chelating activities of the synthesized compounds, BHT and α-tocopherol are shown in Figure **3**. The data obtained from Figure **3** reveal that the compounds **4b** and **4g** demonstrate a marked capacity for iron binding in a concentration-dependent manner, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. The metal chelating effect of the compounds and standards decreased in the order of **4g** > BHT > **4b** > α-tocopherol which were 66.4, 65.7, 65.0, 53.3 (%), at the highest concentration, respectively.

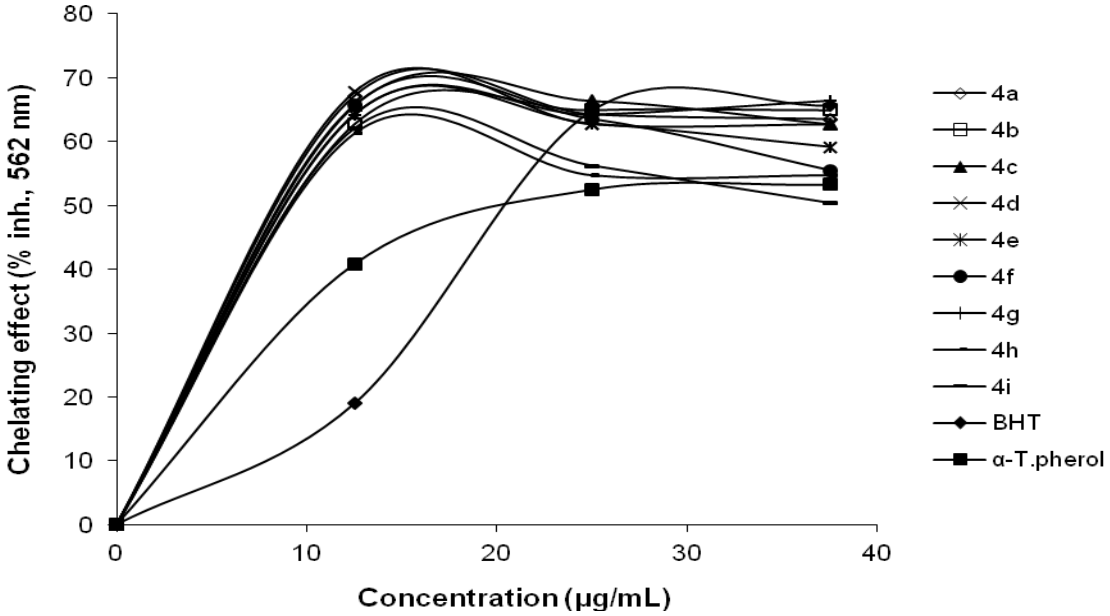


Figure 3. Metal chelating effect of different amount of the compounds 4a-i, BHT and α-tocopherol on ferrous ions

Antimicrobial Activity

The observed data for the antimicrobial activity of **4a-i** compounds were given in Table **1**. The data reveal that, all of the compounds did not display any antimicrobial activity against *Bacillus subtilis* (ATCC 10978). However some of the compounds were found to possess good antimicrobial activity against to the other tested microorganisms. The screening data also indicate that compounds **4a** and **4b** were found to be effective against to three different strains: Bc: *Bacillus cereus* (ATCC-11778), Pa: *Pseudomonas aeruginosa* (ATCC-27853) and Ec: *Escherichia coli* (ATCC-25922).

Table 1. Screening for antimicrobial activity of the 4a-i compounds

Compound	Microorganisms and inhibition zone (mm)					
	Bs	**Bc**	**Pa**	**Kp**	**Sa**	**Ec**
4a	-	23	26	15	18	22
4b	-	24	22	16	15	19
4c	-	14	20	14	13	16
4d	-	14	15	10	13	12
4e	-	10	12	15	11	8
4f	-	14	17	7	8	10
4g	-	18	18	11	10	13
4h	-	21	15	7	11	12
4i	-	16	21	9	11	13

Bs: *Bacillus subtilis* (ATCC-11774), Bc: *Bacillus cereus* (ATCC-11778), Pa: *Pseudomonas aeruginosa* (ATCC-27853), Kp: *Klebsiella pneumoniae* (ATCC-4352), Sa: *Staphylococcus aureus* (ATCC-6538), Ec: *Escherichia coli* (ATCC-25922).

CONCLUSION

The synthesis and *in-vitro* antioxidant and antimicrobial evaluation of new 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives are described. Compound **4b** demonstrates a marked capacity for both antioxidant and antimicrobial activities. Design and synthesis of novel small molecules can play specifically a protective role in biological systems and in modern medicinal chemistry. In this regard, these results may also provide some guidance for the development of novel triazole-based therapeutic target.

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